



A PROCESS FOR PRODUCTION OF ACTIVE BIOMASS OF VIOLA ODORATA IN BIOREACTORS

IITM Technology Available for Licensing

Problem Statement

- *Viola odorata* L. (Banafsha) is an endangered plant with valuable medicinal properties, but its natural propagation is challenging due to seed dormancy and specific growth requirements.
- **Natural sources of bioactive secondary metabolites from this plant are limited**, with variations in composition based on environmental factors.
- In vitro cultures offer a sustainable alternative for continuous production, but they face limitations, such as low yield and long cultivation times.
- **The need is to develop a process for enhanced biomass production of *Viola odorata* L. in *in vitro* cell suspension culture**, ensuring a high yield of bioactive secondary metabolites for malaria treatment.

Intellectual Property

- IITM IDF Ref. 2296
- IN 202241003520

Technology Category/ Market

Category- Bioprocessing & Biotechnology

Applications- Pharmaceuticals, Herbal Medicine and Nutraceuticals.

Industry- Pharmaceuticals and Healthcare

Market- Global bioprocessing technology market is projected to increase from \$18.71 billion in 2022 to \$21.74 billion in 2023, with a CAGR of 16.2%..

Research Lab

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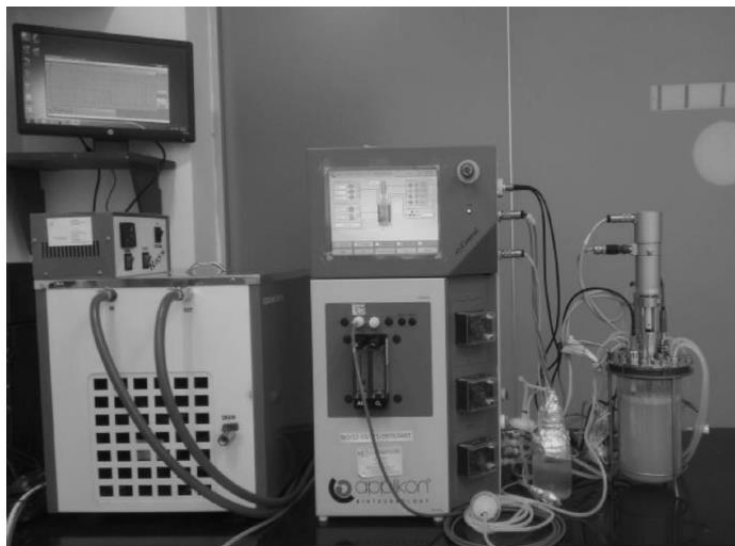


FIG.1. Stirred-tank reactor (STR) for biomass production of *Viola odorata* L.



FIG. 2. Airlift reactor (ALR) for biomass production of *Viola odorata* L.

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Key Features / Value Proposition

•The bioprocess optimally cultivates *Viola odorata* L. in bioreactors, ensuring high biomass yield for the extraction of valuable secondary metabolites.

Enhanced Biomass Production

•Using advanced tools, the process optimizes various parameters, including temperature, pH, and growth regulators, to maximize both biomass and metabolite production.

Multi-Factor Optimization

•The choice of bioreactor design (stirrer tank, air lift, or bubble column) accommodates diverse production needs.

Bioreactor Versatility

•Operate in batch, fed-batch, or continuous mode, with nutrient feeding strategies fine-tuned for efficiency.

Operation Flexibility

•The optimized *Viola odorata* L. cell line VOP-4 ensures maximum biomass production.

High-Yielding Cell Lines

•Extracted metabolites exhibit potent biological activities, rivaling those from natural plant sources with applications in malaria treatment, antimicrobial, anticancer, antioxidant, and anti-inflammatory activities.

Robust Secondary Metabolites

Technology

The invention discloses a bioprocess for the production of biomass of *Viola odorata* L. comprising:

- Preparation of inoculum** comprising suspending in vitro developed callus, in growth medium(A) and growing at optimal culture conditions to form cell suspension culture, from which cells were isolated and used as inoculum;
- Inoculating the inoculum** at a specific density in optimized growth medium(C) contained in bioreactor and culturing the inoculum for 12-50 days under optimal conditions of temperature, pH, light intensity, agitation, aeration and photoperiod in the presence of anti-foaming agent to form biomass;
- Harvesting the biomass** after 12-50 days by filtering the contents of bioreactor through filter paper under vacuum.

The said bioprocess for the production of biomass of *Viola odorata* L, the bioprocess further comprises:

- Washing the harvested biomass** with distilled water to form washed biomass;
- Lyophilizing the washed biomass** to form lyophilized biomass;
- Homogenizing the lyophilized biomass** by macerating in aqueous ethanol (50-70%) for 6 h with intermittent shaking to form homogenized biomass;
- Centrifuging the homogenized biomass** to separate supernatant from cell debris;
- Collecting the supernatant** and lyophilizing the supernatant to form biomass extract powder;
- Storing the biomass** extract powder at -80 °C.

TRL (Technology Readiness Level)

TRL - 4: Technology validated in lab scale.

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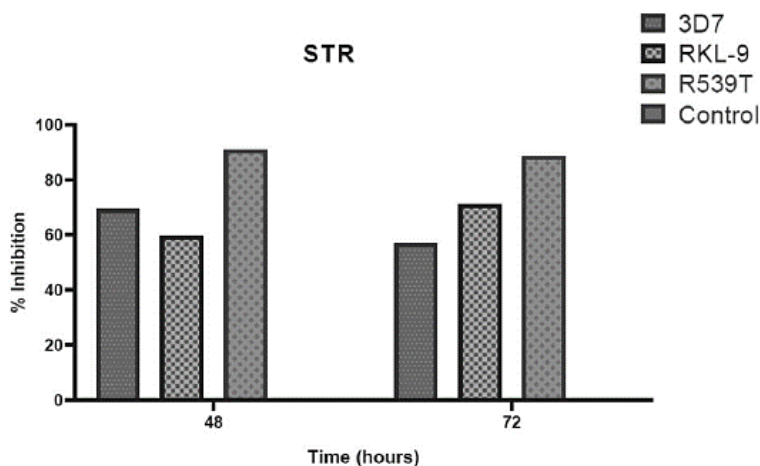


FIG. 3. Anti-plasmodial activity of *Viola odorata* plant extracts. STR cultivated biomass extract inhibits the growth of 3D7 strain, RKL -9 and R539T strains at 48 and 72 hours. The concentration of biomass extract is 250 µg / mL. The initial parasitemia was 1% and 2% HC maintained. No inhibition was observed in control conditions.

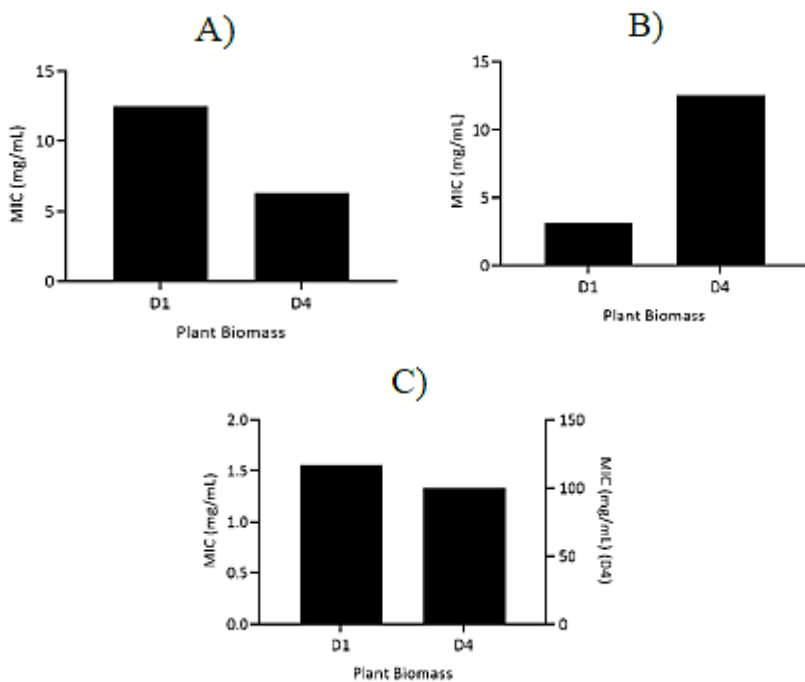


FIG. 4. Antibacterial activity of *Viola odorata* plant extracts. MIC values of natural plant biomass extract (D1) and STR cultivated biomass extract (D4).
A) MTCC 3826 *Haemophilus influenzae*
B) MTCC 655- *Streptococcus pneumoniae*,
C) MTCC 442 - *Streptococcus pyogenes*.

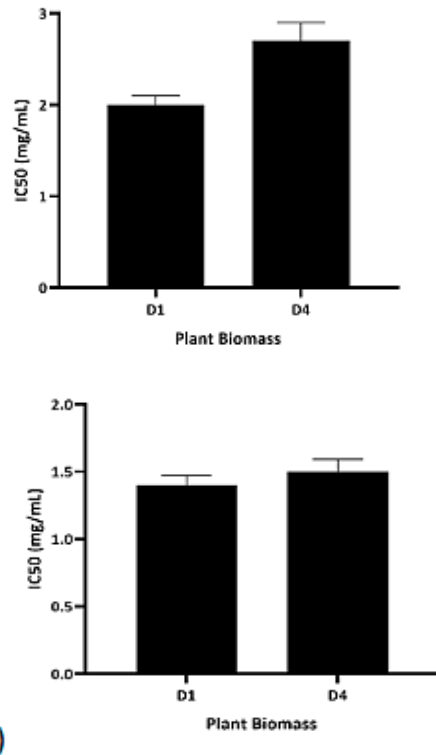


FIG. 5. Cytotoxic activity of *Viola odorata* plant extracts. IC50 values of natural plant biomass extract (D1) and STR cultivated biomass extract (D4).
A) A549 - Lung adenocarcinoma cells,
B) Caco-2 human colorectal adenocarcinoma cells.

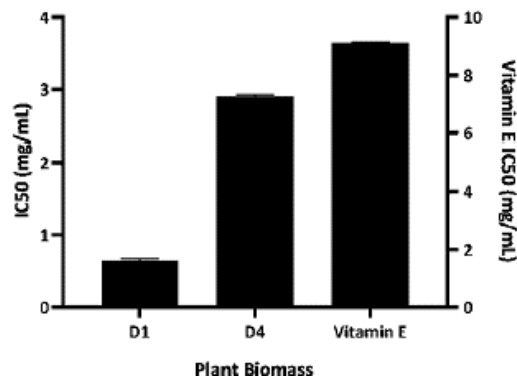


FIG. 6. Antioxidant activity of *Viola odorata* plant extracts. IC50 values of natural plant biomass extract (D1), STR cultivated biomass extract (D4) and Vitamin E.

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