Original Article

Thai Journal of Pharmaceutical Sciences

Moss *Bryum weigelii* spreng improves survival in septic rats induced by cecal ligation and puncture

Ho Viet Hieu[1], Surendra Babu Lagu[2,3], Vinay Bharadwaj Tatipamula[4]

[1]Department of Medical Microbiology and Parasitology, Faculty of Medicine, Duy Tan University, Da Nang, Vietnam, [2]Department of Pharmaceutical Chemistry, AU College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, India, [3]Department of Pharmaceutical Chemistry, Adikavi Nannaya University, Tadepalligudem Campus, Tadepalligudem, Andhra Pradesh, India, [4]Center for Molecular Biology, College of Medicine and Pharmacy, Duy Tan University, Danang 550000, Vietnam

Corresponding Author:
Vinay Bharadwaj Tatipamula, Institute of Research and Development, Duy Tan University, Da Nang 550000, Vietnam.
Phone: +84-774562619.
E-mail: vinaybharadwajtatipamula@duytan.edu.vn

Received: Aug 06, 2020
Accepted: Nov 10, 2020
Published: Aug 01, 2021

ABSTRACT

The present study investigated the phytochemical, high-performance thin-layer chromatography (HPTLC) profiling, total phenolic content, antibacterial activity, acute toxicity studies, hemodynamic parameters, myeloperoxidase (MPO) activity and serum procalcitonin (PCT) levels of hydroalcoholic extract of *Bryum weigelii* (HE-Bw), and its effects on cecal ligation and puncture (CLP) induced sepsis in mice. The preliminary phytochemical screening showed that HE-Bw contains alkaloids, terpenoids, flavonoids, phenolics, and tannins. The HPTLC analysis of HE-Bw identified three unknown components with different R_f values and area percentage. The total phenolic amount for HE-Bw was equivalent to 211.6 ± 0.8 mg of gallic acid per 100 g of dried plant material. At 100 µg/ml concentration, HE-Bw showed antibacterial activities as potent as streptomycin against *Staphylococcus aureus* and *Escherichia coli*. Acute toxicity studies on mice found out that HE-Bw was non-toxic up to 2000 mg/Kg body weight. At both low and high doses, HE-Bw improved hemodynamic parameters such as mean arterial pressure and decreased optical density of blood, while decreased serum MPO activity and PCT levels. Moreover, at a high dose, HE-Bw showed a survival rate of 92.50 ± 3.50% in mice that might be through proinflammatory and bacteremia effects. The results indicate that *B. weigelii* can be a favorable natural source for CLP-induced sepsis treatment in mice.

Key words: Antibacterial agent, Cecal ligation and puncture -induced sepsis, Hemodynamics parameters, High-performance thin-layer chromatography profile, Myeloperoxidase activity, Total phenolic content

INTRODUCTION

Sepsis is one of the major causes of death in intensive care units, and no decisive medical treatment is available against sepsis. Globally, it is the primary cause of death from infection in intensive care units.[1] It is a lethal clinical syndrome that results from the body's dysregulated systemic inflammatory response due to the invasion of pathogens.[2] Sepsis complications are varied and involve coagulation disorders, immune suppression, organ dysfunction, and systemic inflammation.[3,4] Severe sepsis affects the cardiovascular system that causes cardiomyopathy and endothelial dysfunction, which results from adverse effects of substances secreted from pathogens and host cells.[5]

Furthermore, sepsis impairs neutrophil migration and its antimicrobial activity. Inadequate migration of neutrophils into the infection site causes the systemic spread of pathogens, which results in high mortality rates. The initial management of infection in the sepsis requires initiating appropriate and timely antibiotic therapy.[6] However, there is no specific therapy or drug against sepsis. Hence, searching to find a new medication for the management of sepsis is necessary.

Many of the available medicines are derived from herbs, and medicinal plants have long been used to treat various disorders.

Bryum (Bryaceae family) is the largest genus of mosses with around 1000 species all over the world, and well recorded in the flora of Antarctica, India, Iceland, South Africa, Europe, Thailand, and China.[7,8] In traditional medicine, *Bryum* species has wide applications in treating microbial, burn cuts, wounds, and skin disorders infections.[9] Biologically, *Bryum* species reported for anticancer,[10] antimicrobial,[11,12] and antioxidant[13] activities. Chemically, *Bryum* species chiefly contains alkaloids, terpenoids, flavonoids, saponins, and tannins as major constituents.[10] To date, no proper phytochemical and biological investigation have attempted on *Bryum weigelii*. Hence, the current investigation mainly aimed to evaluate the phytochemical analysis, high-performance thin-layer chromatography (HPTLC) profiling, total phenolic content, and antibacterial activity of whole moss *B. weigelii* extract. Furthermore, its protective effects on hemodynamic parameters, myeloperoxidase (MPO) activity, serum procalcitonin (PCT) levels as well as survival rate were evaluated in cecal ligation and puncture (CLP)-induced sepsis in mice.

MATERIALS AND METHODS

Collection

The whole moss of *B. weigelii* Spreng has collected from Aaraku valley, Visakhapatnam, Andhra Pradesh, India in 2019, and a voucher specimen (AU/2019/Moss/1146) has deposited at the Department of Botany, Andhra University, Visakhapatnam, India.

Extraction and isolation

The whole moss was dried and powdered (200 g) and extracted three times with ethanol-water (7:3) at 25°C. All combined and evaporated under low pressure to obtain a hydroalcoholic extract of *B. weigelii* (HE-Bw**,** 5.0 g) preserved in an amber color bottle at 4°C.[14]

Preliminary phytochemical analysis

Preliminary phytochemical analysis on HE-Bw was performed according to the standard practical methods.[15,16] 0.5 g of HE-Bw was dissolved in 15 ml of methanol and filtered using a muslin cloth. The filtrate was subjected to the below phytochemical tests.

Test for alkaloids

To 1 ml of the prepared extract solution, two drops of Mayer's reagent were added. The appearance of a creamy white precipitate indicates the presence of alkaloids.

Test for anthraquinones

To 1 ml of extract solution, a few drops of 10% ammonia solution were added. The appearance of a pink color precipitate indicates the presence of anthraquinones.

Test for bibenzyls

To 1 ml of extract solution, a few drops of aqueous potassium permanganate were added. The appearance of purple color indicates the presence of phenanthrenes.

Test for cardiac glycosides

To 1 ml of prepared extract solution was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the deoxysugar characteristics of cardenolides. A violet ring may appear below the ring, while in the acetic acid layer, a greenish ring may be formed.

Test for coumarins

To 1 ml of 10% sodium hydroxide was added to 1 ml of the prepared extract solution. The formation of yellow color indicates the presence of coumarins.

Test for flavonoids

To 1 ml of prepared solution was treated with a 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.

Test for fluorenones

To 1 ml of prepared extract solution, a few drops of glacial acetic acid and 10% sodium hypochlorite solution were added, a bright yellow fluorescent indicates the presence of fluorenones.

Test for phenanthrenes

To 1 ml of prepared extract solution, a few drops of 2% chromic acid were added. The liberation of intense green color vapors indicates the presence of phenanthrenes.

Test for phenolics

To 1 ml of prepared solution, a few drops of neutral 5% ferric chloride solution were added. Dark green color indicates the presence of phenolic compound.

Test for saponins

The extract (100 mg) is diluted with distilled water and made up to 10 ml. The suspension was shaken in a graduated cylinder for 15 minutes. A 1 cm layer of foam indicates the presence of saponins.

Test for tannins

To 1 ml of extract, 2 ml of 5% ferric chloride was added. The formation of dark blue or greenish-black indicates the presence of tannins.

Test for terpenoids

To 1 ml of prepared solution, 1 ml of chloroform and 2 ml of sulphuric acid were added. The formation of reddish-brown color indicates the presence of terpenoids.

HPTLC analysis

The HPTLC analysis of HE-Bw was performed on CAMAG Linomat 5 instrument. The HPTLC plate was developed using

HE-Bw (10 mg/0.5 ml, HPLC grade ethyl acetate) as per the standard operating protocol mentioned in Deattu group.[17] The developed plate was dried and sprayed with stannic chloride reagent and placed in a CAMAG TLC Scanner and captured image at wavelength 254 nm using a ultraviolet lamp (D2 & W) to visualize spots and peaks.

Assay for total phenolic content

The total phenolic content[18] of HE-Bw was established through the Folin-Ciocalteu reagent. Initially, 500 ml of HE-Bw were mixed with 5 ml of Folin-Ciocalteu reagent 10% (v/v) and 4 ml of sodium bicarbonate solution (1 M). After 15 min of incubation time for the mixture at room temperature, the absorbance of the produced blue color was read by spectrophotometer. Ultimately, the total phenolic content of HE-Bw was calculated from the calibration curve of gallic acid and expressed as gallic acid equivalent, respectively.[19]

Antibacterial activity

In vitro antimicrobial activity of HE-Bw was performed by the cup-plate method.[20] The test bacteria used in this study were two Gram-positive bacteria: *Staphylococcus aureus* (ATCC25923), and *Bacillus subtilis* (ATCC21332) and two Gram-negative bacteria: *Salmonella typhi* (ATCC1408), and *Escherichia coli* (ATCC25922). Mueller Hinton agar plates inoculated with 0.5 McFarland standards of mentioned bacteria were used for this assessment. Test strains are inoculated by spread late technique, and wells were made by sterile cork borer. Accurately, 50 µl (100 µg/ml concentration) of HE-Bw and standard (streptomycin) poured in each well. After 24 h incubation at 37 °C, inhibition zones were measured by a calibrated scale.[21]

Animals

Adult male Swiss albino mice (weighing 25 ± 5 g, age 6–8 weeks) were used in this study. The animals were given food and water *ad libitum* and were housed in the Animal House of Duy Tan University of Medicine and Pharmacy under the standard condition with a temperature of 21 ± 2 °C, the relative humidity (50 ± 10%), and a 12-h light/12-h dark cycle.[22] This study was approved by the Ethics Committee of Duy Tan University of Medicine and Pharmacy (Code: VN.DTU.MP.2020.412).

Acute oral toxicity using organization for economic cooperation and development (OECD) main test 425

Mice were randomly divided into four groups (6 mice in each group). The OECD main test 425 (up-and-down dose procedure) was utilized using doses of 175, 550, 1750, and 2000 mg/kg body weight (b.w) of HE-Bw. The test animals have undergone fasting overnight before administering the extract using oral gavage. The first set of test animals was administered with a dose of 175 mg/kg b.w. When the animal survived after 48 h, the dose given to the next sets of rodents increased a factor 3.2, which was 550 mg/kg b.w. After 48 h of survival, the next test animal was given 1750 mg/kg b.w, then the same cycle was repeated, and the upper bound dose of 2000 mg/kg b.w was given to the test rodents. The testing was ended until the last three animals survived the upper bound dose, and all of the test animals were observed up to 14 days.[23,24]

CLP-induced sepsis in mice

The CLP model[25] was used for the induction of sepsis. At the beginning of the experiment, mice were randomly divided into four groups (six mice in each group). Mice in group 1 (normal control) underwent midline abdominal incision without CLP. Mice in group 2 (CLP) underwent midline abdominal incision with cecal ligation (50%) and punctured to induce polymicrobial sepsis. Mice in groups 3 and 4 received 100 mg/kg b.w (as a low dose) and 200 mg/kg b.w (as a high dose) of HE-Bw orally at 0, 1, 3, 6, and 24 h after CLP operation. Blood samples were obtained from the portal vein. 0.5 ml of blood samples were transferred into laboratory tubes containing pre-autoclaved nutrient broth medium (Sigma-Aldrich, Germany) and put in an incubator at 37°C. The remaining blood samples decanted gently into collection plastic tubes, centrifuged at 3000 rpm for 5 min. Then, serum was obtained, aliquoted into microtubes, and stored at −20°C for biochemical analysis.

Later, mice were anesthetized by intraperitoneal ketamine (60 mg/kg b.w) and xylazine (10 mg/kg b.w). Then, the abdominal region of animals was shaved and sterilized by betadine. The cecum was exposed through a midline abdominal incision and ligated (50 %) with 3/0 silk suture then punctured with a sterile 18-gauge needle. The cecum was gently squeezed, and after a drop of cecal contents was discharged, and the cecum was repositioned into the abdominal cavity. The abdominal wall and skin were closed with 3/0 silk suture. After the surgery, mice received 3 ml warm 0.9% normal saline subcutaneously (s.c) for fluid resuscitation. After mice recovered from anesthesia, they had free access to food and water.

Animal survival rate

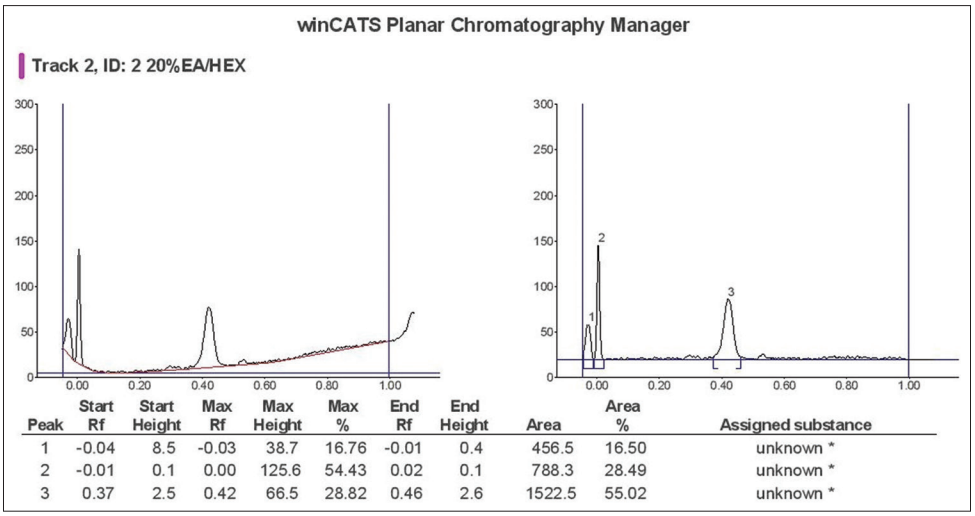
In addition to monitoring the animals for three days, animals' survival rate was reported after 72 h.[26]

Hemodynamic parameters

For measurements of hemodynamic parameters such as arterial blood pressure (ABP), mean ABP (MAP), developed pressure (DP), and heart rate (HR), a polyethylene cannula connected to a pressure transducer that prefilled with heparinized normal saline solution was cannulated into the right common carotid artery.[26]

MPO measurement

The activity of MPO,[27] an abundant enzyme of neutrophils, was assessed as previously described by the Xiao group with minor modification. Briefly, 1 ml of the serum was mixed with 1 mg of hexadecyltrimethylammonium bromide. Then, sonicated for 5 min and centrifuged at 3000 rpm for 10 min at 4°C. 0.1 ml of supernatant was mixed with 2.9 ml of 50 mM phosphate buffer (pH 6.0), containing 0.167 mg/ml *O*-dianisidine dihydrochloride, and 1% hydrogen peroxide. Then, the mixture was incubated for 5 min at room temperature.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.04	8.5	-0.03	38.7	16.76	-0.01	0.4	456.5	16.50	unknown *
2	-0.01	0.1	0.00	125.6	54.43	0.02	0.1	788.3	28.49	unknown *
3	0.37	2.5	0.42	66.5	28.82	0.46	2.6	1522.5	55.02	unknown *

Figure 1: HPTLC chromatogram of hydroalcoholic extract of *B. weigelii*

After adding 0.1 ml of 1.2 M HCl, the change in absorbance was measured at 460 nm using a spectrophotometer.

PCT test

PCT test was measured using immunofluorescence assay.[28] 100 µL of serum sample was placed in PCT fast test kit and measured using a quantitative immunofluorescence analyzer (Getein 1100, Getein Biotechnology Co., Ltd., China).

RESULTS

Phytochemical analysis

The preliminary phytochemical screening of HE-Bw possesses alkaloids, terpenoids, flavonoids, phenolics, and tannins, among the tested class of compounds. Besides, coumarins, phenanthrenes, anthraquinones, bibenzyls, fluorenones, saponins, and cardiac glycosides were found to be absent in the extract.

The HPTLC analysis was evaluated for the progress of the fingerprinting profile of HE-Bw, which was illustrated in Figure 1a and b. The HPTLC chromatogram recorded three unknown substances of peak 1, 2 and 3, with R_f values −0.04, −0.01 and 0.37, respectively, with a percentage area of about 16.67, 54.43 and 28.82%, respectively [Figure 1].

In addition, the total phenolic content was ascertained via the absorbance of HE-Bw and the equation obtained from the standard curve of gallic acid through the Folin-Ciocalteu method. Consequently, the total phenolic value for HE-Bw was equivalent to 211.6 ± 0.8 mg of gallic acid per 100 g of dried plant material.

Acute oral toxicity (OECD main test 425)

HE-Bw was non-toxic up to 2000 mg/kg B.W. of tested mice. There were no significant changes that occurred in the pattern of behavior of the tested animals. No mortality was noted for 14 days. These results present that the extract was non-toxic up to 2000 mg/kg b.w, and the low (1/20th) and high (1/10th) dosage was fixed as 100 and 200 mg/kg b.w, respectively.

Table 1: Antibacterial screening test of HE-Bw

Sample	Zone of inhibition (mm)*			
	Gram-positive		Gram-negative	
	B. subtilis	*S. aureus*	*E. coli*	*S. typhi*
HE-Bw	21.0±0.2	20.8±0.1	20.0±0.1	22.5±0.1
Streptomycin	24.5±0.1	21.3±0.1	19.8±0.1	25.4±0.1

*mean±SD values (n=3), *S. aureus*: *Staphylococcus aureus*, *E. coli*: *Escherichia coli*, *B. subtilis*: *Bacillus subtilis*, *S. typhi*: *Salmonella typhi*, HE-Bw: Hydroalcoholic extract of *Bryum weigelii*

Antibacterial activity

The *in vitro* antimicrobial activity of HE-Bw revealed that it has antibacterial activity against both gram-positive bacteria (*S. aureus* and *B. subtilis*), and gram-negative bacteria (*S. typhi* and *E. coli*). As shown in Table 1, HE-Bw was as potent as streptomycin against *S. aureus* and *E. coli* and showed the same inhibitory effect.

Hemodynamic responses

It was observed that the MAP was significantly decreased from 128.5 ± 3.5 mm of Hg in the normal control group to 57.0 ± 3.0 mm of Hg in the CLP group ($P < 0.05$). There was a significant increase ($P < 0.05$) in the MAP of mice treated with HE-Bw at 100 and 200 mg/kg b.w to 90.7 ± 5.3 and 108.7 ± 3.3 mm of Hg. It was found that ABP from 148.0 ± 4.0 mm of Hg in the normal control group decreased to 83.3 ± 6.7 mm of Hg ($P < 0.001$) in the CLP group. Treatment with HE-Bw at 100 and 200 mg/kg b.w increased the ABP ($P < 0.001$) significantly. DP decreased, and HR increased significantly ($P < 0.05$) in the CLP group, and HE-Bw treated groups showed no significant change [Table 2].

Optical density (OD) of blood at 600 nm

As shown in Figure 2a, the OD of blood significantly ($P < 0.01$) increased in the CLP group compared with the normal control group. On the other hand, the administration of HE-Bw (at both doses) to the septic mice significantly ($P < 0.05$) decreased OD in the blood of animals compared with the CLP group.

Table 2: Effects of hydroalcoholic extract of *B. weigelii* (HE-Bw) on hemodynamic parameters in CLP-induced sepsis after 72 h

Sample	Hemodynamic parameters (mm of Hg)*			
	Mean arterial pressure	Arterial blood pressure	Heart rate	Developed pressure
Normal Control	128.5±3.5	148.0±4.0	209.3±6.7	40.3±1.7
CLP	57.0±3.0[a]	83.3±6.7[b]	246.5±5.5	21.0±3.0[a]
HE-Bw-100	90.7±5.3[c]	113.5±6.5[d]	222.3±5.7	28.0±2.0
HE-Bw-200	108.7±3.3[d]	125.5±4.5[c]	206.0±4.0	32.5±3.5

*mean±SEM values (n=6); one-way ANOVA with Student-Newman-Keuls *post hoc test* was used for wise pair comparison where [a]$P<0.05$, [b]$P<0.001$ *versus* normal control group; [c]$P<0.05$, [d]$P<0.001$ compared with CLP group. HE-Bw-100: hydroalcoholic extract of *B. weigelii* at 100 mg/kg body weight; HE-Bw-200: hydroalcoholic extract of *B. weigelii* 200 mg/kg body weight

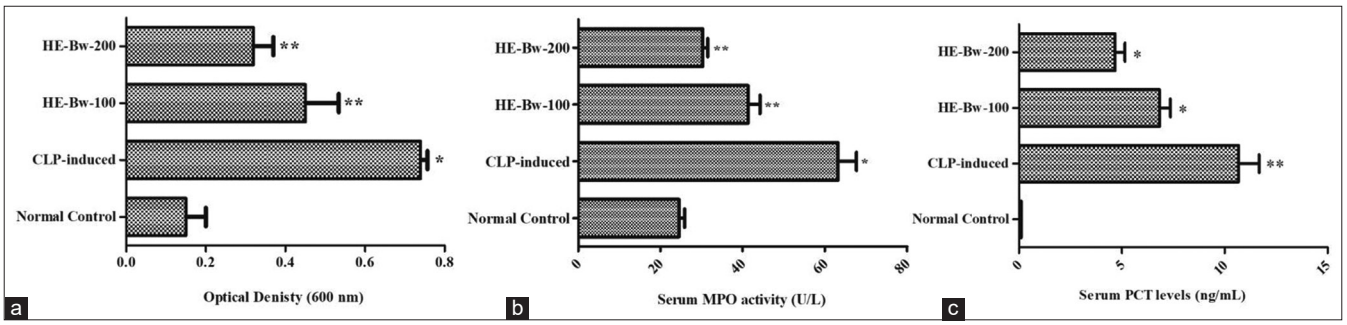


Figure 2: Effect of hydroalcoholic extract of *Bryum weigelii* (HE-Bw) on (a) optical density at 600 nm (b) myeloperoxidase activity (c) procalcitonin levels. Values are mean±SEM (n = 6). *P < 0.05, as compared with the normal control group; ** P < 0.01, as compared with cecal ligation and puncture group using one-way ANOVA with Student-Newman-Keuls *post hoc test*. HE-Bw-100: hydroalcoholic extract of *B. weigelii* at 100 mg/kg body weight; HE-Bw-200: hydroalcoholic extract of *B. weigelii* 200 mg/kg body weight

Serum MPO activity

Animals in the CLP group showed a significant (P < 0.05) increase in MPO activity than the normal control group. The treatment of mice with HE-Bw (at both doses) decreased markedly (P < 0.01) the enzyme activity compared with the CLP group [Figure 2b].

PCT test

CPT treated animals displayed a significant (P < 0.05) increase in serum PCT levels, compared to normal control group [Figure 2c]. At both doses, HE-Bw treated animals markedly reduced (P < 0.05) the serum PCT levels compared with the CLP group [Figure 2b].

Survival rate

To examine the effects of HE-Bw on survival rates, animals were monitored for 72 h after CLP surgery. There was no death of mice in the normal control group after 72 h, and the survival rate was 100.0 ± 0.0%. At 72 h, the survival rate decreased in the CLP group to 40.50 ± 3.67% compared with the normal control group. Treatment of septic mice with HE-Bw with doses of 100 and 200 mg/kg b.w increased the survival rate to 68.84 ± 5.34% and 86.50 ± 3.50%, respectively, compared to the CLP group at 72 h [Figure 3].

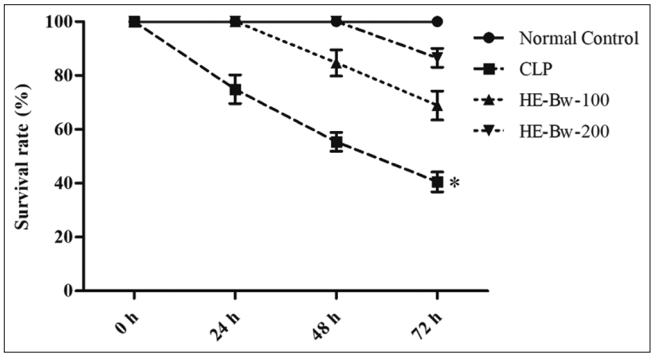


Figure 3: Effect of hydroalcoholic extract of *Bryum weigelii* (HE-Bw) on survival rate after 72 h (n = 6). * P < 0.05, as compared with the normal control group using one-way ANOVA with Student-Newman-Keuls *post hoc test*. HE-Bw-100: hydroalcoholic extract of *B. weigelii* at 100 mg/kg body weight; HE-Bw-200: hydroalcoholic extract of *B. weigelii* 200 mg/kg body weight

DISCUSSION

In the present study, we showed that HE-Bw improved hemodynamic parameters and MPO activity with potent antibacterial activity. In addition, HE-Bw unexpectedly decreased the inflammatory response and mortality rate in mice with polymicrobial sepsis. Preliminary phytochemical screening showed that HE-Bw contains alkaloids, terpenoids, flavonoids, phenolics, and tannins. Furthermore, a good amount of total phenolic content was observed in HE-Bw.

Our results showed that HE-Bw has antibacterial activity against *E. coli* and *S. aureus* [Table 1], which might be related to its phytochemical contents because the antibacterial activities of many alkaloids, flavonoids, and phenolics have been reported by several studies.[29-31] As these bacteria are the predominant cause of sepsis, this edible moss can have beneficial effects against sepsis.

As mentioned, sepsis causes cardiac and endothelial dysfunction by increasing vascular permeability, promoting activation of the coagulation cascade, tissue edema, and compromising regional perfusion in vital organs. Hemodynamic parameters and serum endotoxin to septic shock are two key determinants of sepsis survival. Amelioration of hemodynamics can relieve the severity of organ dysfunction in sepsis rats.[5,32] The hemodynamic monitoring in this study showed attenuation of hemodynamic parameters in the CLP group. This event can lead to misbalancing in tissues oxygen supply/demand and accelerates the process of septic shock. For this reason, apart from antibiotic therapy, hemodynamic stability is essential in the management of sepsis.[6] Administration of HE-Bw to septic mice increased mean arterial and mean blood pressure [Table 2]. Furthermore, at both doses, the OD in the blood of septic mice significantly decreased by the HE-Bw administration compared with the CLP group [Figure 2], which justifies the growth control of microbial infection in the blood.[33]

On the other hand, MPO is the most abundant proinflammatory enzyme stored in the azurophilic granules of neutrophilic granulocytes and is a marker of inflammation initiation in plasma. Thus, increased MPO activity indicates the onset of the inflammatory response and neutrophil infiltration due to the induction of microbial sepsis.[27] While PCT is a promising biomarker for the prediction of bacteremia.[28]

Our results showed that MPO activity and PCT levels increased in the serum of animals with CLP-induced polymicrobial sepsis compared to normal animals. Administration of HE-Bw decreased MPO activity and serum PCT levels, which showed the control of the proinflammatory effect and bacteremia levels, respectively, of *B. weigelii* when compared to the CLP group [Figure 2b]. However, the evidence related to the proinflammatory activities and bacteremia levels of *B. weigelii* is low.

The present study also demonstrated a reduction in survival rate in CLP-induced sepsis in mice [Figure 3]. The reason for high mortality rates in sepsis is the occurrence of a severe injury from CLP-induced sepsis. However, a great improvement in the survival rate of HE-Bw treated groups was noticed in our study. Taken together, the potential mechanism may be an antimicrobial effect of HE-Bw and its ability to neutralize the local and systemic levels of wide-spectrum inflammatory mediators in the serum of sepsis animals. Therefore, the administration of medications-extracted from *B. weigelii* might be useful for the treatment of sepsis.

CONCLUSIONS

To conclude, the present study results are the first report of moss *B. weigelii*, as a good source in the treatment of CLP-induced sepsis in mice. The key phytochemicals responsible for this activity claimed to be alkaloids, flavonoids, polyphenols, and tannins. The results provide evidence that supports the traditional uses of *B. weigelii*. Also, these findings suggest that moss *B. weigelii* can take an account as a good natural source of remedial medicine for sepsis. Hence, the current study results remain useful for further research to identify the potential bioactive molecules from *B. weigelii*.

ACKNOWLEDGMENT

None to acknowledge.

CONFLICT OF INTEREST

No conflict of interest between any of the authors.

REFERENCES

1. Pundir J, Coomarasamy A, Pundir J, Coomarasamy A. Bacterial sepsis in pregnancy. In: Obstetrics: Evidence-Based Algorithms. Cambridge: Cambridge University Press; 2016. p. 87-9.
2. Hayes G, Brisson BA. Sepsis. In: Complications in Small Animal Surgery. Chichester, UK: John Wiley and Sons Ltd.; 2017. p. 8-14.
3. Hund E. Neurological complications of sepsis: Critical illness polyneuropathy and myopathy. J Neurol 2001;248:929-34.
4. Freund HR, Muggia-Sullam M, Melamed E, LaFrance R, Holroyde J, Fischer JE. Septic encephalopathy. In: Advances in Hepatic Encephalopathy and Urea Cycle Diseases. Basel, Switzerland: Karger AGS; 2013. p. 473-83.
5. Funk DJ, Parrillo JE, Kumar A. Sepsis and septic shock: A history. Crit Care Clin 2009;25:83-101.
6. Wheeler AP, Bernard GR. Treating patients with severe sepsis. N Engl J Med 1999;340:207-14.
7. Ingimundardóttir GV, Weibull H, Cronberg N. Bryophyte colonization history of the virgin volcanic island Surtsey, Iceland. Biogeosciences 2014;11:4415-27.
8. Pedersen N, Holyoak DT, Newton AE. Systematics and morphological evolution within the moss family *Bryaceae*: A comparison between parsimony and Bayesian methods for reconstruction of ancestral character states. Mol Phylogenet Evol 2007;43:891-907.
9. Singh M, Singh S, Nath V, Sahu V, Rawat AK. Antibacterial activity of some bryophytes used traditionally for the treatment of burn infections. Pharm Biol 2011;49:526-30.
10. Azuelo AG, Sariana LG, Pabualan MP. Some medicinal bryophytes: Their ethnobotanical uses and morphology. Asian J Biodivers 2011;2:49-80.
11. Sabovljevic A, Sokovic M, Sabovljevic M, Grubisic D. Antimicrobial activity of *Bryum argenteum*. Fitoterapia 2006;77:144-5.
12. Deora GS, Vishwakarma G. Antimicrobial efficacy of moss *Bryum argenteum* (Hedw.) (Bryales: Bryaceae) against plant pathogen *Pseudomonas syringae* (PV.) (*Pseudomonadales*: *Pseudomonadaceae*). J Appl Life Sci 2016;5:1-8.
13. Pejin B, Bogdanovic-Pristov J, Pejin I, Sabovljevic M. Potential antioxidant activity of the moss *Bryum moravicum*. Nat Prod Res 2013;27:900-2.
14. Evans WC. Trease and Evans' Pharmacognosy. Amsterdam, Netherlands: Elsevier Health Sciences; 2009.
15. Tatipamula VB, Killari KN, Prasad K, Rao GS, Talluri MR, Vantaku S, *et al*. Cytotoxicity studies of the chemical constituents from marine algae Chara baltica. Indian J Pharm Sci 2019;81:815-23.
16. Tatipamula VB, Killari KN, Sastry VG, Ketha A. Taxithelium napalense acts against free radicals and diabetes mellitus. Bangladesh J Pharmacol 2017;12:197-203.
17. Deattu N, Suseela L, Narayanan N. Chromatographic analysis of polyherbal extract and formulation by HPTLC and GC-MS methods. J Pharm Res 2013;6:6-10.
18. Tatipamula VB, Killari KN, Gopaiah K V, Ketha A. GC-MS analysis of ethanol extract of *Taxithelium napalense* (Schwaerg) Broth along with its alpha-glucosidase inhibitory activity. Indian J Pharm Sci 2019;81:569-74.
19. Tatipamula VB, Kukavica B. Protective effects of extracts of lichen Dirinaria consimilis (Stirton) D.D. Awasthi in bifenthrin and diazinon-induced oxidative stress in rat erythrocytes in vitro. Drug Chem Toxicol 2020;2020:1-8.
20. Sastry A, Vedula GS, Tatipamula VB. *In-vitro* biological profile of

mangrove associated lichen, *Roccella montagnei* extracts. Inven Rapid Ethnopharmacol 2018;2018:153-8.
21. Tatipamula VB, Vedula GS. Antimicrobial and anti-tubercular activities of isolates and semi-synthetic derivatives of lichen *Ramalina leiodea* (Nyl.) Nyl. J Serb Chem Soc 2019;84:555-62.
22. Paidi KR, Tatipamula VB, Kolli MK, Annam SS, Pedakotla VR. Synthesis of imidazo[1,2-b]pyridazine comprised piperazine, morpholine derivatives as potent antimycobacterial agents with *in vivo* locomotor activity. Antiinfect Agents 2018;15:131-9.
23. Tatipamula VB, Kolli MK, Lagu SB, Paidi KR, Reddy PR, Yejella RP. Novel indolizine derivatives lowers blood glucose levels in streptozotocin-induced diabetic rats: A histopathological approach. Pharmacol Rep 2019;71:233-42.
24. Tatipamula VB, Annam SS, Nguyen HT, Polimati H, Yejella RP. Sekikaic acid modulates pancreatic β-cells in streptozotocin-induced Type 2 diabetic rats by inhibiting digestive enzymes. Nat Prod Res 2021;35:5420-4.
25. Rittirsch D, Huber-Lang MS, Flierl MA, Ward PA. Immunodesign of experimental sepsis by cecal ligation and puncture. Nat Protoc 2009;4:31-6.
26. Ozer EK, Goktas MT, Toker A, Pehlivan S, Bariskaner H, Ugurluoglu C, *et al*. Thymoquinone protects against the sepsis induced mortality, mesenteric hypoperfusion, aortic dysfunction and multiple organ damage in rats. Pharmacol Rep 2017;69:683-90.
27. Xiao X, Yang M, Sun D, Sun S. Curcumin protects against sepsis-induced acute lung injury in rats. J Surg Res 2012;176:e31-9.
28. Su L, Han B, Liu C, Liang L, Jiang Z, Deng J, *et al*. Value of soluble TREM-1, procalcitonin, and C-reactive protein serum levels as biomarkers for detecting bacteremia among sepsis patients with new fever in intensive care units: A prospective cohort study. BMC Infect Dis 2012;12:157.
29. Barbieri R, Coppo E, Marchese A, Daglia M, Sobarzo-Sánchez E, Nabavi SF, *et al*. Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity. Microbiol Res 2017;196:44-68.
30. De Souza AM, De Oliveira CF, De Oliveira VB, Betim FC, Miguel OG, Miguel MD. Traditional uses, phytochemistry, and antimicrobial activities of *Eugenia* species a review. Planta Med 2018;84:1232-48.
31. Arwyn-Jones J, Brent AJ. Sepsis. Surgery 2019;37:1-8.
32. Hotchkiss RS, Monneret G, Payen D. Sepsis-induced immunosuppression: From cellular dysfunctions to immunotherapy. Nat Rev Immunol 2013;13:862-74.
33. McAdow M, Kim HK, DeDent AC, Hendrickx AP, Schneewind O, Missiakas DM. Preventing *Staphylococcus aureus* sepsis through the inhibition of its agglutination in blood. PLoS Pathog 2011;7:e1002307.