

## Studies on Phytochemical Screening and Antibacterial Activity of Rhizome Extracts of *Coleus forskohlii* Briq

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### Abstract

The present study was on the analysis of phytochemical constituents of *Coleus forskohlii* rhizome collected from Thiruvannamalai of Tamil Nadu and testing for its antimicrobial potential against human bacterial pathogens. The dried tubers were extracted using different solvents such as ethanol, aqueous, acetone, chloroform and petroleum ether and screened. The total tannin and saponin content in the extract was estimated. The crude ethanolic solvent extract of *C. forskohlii* was tested for its antimicrobial activity using disc diffusion method. Phytochemical screening of the crude solvent extracts revealed the presence of tannins, phenols, saponins, quinone, flavonoids, cardiac glycoside, terpenoids, steroids, alkaloids, and coumarins. Tannic acid was found in the level of  $53.47 \pm 0.37$  mg TAE/g. Saponin content was  $2.42 \pm 0.63$  mg/g dry weight. The crude ethanolic rhizome extract was tested in three different concentrations (10, 20 and 30mg/ml). Maximum antibacterial activity was noted against *Bacillus subtilis* (15 mm), followed by *Bacillus cereus* (14 mm), *P. aeruginosa* (12 mm) and *S. aureus* (12mm). *E. coli* was not inhibited even at the highest concentration tested (i.e.) 30mg/ml. The minimum inhibitory concentration (MIC) values of the pathogens tested were  $1.4 \pm 0.02$ ,  $1.590 \pm 0.02$ ,  $1.820 \pm 0.02$  and  $2.350 \pm 0.02$  mg/ml respectively to *B. subtilis*, *B. cereus*, *P. aeruginosa*, and *S. aureus*. Similarly, the minimum bactericidal concentration (MBC) for *B. subtilis*, *B. cereus*, *P. aeruginosa*, and *S. aureus* were respectively of  $2.89 \pm 0.02$ ,  $3.833 \pm 0.05$ ,  $3.842 \pm 0.01$  and  $4.856 \pm 0.05$  mg/ml. In time-kill assay 3 Log reduction was found to be around 11 h for *B. subtilis*, around 12 h for *B. cereus* for *P. aeruginosa* around 13 h and above 14 and less than 15 h for *S. aureus*. The study showed the antimicrobial potential of ethanol extract of *C. forskohlii* tuber against human bacterial pathogens and hence it seems to be useful in the preparation of drug formulations.

**Keywords:** *Coleus forskohlii*, phytochemical screening, tannin, saponin, antibacterial activity, MIC, MBC, Time-kill assay.

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## INTRODUCTION

*Coleus* spp. is available in different countries and used for different ailments. About 150 *Coleus* spp. are distributed in different parts of the world in which the *C. forskohlii* is found in Asia, Africa, Australia and Pacific Islands. This plant is an herb belonged to the family Lamiaceae, in which mint and lavenders are also placed. The fragrance is due to the presence of essential oil which is responsible for the aroma. Roots are fibrous in nature. *Coleus* plants have 3000 years old history in Ayurvedic medicines which are in practice to cure heart and lung diseases. As it is having the capacity to break the adipose tissue it is also useful in weight reduction programs. The rate of metabolism is induced to burn fat tissue by stimulation of the enzyme adenylate cyclase, resulting in cyclic AMP synthesis. It is also seemed to be useful in eye care products to reduce pressure in the eyes. In the small intestine, it helps in adsorption of pre-digested foods. It is also used against allergens (i.e.) as an antihistamine drug in diseases like asthma, bronchitis etc. As it improves the cyclic AMP content, it also been used against diseases like hypothyroidism and skin diseases like eczema and psoriasis. It acts on lipid hydrolysis as well as to relax the arterial walls and hence used as angina. Due to its antioxidant and anticancer properties, now focus is on using its extract against secondary or metastatic cancers especially gastric cancers.

In India it is for dysentery, digestive disorders and recently to reduce body weight in human beings<sup>1</sup>. In Brazil also, for a similar purpose, they are used. Ayurvedic medicine recommended this for various disorders like heart disease, abdominal colic, respiratory disorder, burning sensation, insomnia, convulsions, asthma, bronchitis, epilepsy and angina<sup>2</sup>. Its leaves are used as an expectorant, emmenagogue and as a diuretic in various countries. The present study was on phytochemical screening and testing of antimicrobial activity of crude ethanolic root extract of *C. forskohlii* plant.

## MATERIALS AND METHODS

### Phytochemical screening of rhizome extract of *Coleus forskohlii*

*Coleus forskohlii* rhizomes were collected from Tiruvannamalai, phytochemical screening

was done by adopting the described standard methods<sup>3-5</sup>. Visible color change or precipitate formation was taken into consideration for the presence (+) or absence (-) of a particular active constituent.

### Estimation of tannin content in rhizome extracts of *Coleus forskohlii*

Tannin content was quantified by adopting a standard method<sup>6</sup>. 1 g of powdered tubers was extracted thrice with 10 ml of absolute ethanol. From this 1 ml was taken in a test tube and 0.5 ml of Folin-Ciocalteu's reagent was mixed followed by 1 ml of saturated sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) and made up to 10 ml using distilled water. After 30 min. incubation at room temperature it was centrifuged at 6000 rpm for 10 min. and the supernatant was used to read in a UV-Vis spectrophotometer (Systronics) at 725 nm. Tannic acid was used as the standard.

### Determination of saponin in *Coleus forskohlii*

Saponin content was done according to described standard method<sup>7</sup>. In which ethanol, diethyl ether, and butanol were used and washed with 5% aqueous NaCl. Evaporation was done in a water bath to get constant weight which represented the saponin content.

### Antibacterial activity of rhizome extracts of *Coleus forskohlii*

#### Bacterial strains and inoculum preparation

The antibacterial potency activity of ethanol extract of tuber was tested against five different pathogens such as *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* at three different concentrations (i.e.) 10, 20 and 30 mg/ml. Each strain was subcultured in 5 ml of Mueller Hinton broth medium (Hi-media) and incubated overnight at 37°C for 18-24 h to attain viable cell count of  $10^7$  CFU/ml.

#### Antibacterial activity testing using disc diffusion test

Antibacterial activity was measured using the standard method of disc diffusion on agar plates<sup>8</sup>. 0.1 ml of overnight grown each bacterial culture was spread on Mueller Hinton agar (Hi-media) plate surfaces. Paper discs (6mm in diameter) loaded with 20µl of different concentrations (10, 20 and 30 mg/ml) of ethanolic rhizome extract of *Coleus forskohlii* were placed on the agar medium. Plates were incubated at

35°C for 24 h. Antibacterial activity was measured and assessed based on the size of the zone of clearance.

#### Determination of Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) against bacterial pathogens

The Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) were determined by broth/tube dilution method according to the recommendations of the National Committee for Clinical Laboratory Standards Institute<sup>9</sup>. Different concentration of ethanolic plant extract 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781, 0.390, 0.195 mg/ml were prepared in tubes containing 5 ml Muller Hinton broth. Pathogenic bacterial strains (*Bacillus subtilis*, *Bacillus cereus*, *P. aeruginosa* and *S. aureus*) were inoculated in 5ml of Muller Hinton broth (MHB) and incubated at 37°C until the turbidity value of 0.5 McFarland (approximately 10<sup>6</sup>CFU/ml) was attained<sup>10</sup>. 100 µl of each bacterial strain was inoculated into tubes containing different concentrations of plant extract were incubated at 37°C for 24 h and then observed for turbidity. The Tube containing sterile Muller Hinton broth alone was used as a negative control. The MIC was defined as the lowest concentration that completely prevented visible growth after incubation at 37°C for 18-24 h.

After determination of MIC for each bacterium, 100µl from all tubes in which no visible

bacterial growth observed were inoculated into sterile MHB without the plant extract individually. The tubes were then incubated for overnight at 37°C. The MBC is defined as the lowest concentration of antimicrobial agent that kills >99.9% of the initial bacterial population where no visible growth of the bacteria was observed in the Muller Hinton broth tubes.

#### Time-kill assay

Time-kill assays were performed to further evaluate the antibacterial activity of ethanolic extract against all tested pathogens according to the procedure described previously<sup>11,12</sup>. The Overnight culture was used to inoculate fresh Muller-Hinton broth containing 10mg/ml of tuber extract with 10<sup>6</sup> CFU/ml of cells. The culture was incubated at 37°C for 24 h. Surviving bacteria were counted for every 3 h up to 24 h (i.e. 0-24 h). 100µl of samples were subcultured on Muller-Hinton plates and the surviving colonies were counted and expressed as CFU/ml. A bactericidal effect was defined as a  $\geq 3 \log_{10}$  CFU/ml decrease compared to the initial inoculum.

#### Statistical analysis

Each test was performed in triplicates and the results were expressed as mean  $\pm$  standard deviation.

## RESULTS

In the present investigation, rhizome samples were collected from Thiruvannamalai

**Table 1.** Phytochemical screening from rhizome extract of *Coleus forskohlii*

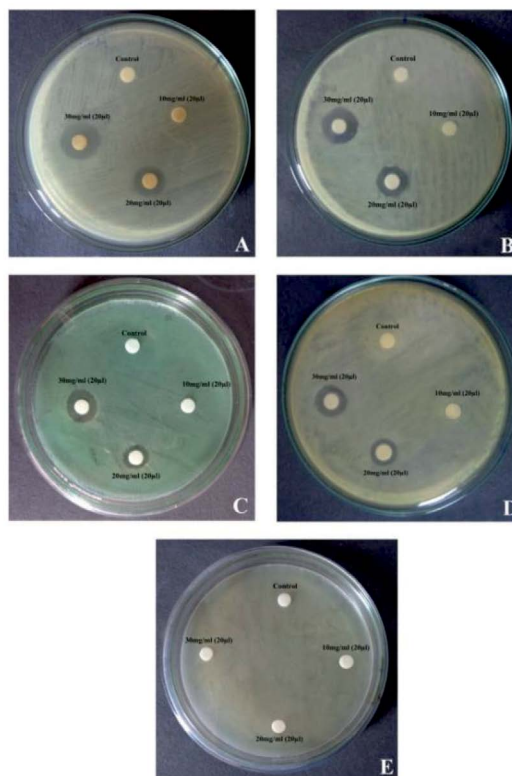
Phytochemicals	Rhizome extract of <i>Coleus forskohlii</i>				
	Ethanol	Aqueous	Acetone	Chloroform	Petroleum ether
Tannin	++	++	++	+	+
Saponin	+	+	+	-	-
Quinone	++	+	+	-	-
Flavanoid	++	+	+	-	-
Phenol	+	+	+	+	+
Glycosides	-	-	-	-	-
Cardiac glycoside	++	-	-	+	+
Terpenoids	++	++	++	-	-
Steroids	++	+	+	-	-
Alkaloid	+	-	++	-	+
Coumarins	++	+	+	+	+

Key: + Positive: ++ Strong positive: - negative

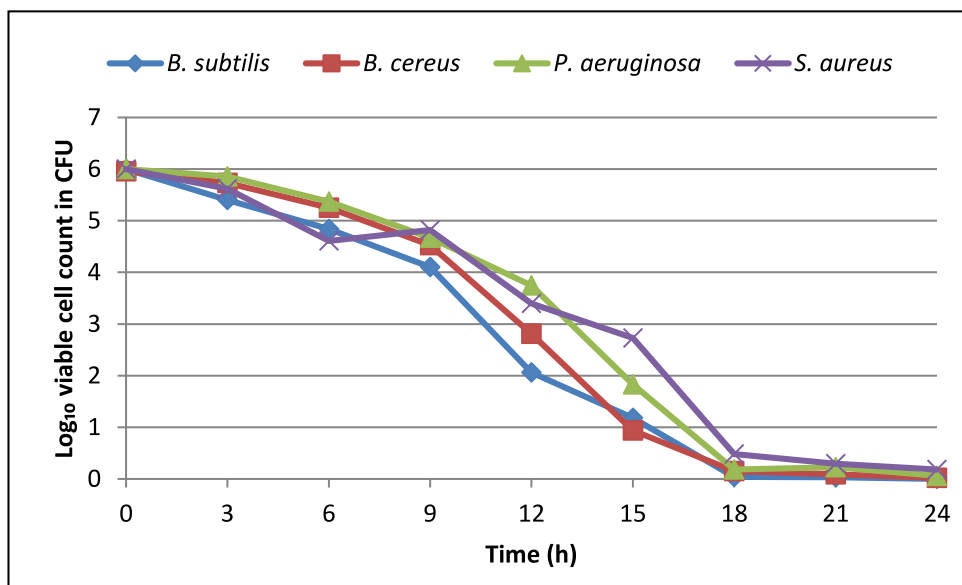
of Tamil Nadu. The dried rhizome sample was extracted with different solvents such as ethanol, aqueous, acetone, chloroform and petroleum ether. Each solvent extract was screened for the presence of phytochemicals. The results revealed the presence of tannins, phenols, saponins, quinone, flavonoids, cardiac glycoside, terpenoids, steroids, alkaloids and coumarins (Table 1). Quantitative estimation of tannic acid content showed  $53.47 \pm 0.37$  mg TAE/g and the saponin content in the *C. forskohlii* was  $2.42 \pm 0.63$  mg/g dry weight (Table 2). When testing the antibacterial activity of crude ethanolic rhizome extract using disc diffusion method against various human pathogens such as *Bacillus subtilis*, *Bacillus cereus*, *P. aeruginosa*, *S. aureus* and *E. coli* at the maximum concentration 30mg/ml, the zone of clearance of 15 mm, 14 mm, 12 mm and 12 mm and 0 mm respectively (i.e.) *E. coli* was not inhibited (Fig. 1 and Table 3).

**Table 2.** Quantitative estimation of tannin and saponin content from rhizome extract of *Coleus forskohlii*

Sample	Tannin content (mg TAE/g)	Saponin content (mg/g dry weight)
<i>Coleus forskohlii</i> rhizome extract	$53.47 \pm 0.37$	$2.42 \pm 0.63$



**Fig. 1.** Antibacterial activity of ethanolic rhizome extract of *Coleus forskohlii* on bacterial pathogens A: *Bacillus subtilis*; B: *Bacillus cereus*; C: *Pseudomonas aeruginosa*; D: *Staphylococcus aureus* and E: *Escherichia coli*



**Fig. 2.** Time-kill assay for tested bacterial pathogens

**Table 3.** Antibacterial activity from rhizome extract of *Coleus forskohlii*

Microorganisms tested	Zone of inhibition (mm)		
	Concentrations of ethanolic rhizome extract used		
	10mg/ml	20mg/ml	30mg/ml
<i>Bacillus subtilis</i> (MTCC No. 100224)	-	12	15
<i>Bacillus cereus</i> (MTCC No. 10221)	-	11	14
<i>Pseudomonas aeruginosa</i> (MTCC No. 14676)	-	12	12
<i>Staphylococcus aureus</i> (MTCC No. 9542)	-	10	12
<i>Escherichia coli</i> (MTCC No. 1563)	-	-	-
Negative control (Ethanol)	-	-	-

Inhibition Zone in diameter (mm)\*

The minimum inhibitory concentration (MIC) values of the tested pathogens viz., *B. subtilis*, *B. cereus*, *P. aeruginosa* and *S. aureus* were respectively of  $1.4 \pm 0.02$ ,  $1.590 \pm 0.02$ ,  $1.820 \pm 0.02$  and  $2.350 \pm 0.02$  mg/ml. Likewise, the minimum bactericidal concentration (MBC) for *B. subtilis*, *B. cereus*, *P. aeruginosa*, and *S. aureus* were respectively of  $2.89 \pm 0.02$ ,  $3.833 \pm 0.05$ ,  $3.842 \pm 0.01$  and  $4.856 \pm 0.05$  mg/ml (Table 4). The time-kill assay for the ethanolic rhizome extract of *C. forskohlii* against the tested human pathogens *Bacillus subtilis*, *Bacillus cereus*, *P. aeruginosa*, and *S. aureus* revealed the viable cells in the range of 0 to  $0.182 \log_{10}$  CFU/ml after 24 hours of incubation. Whereas at 3 hours incubation, it was in the range of  $5.4-5.86 \log_{10}$  CFU/ml and at 12 hours it was ranged from  $2.06-3.75 \log_{10}$  CFU/ml. At 18 hours it was at the range of  $0.04-2.73 \log_{10}$  CFU/ml (Fig. 2). Time-kill assay showed 3 Log reduction around 11 h for *B. subtilis*, around 12 h for *B. cereus* for *P. aeruginosa* around 13 h and above 14 and less than 15 h for *S. aureus*.

**Table 4.** MIC and MBC

Pathogens tested	Minimum Inhibitory concen./ Minimum Bactericidal concen. (mg/ml)	
	MIC	MBC
<i>B. subtilis</i>	$1.4 \pm 0.02$	$2.89 \pm 0.02$
<i>B. cereus</i>	$1.590 \pm 0.02$	$3.833 \pm 0.05$
<i>P. aeruginosa</i>	$1.820 \pm 0.02$	$3.842 \pm 0.01$
<i>S. aureus</i>	$2.350 \pm 0.02$	$4.856 \pm 0.05$

## DISCUSSION

Qualitative phytochemical analysis of the ethanolic extract of *C. forskohlii* rhizome showed the presence of tannins, quinone, flavanoids, cardiac glycoside, terpenoids, steroids, and coumarins in relatively higher level, whereas saponins, phenols, and alkaloids in lower level. In chloroform and petroleum ether extracts, only tannin, phenol, cardiac glycoside and coumarins were present. Other solvents also showed varying results. The solvent used in the extraction procedure seemed to decide the phytochemical component that is derived. Hence to know the components present and to delineate their biological or physiological activities useful phytochemical screening is important<sup>13</sup>.

Earlier studies found *C. forskohlii* rhizome was rich in phenol and flavonoids<sup>14-16</sup>. The present investigation revealed the high content of tannin. The total tannin content in the ethanolic rhizome extract of *Coleus forskohlii* was determined by UV spectrophotometric method and it was found to be  $53.47 \pm 0.37$  mg tannic acid equivalent (TAE) /g (Table 2). The given value is mean  $\pm$  SD for three different determinations. Presence of tannins was reported in various parts like leaves, stems, and roots of popular medicinal plants collected from Northern India<sup>17</sup>.

Saponin content observed in the present work in the *C. forskohlii* was  $2.42 \pm 0.63$  mg/g dry weight. The antibacterial activity of saponin was reported in *Sorghum bicolor* extract<sup>18</sup>. Interestingly it had more inhibitory activity towards Gm positive bacteria as observed in the present study. The

Antibacterial and fungistatic effect had been reported in various plant extracts in earlier work also<sup>19,20</sup>.

Among five pathogens tested four were inhibited by the extract of *C. forskohlii*. Maximum inhibitory activity was found against *Bacillus subtilis* followed by *Bacillus cereus*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. However, it was not inhibitory to *E. coli*. The ethanolic extract of *C. forskohlii* roots collected from Rewa (Madhya Pradesh) was found to be most inhibitory against *B. cereus* (32 mm), followed by *M. luteus* (31 mm), *K. pneumoniae* (30 mm), *S. aureus* (29 mm), *P. aeruginosa* (15 mm) and *E. coli* (15 mm)<sup>16</sup>. In their study also, lowest inhibitory activity was shown against *E. coli*. In another study done on aqueous and methanolic extracts of 12 plants belonging to different families, aqueous extract did not show any activity whereas methanol extract inhibited *B. cereus* and *S. epidermidis*<sup>21</sup>.

When ethanolic extracts of 50 medicinal plants of Nigeria tested, 28 were inhibitory to one or more pathogens tested, which included *B. subtilis*, *E. coli*, *S. aureus*, *P. aeruginosa* and *C. albicans*<sup>22</sup>. Leaves and flowers of *C. hirsute*, extracted using ethanol and dichloromethanol as well as the similar extract of leaves and stems of *C. squalid* were found to inhibit *Staphylococcus* and *Streptococcus*. However only rhizomes were used in the present investigation.

*Coleus caninus* (Roth) Vatke extract showed inhibitory activity against *E. coli* when 50mg/ml of hydro-alcoholic extract was used<sup>23</sup>. Surprisingly they found most inhibitory activity against *E. coli* whereas in the present investigation it was not so, even with 30mg/ml of the rhizome. The methanolic leaf extract of *Acasis nilotica* was inhibitory against *E. coli*, *S. aureus* and *Xanthomonas axonopodis*<sup>24</sup>. Both Gram positive and Gram negative bacteria were found to be inhibited by ethanol extract of the root of this plant. It is true that ethanol, as well as methanol extracts, were more inhibitory compared to other solvents. It is opined that methanol might be more inhibitory compared to ethanol<sup>25</sup>. This might be due to the aromatic nature of the inhibitory compounds<sup>26</sup> which may be easily extracted in organic solvents like ethanol, methanol etc., It was also proved the same through a study on *Leucas aspera*<sup>27</sup>. In general Gram-positive bacteria are more susceptible<sup>28,29</sup>. The cell

wall of Gram-positive bacteria is thinner and made of single layer whereas that of Gram-negative is thicker and is a multilayered structure<sup>30</sup>. However resistance seemed to be strain dependent<sup>31</sup> as *P. aeruginosa* was inhibited and *E. coli* was not.

The antimicrobial activities of ethanolic extract may be due to the presence of phytochemicals identified. Antimicrobial activity of tannins was already reported by many researchers<sup>32-36</sup>. Tannic acid works like a siderophore and it quenches iron and the microorganism may die due to lack of iron to do functions like reduction of the ribonucleotide precursor of DNA, a formation of haem and various other important functions<sup>37</sup>. Inhibition of 15 bacterial pathogens tested by tannic acid was observed<sup>37</sup>. However, in a study, it was not shown due to lack of iron as they added extra iron to the medium<sup>32</sup>. The astringent property of tannin form complexes with enzymes and substrates is another view<sup>37</sup>. They also suggested its action on membrane and complexation of metal ion which are involved in important cellular functions of the pathogens. Tannic acid increased the inhibitory activity of  $\beta$ -lactam antibiotics<sup>32</sup>.

The MIC values of the pathogens tested were  $1.4 \pm 0.02$ ,  $1.590 \pm 0.02$ ,  $1.820 \pm 0.02$  and  $2.350 \pm 0.02$  mg/ml respectively to *B. subtilis*, *B. cereus*, *P. aeruginosa*, and *S. aureus*. Similarly the MBC for the tested pathogens such as *B. subtilis*, *B. cereus*, *P. aeruginosa*, and *S. aureus* were respectively of  $2.89 \pm 0.02$ ,  $3.833 \pm 0.05$ ,  $3.842 \pm 0.01$  and  $4.856 \pm 0.05$  (Table 4).

MIC values were comparable to other studies in related plants, as well as to various other potent medicinal plants<sup>38,32</sup>.

MBC values were ranged from  $2.89 \pm 0.02$  to  $4.856 \pm 0.05$  mg/ml. Similar reports were done in ethanolic extracts of *Coleus aromaticus* (Muniandy *et al.*, 2014). The MBC values were found to be higher than that of MIC. Approximately 2 MIC values in all the pathogens tested. Similar results (i.e.) 2-3 times more MIC for MBC values was reported by many in plant extracts as well as regarding antibiotics also<sup>14,32,36</sup>.

The study results revealed that within 15 to 18 h the pathogens can be killed totally and hence this plant extract can be used for various drug formulations.

The results obtained in the present study



were more potent compared to previous studies in this plant and related species<sup>38,39</sup>. Time-kill assay in the present study showed 3 Log reduction found to be around 11 h for *B. subtilis*, around 12 h for *B. cereus* for *P. aeruginosa* around 13 h and above 14 and less than 15 h for *S. aureus*. Thus the results obtained in the present investigation were appreciable.

## CONCLUSION

The present study clearly indicated the antibacterial potential of an ethanolic crude extract of *C. forskohlii* collected from Thiruvannamalai district of Tamil Nadu.

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## CONFLICT OF INTEREST

The authors declares that there is no conflict of interest.

## AUTHORS' CONTRIBUTION

SS collected plant materials, did laboratory research work, consolidation and interpretation of data, and wrote the manuscript. BVP reviewed and corrected the manuscript.

## FUNDING

None.

## DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

## ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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