

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; SP-8(12): 1351-1357
www.biochemjournal.com
Received: 05-10-2024
Accepted: 11-11-2024

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Antibacterial activity of acetone extract of *Boswellia serrata* Resin in combination with selected phytochemicals against bovine mastitis pathogens

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DOI: <https://doi.org/10.33545/26174693.2024.v8.i12Sq.3359>

Abstract

Mastitis in dairy cattle is a highly prevalent infectious disease, leading to significant economic losses worldwide due to decreased milk quality, reduced yield, and increased production costs. Conventional antibiotic therapy, though effective, is associated with concerns such as drug resistance, milk residues, and high treatment costs. These issues highlight the urgent need for new, natural antibacterial alternatives. Phytochemicals, derived from medicinal plants, are promising candidates due to their antimicrobial properties, lack of side effects, and immunomodulatory potential.

This study evaluated the antibacterial and antioxidant activities of *Boswellia serrata* resin extract against bovine mastitis-causing organisms. The resin extract was assessed for phytochemical constituents, antioxidant potential using the phosphomolybdenum assay, and antibacterial activity via microdilution and biofilm inhibition assays. Its synergistic effects were evaluated in combination with enrofloxacin, amoxicillin, and selected phytochemicals such as thymol, xanthone, and caffeic acid.

Phytochemical analysis revealed the presence of carbohydrates, saponins, triterpenes, phenols, and flavonoids. The resin extract exhibited moderate antioxidant activity, equivalent to 44.82 ± 0.85 µg/ml of ascorbic acid. Minimum inhibitory concentrations (MIC) against *Escherichia coli* and *Staphylococcus aureus* ranged from 5 mg/ml to 1.25 mg/ml, with the lowest MIC observed for *S. aureus*. Synergistic antibacterial effects were observed with xanthone against *E. coli* and additive effects against *S. aureus*. Biofilm inhibition assays confirmed that the resin extract alone significantly reduced biofilm formation, with enhanced inhibition in combination with antibiotics and xanthone.

In conclusion, *Boswellia serrata* resin extract demonstrated notable efficacy against *S. aureus*, likely due to differences in gram-positive and gram-negative cell wall structures. Its additive and synergistic effects with antibiotics and phytochemicals, coupled with its lack of antagonism, underscore its potential as a natural adjunct in mastitis treatment.

Keywords: *Boswellia serrata*, bovine mastitis, phytochemicals, antimicrobial properties

Introduction

The animal husbandry and dairy development sectors are integral to the national economy and the sustainability of Indian farmers. Livestock provide a vital source of employment in rural areas and contribute significantly to the nutritional needs of millions. However, the dairy industry faces numerous challenges, among which mastitis stands out as one of the most economically significant infectious diseases. Mastitis adversely affects both the quality and quantity of milk, leading to losses that include reduced milk production, premature culling of affected animals, increased veterinary expenses, and discarded or downgraded milk.

Mastitis is a complex, multifactorial condition characterized by physical, chemical, and bacterial changes in milk, alongside pathological alterations in glandular tissue [1]. While it can occur in all dairy animals, it is commonly observed that nearly every cow suffers from mastitis at some stage in its life. Cows are particularly at higher risk than buffaloes, with an estimated prevalence rate of around 50% [2]. Animals that have experienced mastitis are unlikely to recover their original milk production levels, resulting in substantial economic losses [3].

Apart from its economic implications, mastitis is a significant public health concern due to medication residues in milk and the potential transmission of harmful organisms to humans

[4]. The condition is caused by multiple pathogens, including *Staphylococcus spp.*, *Streptococcus spp.*, coliforms, *Pseudomonas spp.*, and *Mycoplasma bovis*. The involvement of diverse infectious agents makes mastitis a persistent challenge for veterinarians worldwide [5].

Effective control of mastitis is critical, as contaminated milk can transmit pathogenic organisms to humans and harbor antibiotic residues [6]. The emergence of antibiotic-resistant pathogens has intensified the need for alternative treatments. Over the past two decades, significant attention has been directed toward developing antimicrobial agents from natural sources [7]. Medicinal plants have long been used in traditional animal healthcare for the prevention and treatment of diseases. Their secondary metabolites, including alkaloids, flavonoids, tannins, terpenes, quinones, essential oils, and resins, possess antibacterial activity [8]. These compounds exhibit a broad spectrum of activity [9] and target microbes through mechanisms distinct from conventional antibiotics, making them valuable in addressing resistant pathogens [10].

Plant-based antimicrobials are also associated with fewer side effects and have shown significant therapeutic potential in combating infectious diseases [10, 11]. Furthermore, combining plant extracts with antibiotics often enhances efficacy, producing additive or synergistic effects [13, 14, 15].

Boswellia serrata resin, renowned for its antibacterial properties, has been extensively used in traditional Unani and Ayurvedic medicine. It is reported to be effective in treating conditions such as arthritis, bronchitis, asthma, cough, throat infections, and intestinal disorders. Additionally, it acts as a diaphoretic, astringent, stimulant, expectorant, and diuretic, and is prescribed for syphilitic and pulmonary diseases [16, 17, 18, 19].

In this context, the present study was undertaken to evaluate the acetone extract of *Boswellia serrata* resin with the objectives of phytochemical analysis, evaluation of *in-vitro* antioxidant activity, evaluation of *in-vitro* antimicrobial activity against MTCC and clinical isolates of *Staphylococcus aureus* and *Escherichia coli*, evaluation of *in-vitro* synergism with selected phytochemicals

Materials and Methods

This study investigated the antibacterial activity of acetone extract of *Boswellia serrata* resin (BSR) and its synergistic potential with enrofloxacin, amoxicillin, and phytochemicals (cashew nutshell extract, xanthone, caffeic acid, thymol, and *Ficus bengalensis* extract) against mastitis-causing organisms (*Escherichia coli* and *Staphylococcus aureus*). All the chemicals are of analytical grade and were procured from (Himedia and Merck) Mumbai, India.

The acetone extract of *Boswellia serrata* resin was prepared by soaking powdered resin (20 g) in acetone (1:10 ratio) and stirring overnight at room temperature. The mixture was filtered, and the filtrate was lyophilized. The residue was dissolved in dimethyl sulfoxide (DMSO) to create a stock solution (500 mg/mL), which was further diluted to prepare a working solution (20 mg/mL) (Fig. 1). Stock solutions of enrofloxacin, amoxicillin, cashew nutshell extract, caffeic acid, thymol, xanthone, and *Ficus bengalensis* extract were prepared using appropriate solvents, and working solutions were diluted to suitable concentrations for antibacterial assays.

The BSR extract was screened for phytochemicals, including alkaloids, carbohydrates, saponins, phenols, flavonoids, tannins, and proteins using standard qualitative methods as per Harbone, 1984 [20]. The presence of alkaloids was tested using Dragendorff's reagent, carbohydrates with Molisch's test, saponins with a foam test, phenols with the ferric chloride test, and flavonoids with the lead acetate test. Antioxidant activity was assessed using the phosphomolybdenum assay [21]. Reaction mixtures containing various concentrations of BSR extract, phytochemicals, and standards were incubated at 95°C for 90 minutes, and absorbance was measured at 695 nm. Results were expressed as ascorbic acid equivalents.

Antibacterial activity was evaluated against *E. coli* and *S. aureus* (both clinical isolates and MTCC cultures) using the broth dilution method. Minimum inhibitory concentration (MIC) values were determined by serial dilutions in cation-adjusted Mueller-Hinton broth and assessed for bacterial growth inhibition using iodinitrotetrazolium chloride (INT). MIC was defined as the lowest concentration completely inhibiting visible bacterial growth.

Combined antibacterial activity was examined using the checkerboard assay [22] (Fig.2). Interactions between BSR extract, antibiotics, and phytochemicals were evaluated, and fractional inhibitory concentration (FIC) indices were calculated to classify effects as synergistic (<0.5), additive (0.5–1.0), indifferent (1.0–4.0), or antagonistic (>4.0).

Biofilm inhibition potential of the BSR extract and other phytochemicals at MIC concentrations was assessed using a crystal violet assay [23]. Treated bacterial cultures in microtitre plates were incubated, stained with crystal violet, and de-stained with ethanol. Absorbance at 550 nm was recorded to calculate percentage inhibition. Statistical analysis was performed using SPSS (version 19.0).

Results and Discussion

The acetone extract of *Boswellia serrata* resin revealed the presence of carbohydrates, triterpenes, flavonoids, saponins, and phenols (Table 1). These findings are consistent with previous studies, which reported the presence of carbohydrates [24], boswellic acids [25], flavonoids [26], and phenols [27]. Such phytochemicals are known for their diverse pharmacological activities [28].

The antioxidant activity of *Boswellia serrata* resin and other phytochemicals was assessed through the phosphomolybdenum assay and are showed in Table 2. BSR extract antioxidant activity was equivalent to 44.82 ± 0.85 µg/mL of ascorbic acid. These results align with Hartman *et al.* (2012) [29], who identified limonene and esters in *Boswellia* resin as key contributors to antioxidant activity. Sharma *et al.* (2011) [30] and Gaurea *et al.* (2016) [27] also documented antioxidant activity in *Boswellia serrata* extracts using DPPH radical scavenging assays. The antioxidant activity of other tested compounds, including thymol, cashew nutshell extract, *Ficus* aerial roots, enrofloxacin, caffeic acid, and xanthone, ranged between 33.60 ± 0.31 and 162 ± 1.81 µg/mL of ascorbic acid. Similar activities have been reported by Kavooosi *et al.* (2013) [31] for thymol, Verma *et al.* (2015) [32] for *Ficus* aerial roots, and Sujareon *et al.* (2018) [33] for cashew nutshell extract.

The antibacterial activity of *Boswellia serrata* resin extract alone and in combination with other phytochemicals and respective antibiotics such as enrofloxacin and amoxycillin was evaluated against *Escherichia coli* and *Staphylococcus*

aureus (Table 3). The minimum inhibitory concentration (MIC) for *Escherichia coli* was 5 mg/mL, consistent with Sharma *et al.* (2016) [34], who reported MIC values between 3.125 and 6.25 mg/mL using agar disc diffusion assays. Active components such as acetyl-keto boswellic acid (AKBA) and α - and β -boswellic acids were identified as key antibacterial agents [35, 36]. Similarly, other phytochemicals showed varying MIC values, including cashew nutshell extract (2.5 mg/mL), *Ficus* aerial roots (2.5 mg/mL), xanthone (12.5 mg/mL), thymol (6.25 mg/mL), and caffeic acid (6.25 mg/mL). These results agree with studies by Mustapha *et al.* (2015) [37], Miladiyah and Rachmawaty (2017) [38], and Trombetta *et al.* (2005) [39].

For *Staphylococcus aureus*, the MIC of *Boswellia serrata* resin extract was 1.25 mg/mL, comparable to results from Aman *et al.* (2010) [40] and Sharma *et al.* (2016) [34]. The resin exhibited antibacterial activity due to its bioactive compounds, including boswellic acids and AKBA [36]. MIC values of other phytochemicals against *Staphylococcus aureus* were as follows: cashew nutshell extract (0.07 mg/mL), *Ficus* aerial roots (2.5 mg/mL), xanthone (25 mg/mL), thymol (6.25 mg/mL), and caffeic acid (3.125 mg/mL). These findings are supported by reports on cashew nutshell liquid [33], xanthones [41], and thymol [31].

The synergistic antibacterial activity of *Boswellia serrata* resin extract was evaluated in combination with enrofloxacin against *Escherichia coli* (Table 4), amoxicillin against *Staphylococcus aureus* (Table 5), and phytochemicals. Against *Escherichia coli*, the resin extract exhibited additive effects with enrofloxacin, cashew nutshell extract, thymol, and *Ficus* aerial roots, and synergistic effects with xanthone. The combination with caffeic acid showed indifference. The additive effects are attributed to overlapping mechanisms of action, such as cell membrane disruption by phenolic compounds [42, 43]. Synergism with xanthone likely resulted from the combined disruption of bacterial membranes and inhibition of DNA synthesis [44].

Against *Staphylococcus aureus*, the resin extract showed additive effects with amoxicillin, xanthone, caffeic acid, thymol, and *Ficus* aerial roots. Similar additive antibacterial activity was reported by Rashan *et al.* (2021) [45] for combinations of *Boswellia* oleoresins with conventional antibiotics. The interaction between amoxicillin, which targets cell wall synthesis, and phenolic compounds from *Boswellia serrata* resin supports this additive effect [44, 46].

The biofilm inhibition potential of *Boswellia serrata* resin extract along with other phytochemicals and respective antibiotics enrofloxacin (Table 6) and amoxicillin (Table 7) was assessed using crystal violet assays against *Escherichia coli* and *Staphylococcus aureus*. For *E. coli*, significant biofilm inhibition was observed with enrofloxacin, xanthone, thymol, and combinations of the resin extract with xanthone. Lower inhibition was noted with combinations involving caffeic acid, *Ficus* aerial roots, and cashew nutshell extract. Similar findings were reported by Raja *et al.* (2011) [36], who demonstrated that AKBA could inhibit and disrupt biofilms. *Ficus benghalensis* aerial root extract has also shown biofilm inhibition potential against *E. coli* [47].

For *Staphylococcus aureus*, the highest biofilm inhibition was achieved with cashew nutshell extract, xanthone,

caffeic acid, and combinations of the resin extract with xanthone. Moderate inhibition was observed with thymol, *Ficus* aerial roots, and combinations involving the resin extract with thymol or amoxicillin. Raja *et al.* (2011) [36] and Jorge *et al.* (2019) [48] have similarly reported biofilm inhibition properties for boswellic acids and caffeic acid, respectively.

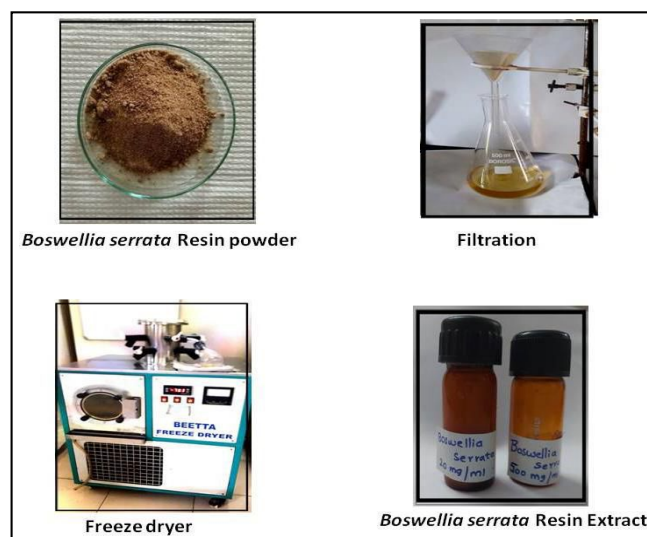


Fig 1: Preparation of stock solution and working solution for antibacterial activity evaluation

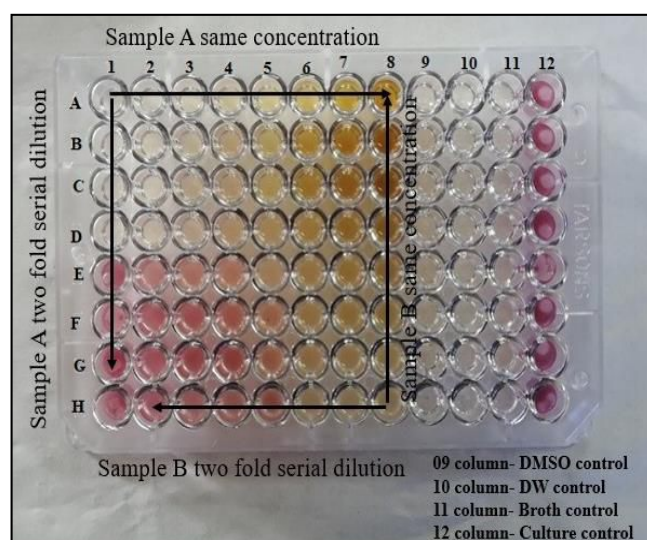


Fig 2: Microtitre plate showing combinations of two test samples adding dilutions and directions in various wells.

Table 1: Phytochemical analysis of *Boswellia serrata* resin (BSR) extract

Constituents	Results
Alkaloids (Dragendroff test)	—
Carbohydrates (Molisch test)	+
Reducing sugars (Benedict's test)	+
Saponins (Foam test)	+
Triterpenes (Salkowski test)	+
Phenols (Ferric chloride test)	+
Flavonoids (Lead acetate test)	+
Proteins (Xanthoproteic test)	-
Amino acids (Ninhydrin test)	-

Tables 2: Antioxidant activity of test compounds by Phosphomolybdenum assay

Test compounds	Concentration	Antioxidant activity equivalent ($\mu\text{g/mL}$)
<i>Boswellia serrata</i> resin extract	0.1 mg/mL	44.82 $\pm$ 0.85
Cashew nutshell extract	0.1 mg/mL	63.6 $\pm$ 0.64
<i>Ficus benghalensis</i> aerial root extract	0.1 mg/mL	54.67 $\pm$ 0.146
Enrofloxacin	0.1 mg/mL	33.605 $\pm$ 0.31
Thymol	0.1 mg/mL	53.80 $\pm$ 0.32
Xanthone	10 mg/mL	162 $\pm$ 1.81
Caffeic acid	0.1 mg/mL	126.14 $\pm$ 0.62

Table 3: MIC Values of *Boswellia serrata* resin extract along with various phytochemicals and Enrofloxacin against Clinical Isolates and MTCC Cultures of *Escherichia coli* and *Staphylococcus aureus*

Test compound	Minimum Inhibitory Concentration against			
	<i>Escherichia coli</i>		<i>Staphylococcus aureus</i>	
	Clinical Isolates	MTCC Cultures	Clinical Isolates	MTCC Cultures
Enrofloxacin ($\mu\text{g/mL}$)	0.03	0.03	--	--
Amoxycillin ($\mu\text{g/mL}$)	--	--	0.3125	0.3125
<i>Boswellia serrata</i> resin extract (mg/mL)	5	5	1.25	1.25
Cashew nutshell extract (mg/mL)	2.5	2.5	0.078	0.078
Aerial roots of <i>Ficus</i> extract (mg/mL)	2.5	2.5	2.5	2.5
Thymol (mg/mL)	6.25	6.25	25	25
Xanthone (mg/mL)	12.5	12.5	6.25	6.25
Caffeic acid (mg/mL)	6.25	6.25	6.25	3.125

Table 4: Fractional inhibitory concentration and the type of interaction between, Enrofloxacin, *Boswellia serrata* resin extract and various phytochemicals against *Escherichia coli* for both MTCC culture and Clinical isolates

Test compound	MIC alone	MIC combination	FIC	Type of interaction
<i>Boswellia serrata</i> Resin extract (mg/mL)	5 mg /mL	2.5 mg /mL	1	Additive
Enrofloxacin (mg/mL)	0.03 μg /mL	0.015 μg /mL		
<i>Boswellia serrata</i> Resin extract (mg/mL)	5 mg /mL	2.5 mg /mL	1.5	Indifference
Caffeic acid (mg/mL)	6.25 mg /mL	6.25 mg /mL		
<i>Boswellia serrata</i> Resin extract (mg/mL)	5 mg /mL	2.5 mg /mL	0.62	Partial Synergy
Xanthone (mg/mL)	25 mg /mL	6.25 mg /mL		
<i>Boswellia serrata</i> Resin extract (mg/mL)	5 mg /mL	5 mg /mL	1	Additive
Cashew nutshell extract (mg/mL)	2.5 mg /mL	0.625 mg /mL		
<i>Boswellia serrata</i> Resin extract (mg/mL)	5 mg /mL	2.5 mg /mL	1	Additive
<i>Ficus</i> aerial root extract (mg/mL)	2.5 mg /mL	1.25 mg /mL		
<i>Boswellia serrata</i> Resin extract (mg/mL)	5 mg /mL	2.5 mg /mL	1	Additive
Thymol (mg/mL)	6.25 mg /mL	3.125 mg /mL		

Table 5: Fractional inhibitory concentration and the type of interaction between Amoxycillin, *Boswellia serrata* resin extract and various phytochemicals against *Staphylococcus aureus*

Test compound	MIC alone	MIC combination	FIC	Type of interaction
<i>Boswellia serrata</i> Resin extract (mg/mL)	1.25 mg /mL	0.625 mg /mL	1	Additive
Amoxycillin (mg/mL)	0.3 μg /mL	0.15 μg /mL		
<i>Boswellia serrata</i> Resin extract (mg/mL)	1.25 mg /mL	0.625 mg /mL	1	Additive
Caffeic acid (mg/mL)	3.12 mg /mL	1.56 mg /mL		
<i>Boswellia serrata</i> Resin extract (mg/mL)	1.25 mg /mL	0.625 mg /mL	1	Additive
Xanthone (mg/mL)	12.5 mg /mL	6.25 mg /mL		
<i>Boswellia serrata</i> Resin extract (mg/mL)	1.25 mg /mL	0.625 mg /mL	1	Additive
Cashew nutshell extract (mg/mL)	0.07 mg /mL	0.03 mg /mL		
<i>Boswellia serrata</i> Resin extract (mg/mL)	1.25 mg /mL	0.625 mg /mL	1	Additive
<i>Ficus</i> aerial root extract (mg/mL)	2.5 mg /mL	1.25 mg /mL		
<i>Boswellia serrata</i> Resin extract (mg/mL)	1.25 mg /mL	0.625 mg /mL	1	Additive
Thymol (mg/mL)	6.25 mg /mL	3.125 mg /mL		

Table 6: Percentage inhibition of biofilm of the test compounds against *Escherichia coli*

Test compound	Concentration	Percent biofilm inhibition
BSR extract	2.5 mg/mL	69.9±0.81 ^C
Xanthone	12.5 mg /mL	81.4± 0.44 ^A
Enrofloxacin	0.03 µg /mL	80.8±0.64 ^A
Thymol	6.25 mg/mL	79.7±0.57 ^B
BSR extract + Xanthone	5 mg/mL +12.5 mg/mL	78.3±0.71 ^B
BSR extract + Thymol	5 mg/mL+ 6.25 mg/mL	75.6±0.67 ^B
Caffeic acid	6.25 mg/mL	73.5 ± 1.53 ^B
BSR extract +Enrofloxacin	5 mg/mL+ 0.03 mg/mL	71.8±0.28 ^{AB}
<i>Ficus</i> aerial root extract	2.5 mg/mL	70.7±0.76 ^{AB}
BSR extract + Caffeic acid	5 mg/mL+ 6.25 mg/mL	70.1±0.62 ^C
BSR extract + <i>Ficus</i> aerial root extract	5 mg/mL+2.5 mg/mL	68.7±0.99 ^C
BSR extract+ Cashew nut shell extract	5 mg/mL +5 mg/mL	66.2±0.38
Cashew nutshell extract	5 mg/mL	No inhibition

Values are mean±SEM oneway ANOVA (SPSS).

Means with different alphabets as superscripts differs significantly ($p<0.05$).

Table 7: Percentage inhibition of biofilm of the test compounds against *Staphylococcus aureus*

Test compound	Concentration	Percent biofilm inhibition
BSR extract	0.15 mg/mL	68.9±0.26 ^C
Cashew nutshell extract	0.07 mg/mL	74.2±0.21 ^A
Xanthone	25 mg/mL	72.2 ± 0.52 ^A
Caffeic acid	3.12 mg/mL	71.5±1.19 ^{AB}
BSR extract + Xanthone	1.25 mg/mL+25 mg/mL	71.2±0.67 ^{AB}
<i>Ficus</i> aerial root extract	2.5 mg/mL	70.14±0.57 ^B
Thymol	6.25 mg/mL	69.7±0.88 ^B
BSR extract+ Cashew nutshell extract	1.25 mg/mL +0.07 mg/mL	68.1±0.89 ^D
BSR extract + Caffeic acid	1.25 mg/mL +3.12 mg/mL	63.6±0.97 ^{DE}
BSR extract + Thymol	1.25 mg/mL +6.25 mg/mL	62.3±0.38 ^E
BSR extract + Amoxicillin	1.25 mg/mL +0.3 µg/mL	61.5±0.72 ^E
Amoxicillin	0.3 µg/mL	59.8± 1.73 ^E
BSR extract + <i>Ficus</i> aerial root extract	1.25 mg/mL +2.5 mg/mL	59.5±0.26 ^E

Values are mean±SEM oneway ANOVA (SPSS).

Means with different alphabets as superscripts differs significantly ($p<0.05$).

Conclusion

In conclusion, *Boswellia serrata* resin extract demonstrated significant antioxidant and antibacterial properties, attributed to compounds such as boswellic acids, AKBA, and phenolic derivatives. The resin exhibited additive effects with enrofloxacin and amoxicillin against *E. coli* and *S. aureus*, respectively, and synergistic effects with xanthone against *E. coli*. It also showed promising biofilm inhibition alone and in combination with other phytochemicals, supporting its potential as a natural adjunct in combating antibiotic resistance and biofilm-associated infections.

Acknowledgments

Authors thank the Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh for funding the research work.

Conflict of interest

There is no conflict of interest with anyone.

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