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Antimicrobial, anti-inflammatory and antioxidant activity of *Beauveria Bassiana*

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Abstract
Beauveria bassiana is a naturally occurring fungus best known for infecting and killing insects. It's widely used in agriculture and pest management as a biological control agent. Generally considered safe for humans, animals, and plants when used as directed, though people handling large quantities may need basic protective measures. If you want, I can explain how to use it on a specific crop or pest problem. But there was very minimal number of studies or research found in the field. In this study, microbial extract was collected from *Beauveria bassiana* then used it for identification of its potential activity in antimicrobial, anti-inflammatory and antioxidant activity. Antimicrobial analysis, Staphylococcus aureus, *Streptococcus pyogens*, *E.coli* and *Klebsiella sp.*, was used and the results were compared with standard antibiotics.

Keywords: *Beauveria bassiana*, antimicrobial, anti-inflammatory, antioxidant activity

Introduction

Beauveria bassiana is one of the entomopathogenic fungi. *Beauveria bassiana* is not directly used to improve human health, but it has potential benefits through research. It produces bioactive compounds (like beauvericin) that show antimicrobial, antifungal, and anticancer properties in laboratory studies. These compounds may help in developing new medicines in the future. However, its medical use is still experimental and not widely applied in clinical practice. The fungus has shown potential in nanoparticle synthesis. Recent studies suggest that *B. bassiana* can be used in the green synthesis of metallic nanoparticles. The SPs act as secondary metabolites and are effective for antimicrobial activity. Antimicrobial components are Oxalic acid, Beauvericin, Bassianin, Tenellin, and Oosporein produced by *Beauveria bassiana.* It has been widely used to control insect pests with insecticidal activity (Alagawany, M 2014) [1].

In the current study *Beauveria bassiana* has *in vitro* antagonistic activity and antimicrobial activity of exo- and endo-metabolites against. *B. cereus*, *B. megaterium*, *Xanthomonas campestris,* and *P. fluorescence.* The GC/MS bioactive compounds are ethanol, butanol, 2-methyl, 2,4-dimethyl-1-heptone, octane, 4-methyl, and β-element. These are used as a biocontrol agent of several bacteria, especially gram-positive bacteria. *Beauveria bassiana* has plant growth-promoting effects and is a protective agent for tomato plants against *B. cinerea, A. alternate*, the pest aphis, and *Macrosiphum euphorbiae.*

The antibacterial activity of *Beauveria bassiana* crude ethyl acetate against aerobic pathogenic bacteria and the sps strongly inhibit the activity of many sps. Apart from this, the endo pathogenic fungi also produce natural antimicrobials against inflammation-related disorders. The anti-inflammatory and anti-thrombotic activities and lipid molecules derived from *Beauveria bassiana* extracts against PAF activities, the presence of lipid bioactives in *Beauveria bassiana* with strong anti-inflammatory and anti-thrombotic properties, and more applications for these endomopathognic fungi. The SPS priority of consumption of carbon sources, specifically sugars, and availability of nutrients. Efficient updates and metabolism of these sugars are critical to the fungus's infectivity and virulence. (Alexandros Tsoupras *et al.*, 2022) [2].

Methodology

Beauveria bassiana has been isolated from soil and identified by macroscopic and microscopic observation. The soil samples were systematically collected from agricultural fields located in the Dharmapuri and Krishnagiri districts of Tamil Nadu. These regions were selected based on their diverse soil types, climatic variations and agricultural significance. Before sample collection, the top surface layer of approximately 20cm was removed to eliminate debris, plant residues, stones and coarse particles were also manually separated to obtain a homogenous fine soil fraction suitable for laboratory analysis. The collected samples were immediately transferred into pre- labelled, sterile polyethylene sampling bags. The samples were stored at ambient temperature for physical and chemical analyses and under refrigeration for microbial studies until further processing (Dannon *et al*., 2020) [3].

Primary screening

Each soil sample was subjected to a serial dilution technique, followed by the spread plate method and study the microbial diversity present in the soil. Initially one gram of soil was aseptically transferred into a sterile 15ml centrifuge tube containing 9ml of phosphate buffer solution, maintaining a 1:9ratio. The suspension was thoroughly mixed using a vortex mixer to obtain a uniform soil suspension. From each dilution tube 0.1ml of the diluted sample was aseptically pipetted and spread evenly over the surface of PDA plates using a sterile L-shaped glass spreader. The PDA medic was selected due to its slightly acidic Ph and high dextrose content, which favour the selective growth of fungi while inhabiting bacterial proliferation. The inoculated plates were incubated at 25°C for 3-5days under aerobic conditions. During incubation fungal growth was periodically monitored. After the incubation period, distinct fungal colonies with varying morphological characteristics such as colour, texture, shape and growth pattern were observed on the agar plate surface (Dereje Geremew *et al*., 2023) [4].

Secondary screening
Individual colonies from the previous plate were carefully transferred to freshly prepared PDA plates to ensure the isolation of pure fungal cultures. The plates were incubated at 25℃ for a period of 3-5 days, allowing adequate time for fungal growth development. Observation of the PDA plates revealed significant variation among the fungal colonies. Several distinct morphological characteristics were noted.

Purification
Single colonies that were initially grown were carefully transferred to freshly prepared PDA plates to obtain pure, young colonies. This step is crucial for ensuring the isolation of individuals fungal strains without contamination from other microorganisms. By transferring well-isolated colonies, researchers can maintain the genetic and morphological consistency of the fungal culture, which is important for subsequent experiment or identification processes.

LPCB Staining
Isolated fungal colonies were subjects to staining with LPCB to facilitate microscopic examination. This step is crucial as it enhances the contrast between the fungal elements and the surrounding medium, allowing for detailed observation of morphological features. The LPCB stain highlights various fungal structures, including hyphae, conidiophores, conidia and enabling precise differentiation between species. The morphological characteristics revealed by LPCB staining vary depending on the fungal species and their development stage. Features such as colony texture, pigmentation, spore arrangement, spore shape & size, septation, and branching patterns were carefully examined.

Mass cultivation and separation
After inoculum preparation, it carried for mass cultivation process. The inoculated broth was incubated in shaker incubator. This process carried up to 20 days for well development of inoculum and during the development period, it monitored then confirmed that the broth cultivated without contamination. After the cultivation, need to confirm that, the organism developed without any contamination. After the proper development period, it forwarded to separation process. Mass cultivated component was poured in separating funnel and equal volume of acetone were added, then mixed well. Place the separating funnel in funnel stand. Allow this set up for three days for separation of components and microbial debrides. After the separation process, the separated components were collected in separate containers.

Protein denaturation assay (PDA)
The anti-inflammatory effect of VM3 strain was assessed using a modified Protein Denaturation Assay (PDA). A reaction mixture was prepared with 0.5 ml of bovine albumin and 2.5 ml of phosphate-buffered saline. The control consisted of 0.5 ml of bovine albumin mixed with an equal volume of distilled water. Various concentrations of bioactive VM3 and Ibuprofen (standard) (100, 200, 300, 400 and 500 µg/ml) were added and incubated at 37ºC for 15 mins in a BOD incubator. The mixture was then heated at 70ºC for 5 mins. Absorbance was measured 660 nm using a UV-Vis Spectrophotometer (Elfalleh *et al*., 2009) [5]. IC50 value for both Ibuprofen and VM3 strain were determined. The percentage of PDA inhibition was calculated using the following formula,
% of PDA inhibition = (Ac-At/Ac) × 100 Ac – Absorbance of positive control, At – Absorbance of test sample.

DPPH Radical Scavenging Assay
The antioxidant activity was determined against DPPH free radical content by DPPH radical scavenging assay. *B.bassiana* extract was diluted with methanol by 10-fold serial dilution. Each concentration of extract (0.5 mL) was incubated with 1.5 mL of 0.1 mM DPPH solution (Hi-media). The absorbance was measured at 517 nm after incubation in the dark at room temperature for 20 min. Methanol was used as the blank solution, while DPPH without extract was used as a control. The absorbance of the DPPH solution A1 and the absorbance of the extract with the DPPH solution A2 were measured. The percentage of DPPH free radical inhibition was calculated as follows:
The antioxidant activity of the extract was assessed by comparing the samples to that of standard gallic acid and is expressed as mg gallic acid equivalent per gram extract (mg GAE/g extract) (Miliauskas *et al*., 2004) [9].

ABTS Radical Cation Decolorization Assay
The radical cation decolorization activity was determined using 2,20-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$) assay. The radical reagent was prepared using 7 mM $ABTS^+$ solution and 2.45 mM potassium persulfate solution and kept in the dark room temperature for 12–16 h to produce $ABTS^+$ radical. The $ABTS^+$ radical was adjusted by dilution with distilled water to obtain an absorbance of 0.700 ± 0.020 at 734 nm. *B.bassiana* extract was diluted with methanol by 2-fold serial dilution. Each concentration of extract (5 µL) or Trolox standard was incubated with 195 µL of $ABTS^+$ solution for 10 min. The absorbance was measured at 734 nm using a microplate reader. Methanol was used as the blank solution, while $ABTS^+$ without extract was used as a control. The absorbance of the $ABTS^+$ solution A1 and the absorbance of the extract with the $ABTS^+$ solution A2 were measured. The percentage of $ABTS^+$ free radical inhibition was calculated as follows:
The antioxidant activity of the extract was assessed by comparing the samples to that of standard Trolox and is expressed as mg Trolox equivalent per gram extract (mg TEAC/g extract) (Elfalleh *et al*., 2009) [5].

Antibacterial activity of microbial extract against of clinical pathogenic bacteria
The extracts were used for identification of its potential antimicrobial activity against few clinical pathogens which was isolated from clinical samples.

Well Diffusion Method
Well cut method was used for the identification of antimicrobial activity of microbial extract. The microbial extraction was used in various concentrations like10µl, 20µl, 30µl, 40µl and gentamycin (10µl) used as control. The microbial extract was added on well which was in MHA medium. The MHA medium which was inoculated with isolated bacterial pathogens. Subsequently the inoculated plates were incubated for about 18 - 24 hours at 37ºC. After incubation the diameter of the circular zones of inhibition were measured.

Results
Anti-inflammatory
Protein Denaturation Assay
Inflammation is a key function in biological processes initiated by various stimuli and noxious factors such as irradiation by ultraviolet light, irritants, infections and cell injury. The main features of inflammation are redness, elevated temperature, pain and alteration in physiological functions at infected sites. In this analysis, anti-

inflammatory activity showed better activity moreover it works well, it produce 74.95% of anti-inflammatory activity in 10 µg/ml of sample.

Table 1: Protein Denaturation Assay

Extract	Concentration µg/ml	Protection% Mean + SD
Test sample	2	58.36 + 0.56
	4	61.41 + 0.74
	6	65.45 + 1.96
	8	69.67 + 0.25
	10	74.95 + 0.65
Standard (Diclofenac sodium)	6	89.56 + 0.98

DPPH Assay

The antioxidant activity was confirmed mainly for polysaccharides present in microbial extract. Few studies have shown the antioxidant properties of cordycepin. The antioxidant activity of microbial extract may also be influenced by other chemical constituents present in fruiting bodies, e.g., ergothioneine, phenolic compounds, carotenoids and selenium. In 100 µl sample concentration, 40.59% antioxidant activity were showed.

Table 2: DPPH Assay

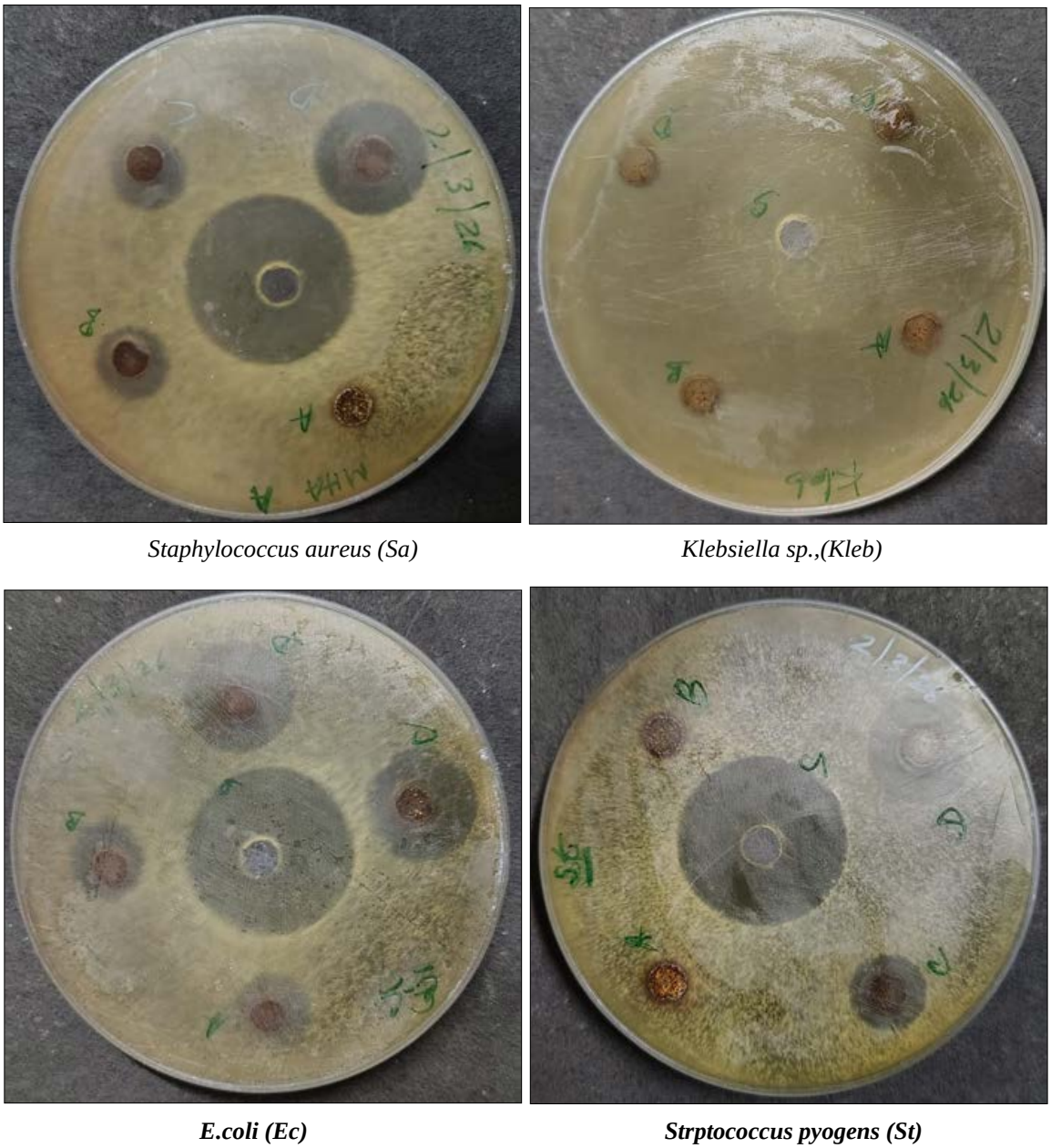
Sample	OD value	Test	DPPH %
25 µl	17.22	15.78	08.36
50 µl	17.22	14.65	14.92
75 µl	17.22	12.74	26.01
100 µl	17.22	10.23	40.59
Standard (50 µl)	17.22	09.56	44.48

ABTS Radical Cation Decolorization Assay

Table 3: ABTS Radical assay

Sample	OD value	Test	DPPH %
25 µl	12.60	11.78	06.50
50 µl	12.60	10.86	13.80
75 µl	12.60	09.23	26.74
100 µl	12.60	08.04	36.19
Standard (50 µl)	12.60	06.42	49.09

Antibacterial Activity



Staphylococcus aureus (Sa) *Klebsiella sp.,(Kleb)*

E.coli (Ec) ***Strptococcus pyogens (St)***

Fig 1: Zone of inhibition of *B.bassiana*

The microbial extraction was used in various concentrations like10µl, 20µl, 30µl, 40µl and gentamycin (10µl) used as control. Those are used for identification of antimicrobial activity of *B.bassiana* extract.

Table 4: Zone of inhibition

	A (05 µl)	B (10 µl)	C (15 µl)	D (20 µl)	S-Standard
Sa	06mm	09mm	13mm	18mm	28mm
St	06mm	06mm	06mm	06mm	29mm
Kleb	09mm	11mm	16mm	17mm	26mm
EC	06mm	06mm	11mm	19mm	26mm

Discussion

In this study *Beauveria bassiana* crude extracts used for identification of its antimicrobial activity against of pathogenic bacterial strains which was isolated from urinary tract infection. The anti-microbial compounds from *Beauveria bassiana* prevent many diseases associated with microbial infections. Beauvericin, Bassianolide, B-tubalin these components are identified and used to many bacterial infections. Antibacterial properties *Beauveria bassiana* associated with many active compounds such as Oosporein, Oxalic acid, Beauvericin, Bassianolide, Bassianin and tenellin. Particular Beauvericin compound virulence of insects and considered antimicrobial activity of many pathogenic bacteria.

This study examined how fats, or lipids, taken from the fungus B. bassiana can fight inflammation and blood clots. The researchers specifically tested how well these lipids could block two common triggers in the body known as PAF and thrombin. To measure this, they used a lab test to see if the lipids could stop blood cells from clumping together. The effectiveness of each lipid was measured by its IC_{50} value, which represents the exact amount of lipid required to reduce the activity of these triggers by half. Essentially, this value helps determine how strong the lipid is at preventing clotting and inflammation, with lower amounts indicating higher potency. (Ippolito Camele *et al.,* 2023) [6]

The fungus B. bassiana is a natural pest killer that relies on 43 specific proteins to transport sugar for energy and growth. Researchers discovered that one protein in particular, BbStp13, is essential for the fungus to develop properly and remain deadly to insects. Without this protein, the fungus struggles to penetrate an insect's outer shell, produces fewer spores for spreading, and cannot maintain a strong cell wall. Furthermore, BbStp13 helps the fungus hide from the insect's immune system, making it a more effective hunter. By understanding how this sugar transporter works, scientists can now develop more powerful biological tools to protect crops from pests. (Jinli Ding *et al,* 2025) [7].

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