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Antimicrobial activity of Myrobalan (*Terminalia chebula* Retz.) nuts: Application in raw skin preservation for leather making

Venkatasubramanian Sivakumar[1*], Resmi Mohan[1], Thirumalaisamy Rangasamy[2] and Chellappa Muralidharan[2]

[1]Chemical Engineering, [2]Tannery Division, CSIR–Central Leather Research Institute, Council of Scientific and Industrial Research, Adyar, Chennai–600020, India

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The antimicrobial property of some plant materials is useful as an eco-benign option. The combination of *Terminalia Chebula* Retz. nuts powder (MP) and common salt (CS) has been employed for better action. The synergistic composition involving MP and CS in 1:1 ratio with 10 % each (% w/w of raw skin) could provide short term preservation of raw skin/hide up to 45 days without any degradation of skin. About 40-50 % (% w/w based on raw skin weight) CS is employed for short term preservation of raw hides/skins in leather processing. The present method based on natural products avoids CS by 75 % and reduces significant total dissolved solids content / salinity in wastewater.

Keywords: Antimicrobial activity, Eco-benign processes, Leather processing, Natural products, Salt preservation, *Terminalia chebula* Retz.

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Introduction

The antimicrobial activity of some of the plant materials has been reported earlier[1]. Earlier report suggests that different plant materials could be used for natural dyeing of leather in an efficient manner[2]. The use of suitable plant materials having antimicrobial activity for preservation of raw skins/ hides, could be an eco-benign option for leather industry. This phytoremediation approach would have tremendous impact as an alternate method for salt based preservation; not only for leather but as a generic approach for similar sort of industrial processes wherein, antimicrobial activity is required. The conventional process using common salt (CS) raises serious environmental concerns such as salinity of ground water and total dissolved solids (TDS) in the wastewater[3], for which there are no viable treatments methods available so far. Alternatives to salt based preservation as reviewed earlier[4] have not provided successful commercial viability so far. The use of natural material chebulic myrobalan (fruit of *Terminalia Chebula* Retz.) has been chosen due to its antimicrobial activity[5] and presently studied for the efficacy in preservation of raw skin[6].

*Correspondent author
Email: vsiva1clri@gmail.com
Phone: 044-24437115

Materials and Methods

Chemicals and raw materials

Nutrient agar (general purpose medium) for the antimicrobial activity study was purchased from Titan Biotech Ltd, India. Natural plant material, myrobalan nuts were procured from Chennai based company M/s Sri Narayanan Traditional Medicine Dealer shop and broken into smaller sizes (~5 mm sizes) using crusher. Then the crushed materials were converted into free flowing powders (0.1 mm) using a Grinding mill. This powdered myrobalan powder (MP) was used for the present experimental work. The soil sample collected from the CSIR-CLRI tannery premises was used as source of microbes for inoculums preparation for antimicrobial activity studies. The discs for disc diffusion studies was prepared by punching the Whatman No. 3 filter paper into 4.5 mm disc form and then sterilized. The CS was used in the present investigation.

Raw skin preservation process

All the % of chemicals used is based on % w/w of raw weight of skins. The powdered myrobalan nuts (50 % w/w) are mixed thoroughly with the CS (50 % w/w) in order to get the synergistic composition product for short term preservation. It was stored in an air-tight container. CSIR-CLRI has developed a product of synergistic composition based on plant materials useful in short term preservation of hides/skins.

The five fresh raw goat skins without hair-slip, each weighing ~2 kg were taken and trimmed. Synergistic composition product (200 g/skin) was applied on the flesh side for short term preservation in such a way to ensure uniform distribution covering entire skin area. Similarly, five raw goat skins were taken for conventional common salt based preservation as control. Then the skins were piled one by one, each pair facing the flesh side and then covered with gunny bags to avoid further contaminations.

Antimicrobial activity studies

The Nutrient agar medium for the study was prepared by dissolving nutrient agar in distilled water and autoclaved for 15 min at 121 °C, 15 psi. Then 25 mL of this sterilized media was poured on each sterilized petriplate. After the solidification of agar, 1 mL of soil sample was spread on the agar plate. A time period of 30 min was given for drying. The antimicrobial activity test of the MP and synergistic composition of MP and CS was determined using well diffusion and disc diffusion method. For the antimicrobial activity test 5 or 10 % (w/v solution in water) of powdered MP was taken and filtered with whatman filter paper. For control process agar plates inoculated with suitable dilution of soil sample were kept in the incubator without any MP or CS at 37 °C. The result of control was compared with the experiments. Various experiments were conducted by adopting disc diffusion or well diffusion method with different concentrations of MP or CS solutions using variation in dilution of soil sample as inoculums.

Disc diffusion method

In disc diffusion method, sterilized filter paper discs were dipped in the various concentrations of MP or CS and synergistic composition solution for 2 h. The plant material impregnated discs were kept on the surface of the agar and 2 drops (60 µL) of the same solution was dropped on the disc in order to keep the disc wet. Then these plates were incubated at 37 °C for 24 h in inverted position. The diameters of the inhibition zones were measured (in cm). The disc diffusion method was used for the antimicrobial activity studies of 5 or 10 % (w/v solution in water) of individual MP and CS and as well as synergistic combination (5 % MP + 5 % CS as w/v solution).

Well diffusion method

For well diffusion assay, a small well (9 mm diam. holes cut in the agar gel) was made in the middle of the inoculated agar plate and 500 µL solution of MP/CS was filled in the well. Then the plates kept in the incubator at 37 °C.

Anti microbial activity of 10 % salt and plant material

The antimicrobial activity of MP was compared with the activity of CS by using disc and well diffusion method. For the comparative studies, 10 % (w/v) of each CS and MP solution were prepared and the disc impregnated in each of these solutions was used for the disc diffusion method. For the well diffusion method 500 µL of these solutions were used and 10^{-4} dilution of soil sample was used as inoculum. The plates were incubated at 37 °C.

Antimicrobial activity of synergistic composition

The individual concentration of 5 and 10 % (w/v) CS and MP were prepared. In addition, synergistic combination (5 % MP + 5 % CS, w/v) was also prepared. The antimicrobial activity of synergistic composition was studied using disc diffusion method and the results were compared with 10 and 5 % (w/v) of individual MP and CS, respectively. The soil sample of 10^{-6} dilution was used as inoculum for the study.

Results and Discussion

Antimicrobial activity of 10 % salt and plant material

After 24 h of incubation, no inhibition zone was found in control process and CS containing petriplates in either disc or well (Plate 1a,b,e). In both disc and well diffusion method of MP extract, an inhibition zone was formed and microorganisms grew around the inhibition zone as shown in Plate 1c,d. The results

Plate 1—Comparative antimicrobial activity studies using 10 % MP extract and CS by using disc and well diffusion method. a) Control, b) CS (disc diffusion), c) MP extract (disc diffusion), d) MP (well diffusion) and e) CS (well diffusion).

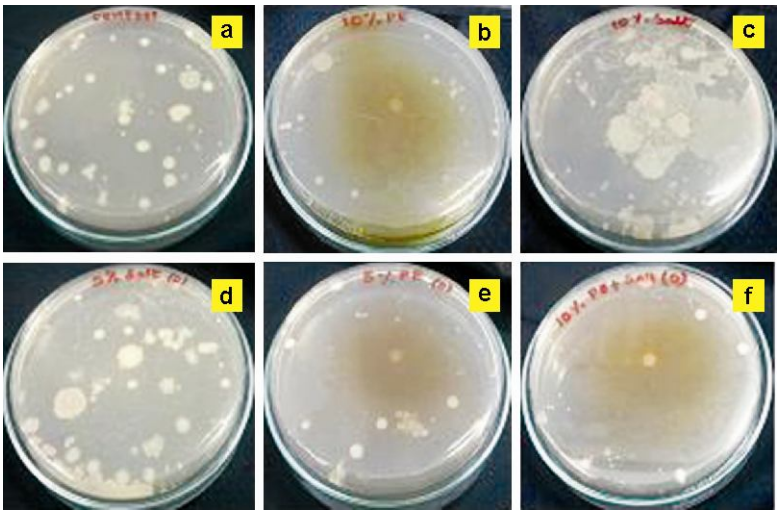

Plate 2—Comparative antimicrobial studies of MP and CS at different concentration using 10^{-6} dilution of soil sample. a) Control, b) 10 % MP, c) 10 % CS, d) 5 % CS, e) 5 % MP and f) 5 % CS + 5 % MP (synergistic composition).

Table 1—Area of inhibition zone formed for synergistic composition, myrobalan powder and common salt

Concentration	Diameter of inhibition zone (cm)	Area of inhibition zone (cm^2)
5 % MP + 5 % CS	6	28.26
5 % CS	No inhibition zone	-
5 % MP	3.5	9.61
Control	No inhibition zone	-
10 % MP	4.2	13.84
10 % CS	No inhibition zone	-

MP-Myrobalan powder, CS-Common salt

indicate that low concentration of MP has better activity against almost all microorganisms compared to CS which needs 40-50 % of concentration for the antimicrobial activity. Because of the low concentration of CS, salt tolerant (halophilic) microorganisms might have grown around the CS impregnated disc and well.

Antimicrobial activity of synergistic composition

Table 1 shows the area of the inhibition zone formed during the course of the observation. After 24 h of incubation, result shows that 10 % (5 % MP + 5 % CS, w/v) synergistic composition has more area of inhibition zone when compared with 10% (*w/v*) and 5% (*w/v*) individual MP and CS. This show that even in low concentration of salt, MP has better antimicrobial activity due to synergistic effect with CS as shown in Plate 2. Thus, it can be used for the preservation of raw hides and skins.

Raw skin preservation

Raw goat skins were preserved for 45 days using 10 % of MP and CS synergistic composition without any sign of putrefaction or bad odor of the skins. Raw skins didn't show sign of hair–slip. The antimicrobial activity of plant extracts involves lysis by degradation of bacterial cell wall, damage to cytoplasmic membrane proteins, the binding of proteins, leakage of cell contents, coagulation of cytoplasm, inhibition of various cellular processes, increase in plasma membrane permeability and impairment of energy or synthesis of structural components in microbial cells[6-8]. The antimicrobial components in MP in combination with dehydrating ability of CS could have contributed to the observed good antimicrobial activity in 10 % (5 % MP + 5 % CS, w/v) synergistic composition.

Conclusion

Raw goat skins were preserved for 45 days using the myrobalan based low salt method without any sign of skin putrefaction. The properties of experimental leather were found to be comparable with control leather. The antimicrobial activity suggests that synergistic composition of MP and CS may have a better activity against wide range of microorganisms as compared to individual effect of either MP or CS.

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References

1 Xia D Z, Yu X F, Zhu Z Y and Zou Z D, Antioxidant and antibacterial activity of six edible wild plants (Sonchus spp.) in China, *Natur Prod Res*, 2011, **25,** 1893-1901.

2 Sivakumar V, Vijayeeswaree J and Lakshmi Anna J, Effective natural dye extraction from different plant materials using ultrasound, *Ind Crops Prod*, 2011, **33,** 116-122.

3 Sivakumar V, Sundar V J, Rangasamy T, Muralidharan C and Swaminathan G, Management of total dissolved solids in tanning process through improved techniques, *J Clean Prod*, 2005, **13,** 699-703.

4 Kanagaraj J and Chandra Babu N K, Alternatives to salt curing techniques, *J Sci Ind Res*, 2002, **61,** 339-348.

5 SuryaPrakash DV, Sree Satya N, Sumanjali A and Meena V, Pharmacological Review on *Terminalia Chebula*, Int J Res Pharm Biomed Sci, 2012, **3(2)**, 679-683.

6 Burt S A, Essential oils: their antimicrobial properties and potential applications in foods– A review, *Int J Food Microbiol*, 2004, **94,** 223-253.

7 Walsh S E, Maillard J Y, Russel A D, Catrenich C E, Charbonneau A L and Bartolo R G, Activity and mechanism of action of selected biocidial agents on gram positive and negative bacteria, *J Appl Microbiol*, 2003, **94,** 240-247.

8 Benli M, Yigit N, Geven F, Guney K and Bingol U, Antimicrobial activity of endemic *Crataegus tanacetifolia* (Lam.) Pers and observation of the inhibition effect on bacterial cells, *Cell Biochem Funct*, 2008, **24,** 844-851.