Review article

Aqueous antiseptic formulations with a neem and pine oil blend

Abstract

This study shows that a blend of pine and neem seed oils was used as a natural ingredient in a liquid disinfectant to combat bacteria. The liquid disinfectant that was produced was found to possess antibacterial qualities against Staphylococcus aureus and not on Escherichia coli as obtained from the analysis. When applied against Staphylococcus aureus, the produced liquid disinfectant at concentrations of 50 and 100 mg/ml demonstrated a larger zone of inhibition as commercial liquid disinfectant, according to the analysis, but it had no effect on Escherichia coli. In contrast, the lower concentrations of 25 and 12.5 mg/ml were less effective against Staphylococcus aureus. This new product is a natural liquid disinfectant that is free of chemicals like chloroxylon, artificial colorant, and sodium sulfate (SLS).

Volume 8 Issue 4 - 2024

Ajadi IK

Chemical Engineering Department, Ahmadu Bello University Zaria, Nigeria

Correspondence: Ibraheem Kazeem AJADI, Chemical Engineering Department, Ahmadu Bello University Zaria, Kaduna, Nigeria, Tel 08052325309, Email kazeemolawale634@gmail.com

Received: September 26, 2024 | **Published:** November 19, 2024

Introduction

Since the eighteenth century, antimicrobial agents have been used to treat infectious diseases. The screening of natural products as a possible source of novel antimicrobial substances has gained attention during the last 20 years.[1,2] Numerous substances derived from plants have demonstrated antimicrobial properties through scientific validation. Accordingly, studies carried out in a number of nations with exceptionally high plant diversity have demonstrated the efficacy of various substances in regulating the growth of microorganisms, as documented in the works of Pessini et al., Souza et al., Fiuza et al., and numerous other researchers.[3–5]

Neem leaf extract contains nimbidin, cyclic trisulphide, cyclic tetrasulphide, and polyphenolic flavonoids, which support antibacterial, antifungal, and anticancer activities. It is also rich in antioxidants, which help develop new skin cell tissues. In Ayurvedic medicine, neem leaf has been used to treat leprosy, eye problems, epistaxis, intestinal worms, anorexia, biliousness, and skin ulcers.[6] Triglycerides are the main lipid type found in neem oil. High concentrations of triglycerides, calcium, vitamin E, and essential fatty acids are found in neem oil. Owing to its elevated concentration of essential fatty acids and vitamin E, neem oil deeply penetrates the skin to mend the microscopic fissures brought about by extreme dryness. The main fatty acid in neem kernel oil is oleic acid (52.8%). Linoleic acid (2.1%), palmitic acid (12.6%), stearic acid (21.4%), and other lower fatty acids (2.3%) come next.

Ointments, toothpaste, emulsions, liquors, hair and skin care products, natural and organic cosmetics, and medicinal cosmetics are all made with neem oil.[7] However, using dried neem seeds, neem oil can be extracted chemically (solvent extraction) or mechanically (hot or cold process). Through cold pressing, the best neem oil with the majority of phytoconstituents intact is obtained. According to Ramakrishna et al.,[8] cold press oil has a milder scent and a lighter color.

The Pinaceae family, which includes the Scots pine, or Pinus sylvestris, is native to Northern and Eastern Europe. Its birthplace is Eurasia. With more than 250 species spread across the globe, the genus Pinus is the most common coniferous plant, according to Zafar et al. (2010).[9] The tree is between 25 and 40 meters tall, with evergreen, fragrant, needle-like blue-green leaves that are arranged spirally or alternately, measuring 3 to 5 centimeters.[10] Maciąg et al. claim that P. sylvestris is the most versatile pine species in the world, having a vast range of applications.[10]

The tree serves as a raw material for the paper industry in addition to being an attractive plant that lessens erosion.[11] P. sylvestris oil's anti-parasitic, anti-viral, anti-allergenic, antispasmodic, anti-hyperglycemic, anti-inflammatory, and expectorant qualities make it useful in a wide range of pharmaceutical applications.[12] Additionally, P. sylvestris terpenic oil is utilized in the chemical, fragrance, and food additive industries.[12] There have also been reports of P. sylvestris oil's insecticidal and larvicidal qualities.[13] The goal of this study was to create a liquid disinfectant that would replace synthetic disinfectants and help control infections by combining neem and pine oil. To determine the liquid disinfectant quality, the physico-chemical properties and antibacterial activity were evaluated.

Methods

Liquid disinfectant preparation

Research on the manufacturing of liquid disinfectant has been carried out at Ahmadu Bello University's Chemical Engineering Laboratory. Neem oil (20 ml) was heated for 5 minutes at 70°C. A thermometer was used to take the temperature. Four grams of KOH were combined with 10 milliliters of distilled water and left to dissolve. Using a hydrometer, the concentration of the solution was checked. The lye-water solution was prepared using the mixture. The lye-water solution was gradually added after the heated oils had been thoroughly mixed and made clear. The heated oils were combined with the lye-water solution in a beaker, and the mixture was heated using a magnetic stirrer until it reached homogeneity. After adding 300ml of distilled water to the mixture, the temperature was raised to 100°C until a clear solution was obtained. After adding 10 milliliters of pine, the mixture was left for an additional three minutes. After being meticulously packaged, the liquid disinfectant was put into containers for examination.

Soap characteristics

The following factors are used to characterize the liquid disinfectant: pH, color, and antibacterial activity.

PH

Within a small baker was five milliliters of natural liquid disinfectant. We verified the pH of the liquid disinfectant solution using a calibrated pH metre.

Color

The liquid disinfectant solution form a milk like color

Antibacterial activity

Culture media:

One culture medium that is used is Mueller Hinton agar (MHA). Potato dextrose agar (PDA), nutrient agar (NA), Muller-Hinson broth (MHB), and minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) assays were carried out using these media. To achieve sterility, each medium was prepared according to the manufacturer's instructions and autoclaved for 15 minutes at 121 degrees Celsius.

Determination of inhibitory activity (sensitivity test) of the extract using agar well diffusion method:

Using sterile swab sticks, the standardized inoculate for the bacterial isolate was streaked on sterile Mueller Hinton and potato dextrose agar plates, respectively. Using a sterile cork borer, four wells were punctured on each inoculated agar plate. The well was appropriately labeled with the concentrations of the prepared extract 100, 50, 25, and 12.5 mg/mL, respectively in mind. A volume of about 0.2 milliliters was used to fill each well with extract. The inoculated plates containing the extract were left on the bench for about an hour to allow the extract to diffuse on the agar. Potato dextrose agar plates were incubated for about three to five days at room temperature, while Mueller Hinton agar plates were incubated for twenty-four hours at 37°C. After the incubation period, the plates were inspected for signs of inhibition, which appeared as a distinct zone of inhibition (an area surrounding the wells without any growth).[14]

Determination of minimum inhibitory concentration (MIC):

The tube dilution method was utilized to find the extract's minimum inhibitory concentration, with Mueller Hinton broth serving as the diluent. When the extract was tested during the sensitivity test, it was serially diluted in test tubes containing Mueller-Hinton broth to the lowest concentration that showed inhibition for each organism. The standardized organisms were added to each tube containing the broth and extract. The inoculation tubes were then incubated at 37°C for a full day. At the end of the incubation period, the tubes were examined or observed using turbidity as a criterion to see if growth had taken place. The lowest concentration in the series that showed no turbidity or discernible growth indicators was called the minimum inhibitory concentration (MIC). The result was also noted.[15]

Determination of minimum bactericidal concentration (MBC):

Using the minimum inhibitory concentration (MIC) result, the minimum bactericidal concentration (MBC) of the extract was computed. Using a sterile wire loop, the test tube(s) that did not show turbidity (clear) in the MIC test were dipped into, and a loopful was taken out and streaked on sterile nutrient agar plates. The plates were incubated at 37°C for 18 to 24 hours. At the end of the incubation period, the plates were examined or observed for the presence or absence of growth. This is to determine whether the antimicrobial properties of the extract are bacteriostatic or bactericidal.[15]

Result and discussion

This study investigated the antimicrobial activity of neem and pine oil to develop pharmaceutical formulations with antimicrobial properties. In addition to their good activity, the oils were chosen to provide less expensive formulations.[16]

Neem and pine oil were used in the formulation of the preparations as an antiseptic agent. Since the liquid disinfectant was significantly less expensive than the traditional one, it also demonstrated significant antiseptic activity. In the agar diffusion tests, the liquid disinfectant demonstrated efficacy as an antiseptic against the two microorganisms tested, as indicated in Table I. When a product (using steel templates with 6 mm diameter wells) produces an inhibition zone of 8 mm or more, it is considered an antiseptic.[17]

Among all the parts of the plant, the oil in neem tree seeds is thought to possess a wide range of antibacterial activity, making it one of the most important sources of antibacterial drugs. Neem seed oil has potential applications as a chemical replacement for certain components in liquid disinfectants with advantageous therapeutic qualities, as demonstrated by the blends of neem and pine oil's antibacterial qualities, which outperformed a commercial antiseptic.[18,19] The best blending ratio for the antiseptic, according to the previous study, was found to be 20:80 of neem seed oil to eucalyptus oil.[20]

Because of the pine oil, the liquid disinfectant in this study has a faintly nutty and piney scent. It has a milky transparency.

The qualities of the liquid disinfectant were categorized based on three factors: pH, color, and antibacterial activity. The physico-chemical properties of liquid disinfectants are actually what matter (Table 1 and Figure 1).

Figure 1 Liquid antiseptic produced from blend of neem and pine oil pH.

Citation: Ajadi IK. Aqueous antiseptic formulations with a neem and pine oil blend. *Material Sci & Eng.* 2024;8(4):129‒132. DOI: 10.15406/mseij.2024.08.00245

Table 1 Physicochemical properties of prepared liquid disinfectant and commercial disinfectant

Characteristics	Prepared liquid disinfectant	Commercial liquid disinfectant
pH	7.8	9
Solubility	Soluble	Soluble
Color	Milky	Light yellow

The pH value of the liquid disinfectant, which is between 4 and 10, complies with SNI (2588:2017) standards. The formulated liquid disinfectant is within the allowed pH range, as shown in Table 1 above. More acidic substances have a pH of less than 7, and more alkaline substances have a pH of greater than 7. Seven is regarded as the neutral pH. The pH of the commercial control, which is also 9, and the pH of the liquid disinfectant used in this study are similar.[21]

Human skin has an acidic pH of 5.4 to 5.9, which is important for its ability to defend against microorganisms. Liquid disinfectant and other alkaline materials neutralize the body's defense layer, which acts as a barrier against microorganisms. Moreover, an overly alkaline pH may harm the acid mantle and the lipid lamellae of the epidermis. This may cause allergens and irritants to penetrate the skin, increasing trans-epidermal water loss and contributing to dry skin. According to Uzwatania et al.,[21] liquid disinfectant produced has a pH that is safe for skin (Table 2).

Table 2 Determination of inhibitory activity (sensitive test) of the medicated soap on the test organisms

Test organisms	Zone of inhibition (mm) at varying conc. (mg/ml) of the soap					
	100	50	25	12.5	Commercial liquid disinfectant	Control Cip (10mg)
Staphylococcus aureus,	17	16	13	10	17	22
Escherichia coli	0	0	0	0	9	19

The susceptibility of Staphylococcus aureus and Escherichia coli to liquid disinfectant concentrations is displayed in Tables 2 and 3. When used against Staphylococcus aureus, the produced liquid disinfectant at concentrations of 50 and 100 demonstrated a larger zone of inhibition as commercial liquid disinfectant, according to the analysis; however, it had no effect on Escherichia coli. In contrast, the lower concentrations of 25 and 12.5 were less effective against Staphylococcus aureus. Escherichia coli is likewise not significantly affected by commercial disinfectants; the table shows that the liquid disinfectant that was created had the strongest inhibitory effect on S. aureus. A high value denotes the susceptibility of the microorganism, or its incapacity to multiply when the liquid sample is present. This suggests that when the liquid disinfectant sample is at that concentration, it works well against the bacteria. A low value suggests that a higher dose of the liquid disinfectant sample is needed to stop growth at that concentration (Table 3).

Table 3 Determination of inhibition concentration (MCC) and determination minimum bacteriocidal concentration (MBC)

Test organisms	MIC	MBC
Staphylococcus aureus	12.5	25
Escherichia coli	0	0

Table 3 displays the liquid soap's analysis of the minimum inhibitory concentration test. The results show that the sample had an impact on Staphylococcus aureus. The minimum inhibitory concentration (MIC) of Staphylococcus aureus is 12.5, while that of Escherichia coli was 0. For Staphylococcus aureus, the minimum bacteriocidal concentration (MBC) was 25, while Escherichia coli showed no reaction to this.

In addition, formulation's stability must be evaluated. The stability study offers details on how the product performs at various time intervals while accounting for potential environmental exposure from the point of production until its expiration date. It was found that the formulations did not significantly alter under stress conditions and storage.

Conclusion

This study shows that a combination of pine oil and neem seed oil could be used as a natural ingredient in a liquid disinfectant to combat bacteria. The liquid disinfectant that was produced was found to possess antibacterial qualities against Staphylococcus aureus. This is a new product, a natural liquid disinfectant derived from neem and pine oils, instead of chemicals like sodium sulfate (SLS), artificial colorant, or chloroxylon, etc. More research is required to improve the liquid disinfectant's quality.

Acknowledgments

None.

Funding

None.

Conflicts of interest

Author declares that there are no conflicts of interest.

References

1. Silva MTG, Simas, SM, Batista TGFM, et al. Studies on antimicrobial activity, *in vitro*, of *Physalis angulata* L. (Solanaceae) fraction and physalin B bringing out the importance of assay determination. *Mem Inst Oswaldo Cruz*. 2005;100(7):779–782.
2. Suffredini IB, Paciencia MLB, Nepomuceno DC, et al. Antibacterial and cytotoxic activity of Brazilian plant extracts– Clusiaceae. *Mem Inst Oswaldo Cruz*. 2006;101(3):287–290.
3. Pessini GL, Holetz FB, Sanches NR, et al. Avaliação da atividade antibacteriana e antifúngica de extratos de plantas utilizados na medicina popular. *Rev Bras Farmacogn*. 2003;13(Suppl 1):21–24.
4. Souza GC, Haas APS, Poser GLV, et al. Ethnopharmacological studies of antimicrobial remedies in the south of Brazil. *J Ethnopharmacol.* 2003;90(1):135–143.
5. Fiúza TS, Morais SMTS, Paula JR, et al. Evaluation of antimicrobial activity of the crude ethanol extract of *Eugenia uniflora* L. leaves. *Rev Ciênc Farm Básica Apl.* 2008;29(3):245–250.
6. Alzohairy MA. Therapeutics role of *Azadirachta indica* (Neem) and their active constituents in diseases prevention and treatment. *Evid Based Complement Alternat Med*. 2016;2016:7382506.
7. Chatterjee A, Pakrashi S. The Treatise on Indian medicinal plants. Publications and directorate, New Delhi. 1994.
8. Ramakrishna G, Prasad NBL, Azeemoddin G. Cold processing neem seed, JNTU. Oil. 1993.
9. Zafar I, Fatima A, Khan SJ, et al. GC–MS studies of needles essential oils of *Pinus roxburghaii* and their antimicrobial activity from Pakistan. *Elect J Environ Agri Food Chem*. 2010;9(3):468–473.

10. Maciąg A, Milaković D, Christensen HH, et al. Essential oil composition and plant–insect relations in Scots pine (*Pinus sylvestris* L.). *Food Chem Biotechnol*. 2007;71:71–95.

11. Bergström B. Chemical and structural changes during heartwood formation in *Pinus sylvestris*. *Forestry*. 2003;76(1):45–53.

12. Silori GK, Kushwaha N, Kumar V. Essential oils from pines: chemistry and applications. In: Malik S (ed) *Essential oil research, trends in biosynthesis, analytics, industrial applications and biotechnological production*. Springer, Cham. 2019.

13. Fayemiwo KA, Adeleke MA, Okoro OP, et al. Larvicidal efficacies and chemical composition of essential oils of *Pinus sylvestris* and *Syzygium aromaticum* against mosquitoes. *Asia Pac J Trop Biomed*. 2014;4(1):30–34.

14. Girish, HV. Antibacterial activities of some important medicinal plants on human pathogenic bacteria- a comparative analysis. *World Appl Sci J*. 2008;5(3):267–271.

15. Andrew AM. Determination of a minimum inhibitory concentrations and minimum bacteriocidal concentration. *J Antimicrob Chemother*. 2001;48(1):5–16.

16. Moreira, TM, Moreira, RRD, Sacramento LVS, et al. Histochemical, phytochemical and biological screening of *Plinia caulifora* (DC.) Kausel, Myrtaceae, leaves. *Rev Bras Farmacogn.* 2010;20(1):48–53.

17. Roessler WG. Methods of testing antiseptics. In: Block SS. *Desinfection, sterilization and preservation*. 3 Ed. Philadelphya: Lea & Febiger. 1983;100–106.

18. Ameh AO, Muhammad JA, Audu HG. Synthesis and characterization of antiseptic soap from neem oil and shea butter oil. *Afr J Biotechnol*. 2013;12(29):4656–4662.

19. Mensah EE, Firempong CK. Chemical characteristics of toilet soap prepared from neem *(Azadirachta indica* A. Juss) seed oil. *Asia J Plant Sci Res*. 2011;1(4):1–7.

20. Bello A, Abdulazeez A, Abdulazeez A, et al. *Mikrobiologi Kedokteran*. 25th Ed, Jakarta: EGC. 2012;168–194.

21. Uzwatania F, Ningrum RS, Resti OS. Formulation of liquid hand soap made from neem seed oil and lemongrass essential oil. *Indonesia J Appl Res (IJAR)*. 2020;1(3):155–162.