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Potential Use of Mosambi Peel-Based Bioenzymes for the Wound Management in Animals

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ABSTRACT

Wound healing is one of the major issues faced by animal rearers. The common afflictions are horn and foot injuries in animals. The therapeutic treatment involves the usage of costly antibiotics, which not only causes resistance to antibiotics but also alters the gut microflora. The present study was carried out with the objective to produce mosambi-peel based bioenzymes having antioxidant properties and further exploited for animal wound healing. The bioenzymes were characterized with respect to physico-chemical, enzymatic, color and microbiological profile. The presence of various antioxidant compounds including pterostilbene, hesperetin, aloin A, ononin, quercetin 3,7-dirhamnoside, isoorientin 2'-O-rhamnoside, saponarin was confirmed by LC-MS. Further, the bioenzyme solution sprayed on animal wounds stopped bleeding within 2-3 minutes and showed normal healing indicated by granulation tissue without pus pocket formation or any other side effects even in absence of any antibiotic treatment. In addition to this, it also acted as fly repellent for 5-6 h. Thus, the mosambi-peel based bioenzymes could serve as a cost-effective alternative to swap antibiotic therapy for wound healing in animals. Furthermore, the issue of solid waste management will be addressed by valorization of horticultural waste to bioenzymes.
Keywords: Bioenzymes, Waste management, Biotherapeutics, Mosambi peel, Wound management.

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INTRODUCTION

India is an agrarian economy where most of the population survives on agriculture and animal rearing for livelihood. In context to animals, wounds/injuries have remained a threatening problem (Bhatt *et al.,* 2022). Animals become furious and are likely to develop self-inflicting injuries as well. The common inflictions are horn and foot injuries. The therapeutic management of wounds and injuries involve use of antibiotics and dressing with antiseptic solutions. Since, the healing of horn and foot injuries are quite slow, there is requirement of prolonged use of antibi-

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otics which are associated with problems like resistance to antibiotics and altered gut microflora (Arora and Chandel, 2023; Arora *et al.,* 2024; Mu *et al.,* 2024). Moreover, the cost of antibiotic treatment is very high and need of prolonged treatment further increases the cost. The minimum cost of antibiotic treatment for 5 days is Rs. 1000-1500/- per animal depending upon the choice of antibiotics.

Alternatively, use of bioenzymes is a new gateway for mitigating this issue. Bioprocessing of horticultural waste to produce bioenzymes is the most striking approach in the awake of waste management and environmental issues (Gupta *et al.,* 2024; Kurniawan *et al.,* 2024). Furthermore, the presence of cocktail of enzymes may open a new area as a swap for antibiotic therapy for wound healing. In addition, the spent waste could be recycled for the production of bioenzymes, thereby, making a zero waste technology. Ding *et al.* (2022) exclusively reviewed the mechanisms of bioenzyme-based nanomedicines for chemo, radio and immune-therapies.

However, the exploitation of bioenzymes for animal wound healing has not been reported so far. In order to avail the animal health benefits, a comprehensive study for preparation and characterisation of bioenzymes and further applications as biotherapeutics is required. To the best of our knowledge, study on application of bioenzymes for animal wound healing is not available in literature. The present study was carried out with an objective to characterize mosambi peel-based bioenzymes for physicochemical, enzymatic, color, microbiological and biochemical profile. Further, the bioenzymes were evaluated for healing on fresh/chronic wounds in animals.

MATERIALS AND METHODS

Bioenzyme production and characterization: Mosambi peels, sugar and water were combined in the ratio of 3:1:10 in a 2-litre airtight container and bioenzymes were produced by the method of Janarthanan *et al.* (2020). The bioenzymes were evaluated for physic-chemical, enzymatic, color, microbiological and biochemical profile.

The mosambi-peel based bioenzymes were evaluated for color (Cheng *et al.,* 2018). pH, total soluble solids, concentrations of citric acid, acetic acid, tartaric acid and lactic acid were estimated by following the methods as mentioned in AOAC (2005). The concentrations of total sugars, reducing sugars and protein were estimated by the method of Dubois *et al.* (1956), Miller (1959) and Lowry *et al.* (1951), respectively. Further, the bioenzymes were accessed for various enzymatic assays for amylases (Miller, 1959), Exo-glucanases (Toyama and Ogawa, 1977), xylanases (Bailey *et al.,* 1992), pectinase (Katan and Phaff, 1959). Microbiological analysis was carried out on general purpose and selective media by serial dilution method. Biochemical analysis with FTIR and LC-MS analysis were outsourced.

Animal wound healing: A total of 8 buffalo bulls having various types and grades of wounds were selected for treatment with bioenzymes (Fig. 1). Bioenzymes were applied as spray twice a day on both fresh and old wounds and clotting time was noted. Blood clotting in fresh injuries and granulation tissue and pus pocket formation in chronic wounds were evaluated. The fly repellent property of bioenzyme was also evaluated. During the course of bioenzyme treatment, no antibiotic was used.

Fig. 1: Fresh and chronic injuries in animals.

Statistical analysis

All the experiments were done in triplicates and results were expressed as mean±standard deviation.

RESULTS AND DISCUSSION

Mosambi-peel based bioenzyme production and characterization: Bioenzymes are the liquid formulations produced by anaerobic fermentation of solid organic wastes (Adetunji *et al.,* 2023). Fruit peel is the principal waste product from food processing industries using fruits as raw material. In the present study, bioenzymes were produced using mosambi peels in 2-liter capped PET bottles. After a period of 3 months, the peels settled at the base of bottles. The bioenzyme was filtered using muslin cloth and stored in air tight containers. The average yield of bioenzyme generated was found to be 32.93 %. Table 1 shows the physicochemical, enzymatic, color and microbiological profile of the bioenzymes produced. The pH and total soluble solids were 3.89 and 2.4 °Bx, respectively. The enzymatic activity for exoglucanase, xylanase, amylase and pectinase were recorded to be 5.67, 95.93, 1.49 and 291.63 Units/mL, respectively. Total Bacterial Count and Total Fungal Count were found to be 7.65 and 2.60 log_{10} cfu/mL.

Several researchers have reported the production of bioenzymes from organic wastes. Neupane and Khadka (2019) evaluated enzymatic potential of bioenzymes from fruit and vegetable waste. The bioenzymes produced were found to have amylase, caseinase, protease and lipase activity. A vast array of applications of bioenzymes has been reported in the literature. Zhang *et al.* (2024) developed a bioenzymes based strip for detection of malathion in water with a sensitivity of 0.209 ng mL^{-1}. Sethi *et al.* (2021) illustrated several applications of bioenzymes including natural biofertilizer, natural pest repellent, natural soil amendment, plant growth promoter, natural immunity booster in animals, natural disinfectant and natural air purifier. Lakra *et al.* (2022) prepared bioenzymes from peels of various fruits and vegetables and reported their application in bio-composting, soil stabilization, treatment of contaminated water and as natural cleansers. However, the animal wound healing properties of bioenzymes have not been reported so far.

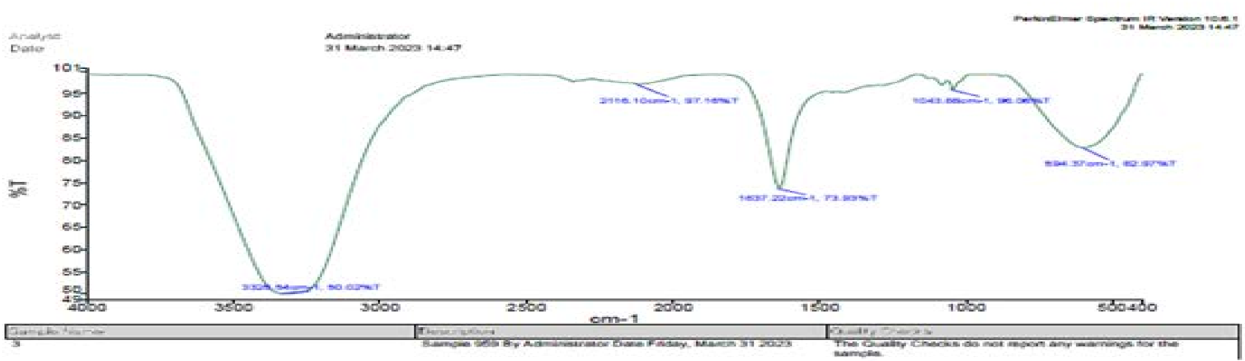
Table 1. Characterization of mosambi-peel based bioenzymes.

Physico-chemical Profile		Enzymatic activity (Units/mL)		Color Profile		Microbiological Profile (log10 cfu/mL)	
pH	3.89±0.07	Exoglucanase	5.67	L	33.03±0.81	TBC	7.65± 0.58
TSS (°Bx)	2.4±0.00	Xylanase	95.93	a*	-1.87±0.12	TFC	2.60± 0.20
Citric acid (%)	3.52±0.02	Amylase	1.49	b*	5.12±0.15	TAC	Absent
Acetic acid (%)	3.47±0.23	Pectinase	291.63	ΔE	35.92	LAB	3.34±0.25
Tartaric acid (%)	7.61±0.09			Hue angle	1.22	Rhizobium sp.	4.48±0.34

Lactic acid (%)	1.48±0.77	Pseudomonas sp.	2.46±0.19
Total Sugars (mg/mL)	21.22±0.25	DBC	4.18±0.32
Reducing Sugars (mg/mL)	9.11±0.04		
Proteins (mg/mL)	6.81±0.04		

Abbreviation: DBC- Diazotrophic bacterial count ; LAB- Lactic acid bacteria; TAC- Total Actinomycetes Count; TBC- Total Bacterial Count; TFC- Total Fungal Count

In the present study, the bioenzymes were characterized biochemically by Fourier Transform Infrared Spectroscopy (Fig. 2) and Liquid Chromatography –Mass Spectrophotometry (Table 2). Some of the important compounds with antioxidant properties were identified during LC-MS analysis including pterostilbene, hesperetin, aloin A, ononin, quercetin 3,7-dirhamnoside, isoorientin 2'-O-rhamnoside, saponarin. The presence of antioxidant rich moieties plays a significant role during wound healing.



Functional Group	Citrus Bioenzyme Absorption (cm^{-1})	Compound Class
N-H stretching	3325.54	Aliphatic primary amine
CΞC stretching	2116.10	Alkyne
C=C stretching	1637.22	Alkene
CO-O-CO stretching	1043.88	Anhydride
C-H bending	594.37	Benzene derivative

Fig. 2: FTIR spectrum of mosambi peel-based bioenzymes.

Table 2. Major compounds present in mosambi-peel based bioenzymes identified by LC-MS.

Spectrum graph peaks (m/z)	m/z	Retention time	Compound identified	Property
153.0214	153.0221	18.1251	Mercaptopurine	Anticancer Immunosuppressive
279.0987	279.0979	15.43951667	Dihydropteroic acid	Intermediate in folate synthesis
		15.43951667	Pterostilbene	Antioxidant Neuroprotective Anti-inflammatory
		15.43951667	(3S)-3-(4-Methoxyphenyl)-7-chromanol	Mimic or modulate endogenous estrogens
303.089	303.0874	12.7842	(+/-)-Hesperetin	Antioxidant Anti-inflammatory Anticarcinogenic Neuroprotective

		12.7842	Homoeriodictyol	Anticancer Therapeutic agent
312.0997	312.1026	23.0641	L-gamma-Glutamyl-N-allyl-D-cysteinylglycine	Anti-inflammatory Antioxidant
328.0937	328.0966	24.61695	Aphidicolin	Antiviral Antimitotical
418.13	419.1359	25.60968333	Aloin A	Anti-inflammatory Anticancer Antibacterial Antioxidant
		25.60968333	Neoisoliquiritin	Anti-depressant Anti-tumor-promoting Anti-inflammatory
425.1223	425.1985	13.4359	Schisanhenol	Anti-tumour
431.1357	431.1358	23.43558333	Ononin	Antioxidant Antidiabetic Antiobesity Neuroprotective Cardioprotective Antiviral Anti-inflammatory
596.1667	595.1666	14.19718333	Quercetin 3,7-dirhamnoside	Wound healing potential
		14.19718333	Isoorientin 2”-O-rhamnoside	Antioxidant Anti-inflammatory
		14.19718333	Saponarin	Antioxidant Antidiabetic Hepatoprotective
		14.19718333	Nictoflorin	Antioxidant
		14.19718333	Luteolin-7-O-neohesperidoside	Antibacterial
		14.19718333	Isovitexin 2”-O-beta-D-glucoside	Antidiabetic
633.1743	633.1781	14.28861667	Hesperidin	Anti-inflammatory Antioxidant Anti-cancer
		14.28861667	Neohesperidin	Neuroprotective Anti-inflammatory Antidiabetic Antimicrobial Anticancer
673.246	673.2463	15.7805	Limonin glucoside	Insecticidal Antibacterial Antifungal Antimalarial Anticancer Antiviral
		15.7805	Thiomarinol B	Antimicrobial
675.2627	675.2628	17.19355	Crocetin diglucosyl ester	Antioxidant Antitumor
		17.19355	Crocin 3	Anticancer Antioxidant
758.5676	758.5657	33.9956	Lecithin	Antioxidant

Effects of bioenzyme spray on wound healing: Preliminary trial on spray of bioenzymes over fresh or chronic wounds indicated a normal healing in the absence of any antibiotic therapy. The fresh wounds stopped bleeding within 2-3 minutes and showed normal healing indicated by granulation tissue without pus pocket formation. In addition to this, fly repellent property of bioenzymes was also observed on the sprayed area, thereby, providing a relief to the animals. Bioenzyme spray over the wound twice a day provided a relief from flies for whole day. Spray of bioenzymes over the chronic wounds of animals also indicated normal healing. Moreover, it did not show any side effects / tissue reaction or local irritation. It indicated that bioenzymes may act as a potential swap for antibiotic therapy for wound management in animals.

CONCLUSIONS

Preliminary trial on bioenzyme spray indicated a cost-effective alternative to antibiotic therapy for wound management in animals. The production of bioenzymes from horticultural waste may address the issue of solid waste management.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest for this article.

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