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Evaluation of *Conocarpus erectus* against multidrug resistant *Staphylococcus aureus*: Cell to animal study

Muzzamil Waheed[1], Arifa Mehreen[2], Saiqa Ishtiaq[3], Muhammad Saleem[1,3]*, and Ummarah Kanwal[3]*

[1]Government College University Faisalabad, Pakistan
[2]Institute of Molecular Biology and Biotechnology, University of Lahore, Pakistan
[3]Punjab University College of Pharmacy, University of Punjab, Allama Iqbal Campus, Lahore, Pakistan

Abstract: Antibiotic resistant infections by *Staphylococcus aureus* (*S. aureus*) in high risk patients is critical challenge for all clinicians across globe. In an effort to achieve robust bactericidal effect, therapeutic approach based on antimicrobial plant extract of *Conocarpus erectus* (*C. erectus)* been assessed *in-vitro* and *in-vivo* against *S. aureus* resistant clinical strains isolated from burn patients and antibiotic susceptibility was conducted using Kirby-baur disc diffusion technique. *C. erectus* plant extract obtained and characterized for phytochemical constituents, its hemocompatibility and for antioxidant potential. Minimum inhibitory concentration studied for *C. erectus* extract against multidrug resistance (MDR) *S. aureus* clinical isolates *in-vitro* and in rat's sepsis model. Therapeutic activity along acute toxicity was evaluated in rat's model. *C. erectus* extract showed marked antioxidant activity attributed to its phenolic components predominately along others. Hemocompatibility results were significantly different ($p<0.05$) compared to vancomycin (positive control). Statistically *s*ignificant reduction in bacterial colony count ($p<0.05$) observed in rat's sepsis model with *C. erectus* treated group vs. controls. *C. erectus* extract offered higher bactericidal effect both *in-vitro* and *in-vivo* along no acute toxicity at therapeutic dose. We infer that it can serve as alternative promising treatment option against antibiotic resistant against MDR *S. aureus* strains.

Keywords: *S. aureus*, *C. erectus* extract, multidrug resistant, acute toxicity.

INTRODUCTION

The critical healthcare challenge faced across globe are bacterial infections resistant to antibiotics (Andersson and Hughes, 2010), (Aslam *et al.*, 2018). Multidrug resistant (MDR) bacteria worldwide are rapidly increasingly and becoming major threat of 21st century. It has drawn tremendous attention of media and global surveillance authorities (Nii-Trebi, 2017). Worldwide over 13 million deaths occurring every year are ascribed to new infectious diseases emergence or re-emergence of resistance strains of prevailing pathogens (Nii-Trebi, 2017). Burn wounds are more prone to infections leading up to 75% deaths in burn wards comparing to surgical wounds, osmotic shock and hypovolemia (Agnihotri *et al.*, 2004), (Zhang *et al.*, 2021). From burn injuries commonly isolated microorganisms include *S. aureus, Pseudomonas aeruginosa (P. aeruginosa), Staphylococcus pyogenes (S. pyogenes)* (Halstead *et al.*, 2018). *S. aureus* was isolated more frequently comparing others from burn patients. The susceptibility of *S. aureus* differs in countries where antibiotics irrational use is common causing reduce efficacy of antimicrobial agents (Bilal *et al.*, 2021), (Bukhari *et al.*, 2004). Over the time *S. aureus* has acquired significance and appeared as super bug from an innocuous organism. It exhibited both intrinsic and acquired resistance towards available treatment options (Harding *et al.*, 2018). Infectious Diseases Society of America has ranked *S. aureus* in top six hit list of pathogenic microbes against which treatment options are not available or very limited (Cho *et al.*, 2017). Methicillin resistant *S. aureus* (MRSA) due to multidrug resistance has become a major public health threat causing both hospital and community acquired infections. MRSA is leading cause of skin and soft tissue infections, bacteraemia, endocarditis, bone and joint infections posing challenging clinical threat with persistent inclined rate of morbidity and mortality (Kshetry *et al.*, 2016). MRSA showed resistance to even last resort antibiotics including vancomycin (Ahmed *et al.*, 2016). Successful therapeutic approaches are still challenging and demanding assessment of novel antimicrobials agents, adjunct care parameters along source control (Turner *et al.*, 2019).

Medicinal plants on account of safety, multi targeting potential and inexpensive comparing to synthetic agents offers promising approach for exploring more drugs (Anand *et al.*, 2019). *C. erectus* extract and its bioactive constituents has been explored via biological assays by scientists tried to justify its biological actions (Rehman *et al.*, 2019). In this study, *in-vitro/in-vivo* antimicrobial efficacy of *C. erectus* leaves extracts against MDR *S. aureus* infections isolated from burn patients was evaluated. Current study findings may serve as translational link for clinical trials for assessment of

**Corresponding author:* e-mail: ummarahkanwal@gmail.com; saleem2978@hotmail.com

efficacy and safety of *C. erectus* extracts after substantial testing and guidance for further studies leading to clinical evaluation to determine safety margins to make it a future drug delivery system.

MATERIALS AND METHODS

Clinical isolates of multidrug resistant S. aureus

Clinical isolates of MDR *S. aureus* used in this study were taken from burn wounds of patients admitted at Allied Hospital Faisalabad, Pakistan after taking consent from Ethical Review Committee (ERC) Government College University Faisalabad (Ref No: GCUF 767/2017). Antimicrobial susceptibility was conducted for erthromycin, clindamycin, methicillin, vancomycin, levofloxacin and moxifloxacin by standard disc diffusion method (Organization, 2003). Interpretation of results were recorded as sensitive and resistant according to clinical and laboratory standard institute guidelines (Wayne, 2015).

Preparation of plant extract

Plant sample was collected from botanical garden of Government College University Faisalabad and identified, confirmed by taxonomist Associate Professor Dr. Uzma Hanif at Department of Botany, GCU Lahore and specimen has been kept in GC University herbarium museum, Lahore under voucher number via GC-Herb-Bot. 1938. Plant leaves were washed, dried in shade, powdered and used for extraction. Fifty grams of air-dried and coarsely powdered plant leaf material was extracted successively with 200 ml each of hexane, ethyl acetate, methanol and water in increasing order of their polarity using buchner funnel and whatman No. 1 filter paper for 12 h and stored at 25°C (Dahiya and Purkayastha, 2012).

Characterization of plant extract

Phytoconstituents screening of extracts

Thin Layer Chromatography (TLC) (Fluka, silica gel F 254) was utilized for extract fractional separation analysis using 10µl ethyl acetate *C. erectus* extracts according to reported method (D'Sousa'Costa *et al.*, 2015). Eluted plates were visualized at 360 nm in ultraviolet light and sprayed with freshly prepared vanillin spray reagent to check separated compounds. Phytoconstituents presence in extract was evaluated to detect phenols, flavonoids, alkaloids, tannins, glycosides (Yasin and Al-Azawi, 2019).

In-vitro bactericidal activity

MDR clinical isolates of *S. aureus* with their antibiotic resistance profiles performed in above section were used. Standard strains *S. aureus* ATCC 25923 was used for quality control. All test strains were kept on nutrient agar slants (Hi-Media Laboratories Pvt. Limited, Mumbai, India) at 4°C and sub-cultured 24h prior to their testing. For antibacterial activity assay these bacteria served as test pathogens. Ethyl acetate *C. erectus* extracts antibacterial potential was evaluated by agar well diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS) (Wayne, 2015). Standard antibiotic discs and distilled water served as positive and negative controls respectively. After incubation zone of inhibition diameter was measured in mm. Experiment was conducted in triplicate.

Minimum inhibitory concentration

Based on preliminary screening ethyl acetate *C. erectus* extracts that exhibited antimicrobial potential was further evaluated for minimum inhibitory concentration (MIC). Vancomycin was kept as control. Different dilutions of ethyl acetate *C. erectus* extract was prepared in range of (0.01-60µg/ml). Microplate Reader (Biotek Model FL×800, USA) was used for recording of results after incubation period of 24 h at 37ºC.

LC-MS/MS Analysis

The ethyl acetate *C. erectus* extracts fractions demonstrated antimicrobial activity were analyzed for bioactive compounds identification by LC MS/MS analysis (LTQ XL, Thermo Electron Corporation, USA via Electrospray ionization (ESI) interface). The ionization source temperature was set at 250°C, voltage of source and lens set at 3 kV and 70V respectively. All the data was analyzed by Xcalibur software version 2.0.7 (Thermo Fisher Scientific USA). The spectrums of observed compounds in ethyl acetate *C. erectus* extracts was examined and compared with Sci Finder, Metlin, Mass bank and NIST library.

Free Radical scavenging activity by DPPH

Ethyl acetate *C. erectus* extracts DPPH radical scavenging capacity was studied as reported earlier (Shirwaikar *et al.*, 2006). Samples were diluted in ethanol ranging from 0.1-1.0µg/ml. DPPH (1.0 ml) plus ethanol (2.5 ml) kept as negative control and ascorbic acid as positive control. Absorbance of each dilution was recorded at 518 nm after 30 min of reaction in dark and at 25ºC. DPPH scavenging ability was expressed as IC50. The experiment was run in triplicate and antioxidant capacity was calculated as:

$$\%\ \text{Inhibition} = \frac{100 - (\text{Abs control} - \text{Abs sample})}{\text{Abs control}} * 100$$

where Abs sample is absorbance of sample; Abs control is absorbance of control.

In-vitro biocompatibility assay

Ethyl acetate *C. erectus* extracts biocompatibility was conducted using RBCs of rat. Blood sample was withdrawn for rat RBCs isolation, centrifuged at 1500 rpm for 5 min and supernatant was discarded and RBCs were washed thrice using sterile Dulbecco phosphate buffer saline (PBS). RBC pellet was purified and diluted 9 times (v/v) using PBS. 100µl RBC suspension was added in each well of 96-well plate (Nadhman *et al.*, 2015) and

treated with varying concentrations of *C. erectus* as test sample vs vancomycin taken as positive control. Samples were allowed to incubate at 37ºC for 2 h followed by centrifugation. Supernatant was collected and absorbance was measured at 485 nm using Microplate Reader (Biotek Model FL×800, USA). Percent hemolysis was calculated.

$$\% \text{ Hemolysis} = \frac{(\text{Absorbance of sample} - \text{Absorbance of negative control})}{(\text{Absorbance of positive control} - \text{Absorbance of negative control})}$$

Animals

Rats of age 7-9 weeks and weight 180-200 g taken from Department of Pharmacy Government University College of Faisalabad, Pakistan for this study. Prior to study an ethical approval was obtained from Animal Ethics Committee, Government College University of Faisalabad, Pakistan (Protocol No AEC/GUCF-675/2017). All the experiments in animals in this work were undertaken in accordance with International Conference on Harmonization ICH guidelines (ICH, 2018).

Multidrug Resistant Sepsis Model in Mice and therapy

Induction of sepsis in rats

To determine therapeutic efficacy of ethyl acetate *C. erectus* extracts in rats initially sepsis model was developed. *S. aureus* isolated from burn patients that was resistant to all tested antibiotics was selected for sepsis induction in rats. For animal inoculation freshly prepared bacterial suspension was used and stored at 2-4ºC. Four groups (n=6 per group) of rats were taken Group I (untreated) given normal saline was kept as negative control, while animals in Group II and IV were given single dose with 40µl of inoculum at different concentrations for sepsis induction, Group II (3×10^8 CFU/ml) Group III (5×10^8 CFU/ml) and Group IV (6×10^8 CFU/ml). Animals were monitored for morbidity and mortality for duration of 7 days. At 8th day rats were anesthetized and blood sample was collected through intra cardiac puncture and stored at 4°C in EDTA vials. The experiments were conducted in triplicate.

Therapeutic monitoring of sepsis model

To determine therapeutic potential of ethyl acetate *C. erectus* extracts group that has received 5×10^7 CFU/ml inoculum in sepsis model study was selected for subsequent experiments. Three groups; n=6 were taken (Group I) untreated animals as negative control, (Group II) dosed with vancomycin as positive control, (Group III) given *C. erectus* extract at dose of 200mg/kg in accordance to reported earlier at clinical significant dose for period of 5 days on daily basis (Arshad *et al.*, 2017). At the end period rats were anesthetized and blood was drawn by cardiac puncture, stored at 4ºC in EDTA vials from control and test groups.

Acute oral toxicity

Acute oral toxicity of plant extract was evaluated in rats for 14 days to establish LD50 (median lethal dose) according to Acute Toxic Class Method (OECD 2001) for acute toxicity test of single dose (Guideline 423). Healthy male rats 188-200 g and 8-10-week age, were used. Rats were divided into 3 groups n=6 and were kept under standard conditions of food and water under controlled environment. Group 1 administered with normal saline served as negative control, Group II received therapeutic dose of 200mg/kg *C. erectus* studied in above section. Group III received vancomycin at dose of 10mg/kg (Bruniera *et al.*, 2014). The rats were kept under observation for 24 h for any change in body weight and visual observations for mortality, behavior pattern, physical appearance changes and signs of illness were conducted daily throughout the week.

Organ to body ratio

Organ weight change was measured for toxicity evaluation after exposure for specific time. The weights of organs from treated groups were compared with control group and body mass index was calculated using formula (Sohail *et al.*, 2017).

$$\text{Organ} - \text{body weight index } (\%) = \frac{\text{Wet organ weight}}{\text{Body weight}} * 100$$

STATISTICAL ANALYSIS

All experiments were conducted in triplicates and data are presented as mean ± SD. Graph Pad Prism 7.0 was employed to compare all parameters using two-way ANOVA with *post-hoc* Tukeys test. Significance was considered at p value (<0.05).

RESULTS

Antibiotics susceptibility of bacterial isolates performed on erythromycin, clindamycin, methicillin, vancomycin, levofloxacin, moxifloxacin by disc diffusion method. Antibiogram of studied isolates revealed that *S. aureus* was resistant to all antibiotic applied. While the isolates were sensitive and resistant to both vancomycin used in this study.

Ethyl acetate *C. erectus* extracts fractional separation analysis by Thin Layer Chromatography (TLC) visualization at 360 nm revealed presence of various compounds (fig.1). Selected extract phytochemical analysis revealed existence of phenols, alkaloids, flavonoids and tannin as well.

Antibacterial activity of ethyl acetate *C. erectus* extract of leaves determined by agar well diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS) revealed comparable results to vancomycin served as positive control. Ethyl acetate *C. erectus* extract showed comparable zone of inhibition at all concentrations against MRSA isolates to vancomycin (fig.2).

Ethyl acetate *C. erectus* extract mass spectrum showed characteristic peaks at 169.08 (Gallic acid), 301.08 (Quercetin), 285.08 (Luteolin), 329.08 (5-hydroxy-3′4′7 trimethoxy-flavanone), 343 (Caffeoyl-O-hexoside), 423 (Equisetumpyrone) in (fig.3).

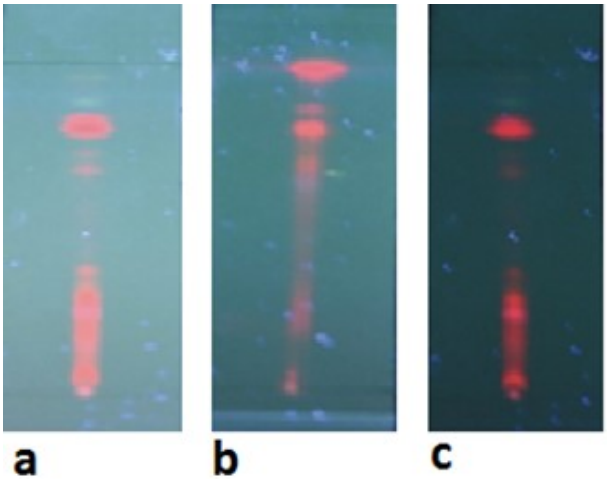

Fig. 1: *C. erectus* extract separated on TLC plates separated on TLC plates by using (a) Acetone: n-Hexane; Ethyl acetate (45:35:20) (b) Acetone: n-Hexane; Ethyl acetate (40:35:25) (c) Acetone: n-Hexane; Ethyl acetate (35:40:25) visualized at 360 nm.

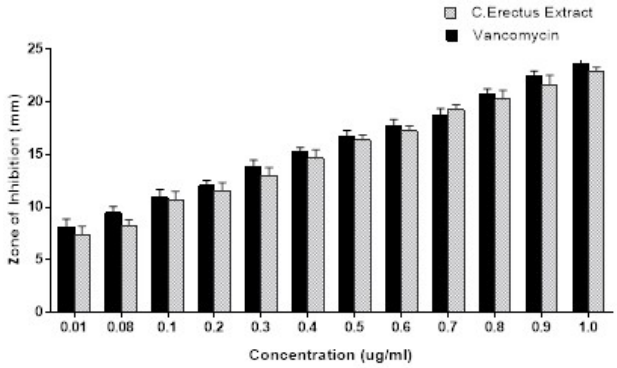

Fig. 2: *In-vitro* bactericidal activity of ethyl acetate *C. erectus* extract vs vancomycin. All cells were incubated for 24 h and activity was measured by ELISA Reader *(P <0.05).*

Radical scavenging activity of different concentrations of ethyl acetate extracts of *C. erectus* showed different activity with IC50 values. The highest showed antiradical activity toward DPPH was at IC 0.5µg/ml (fig. 4).

Percent hemolysis of ethyl acetate *C. erectus* extract and vancomycin at all concentrations presented in fig. 6. *C. erectus* extracts showed 0.35% hemolysis at its highest concentration, 1.0µg/ml showing biocompatibility with RBCs. Vancomycin demonstrated significantly higher (*p<0.01*) hemotoxicity.

In-vivo sepsis model was optimized in rats using inoculums of varying concentrations (3×10^8 CFU/ml, 5×10^8 CFU/ml, 6×10^8 CFU/ml *S. aureus)*. Animals treated with 3×10^8 CFU/ml showed no significant change in weight and no mortality was observed in this group. The rats inoculated with 5×10^8 CFU/ ml presented lethargic condition, increase body temperature and loss in body weight. While animals inoculated with higher dose of 6×10^8 CFU/ml, all rats showed adverse clinical signs of raised body temperature, body weight loss, and 50% animals died by 4th day. The remaining rats were sacrificed at 8th day. All doses effected hematological parameters (Table 1). After 24 h of incubation at 37°C, colony forming unit per ml (CFU/ml) was calculated and represented as $\log_{10}$ value.

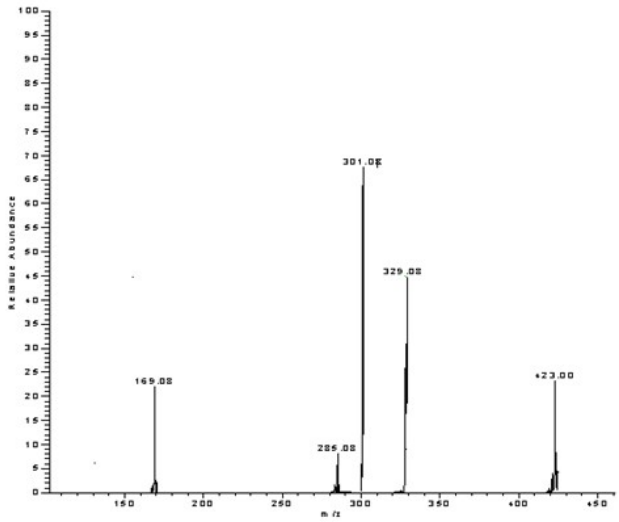

Fig. 3: Mass spectrum of ethyl acetate *C. erectus* extract.

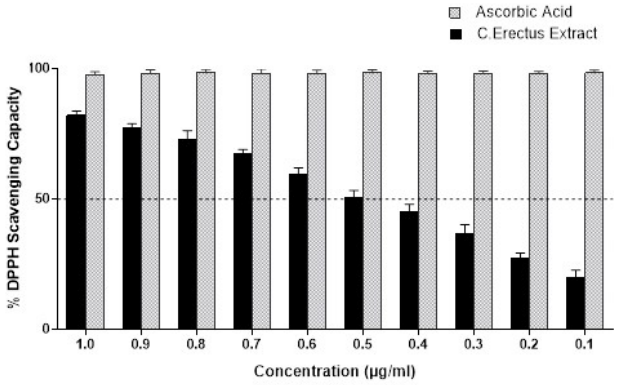

Fig. 4: DPPH scavenging assay of ethyl acetate *C. erectus* extract and standard ascorbic acid *(P <0.05*).

Animals treated with of 5×10^8 CFU/ml that presented lesser mortality was utilized for therapeutic monitoring study. Body weight of rats was significantly less in vancomycin treated positive control group, nevertheless, in ethyl acetate *C. erectus* extract treated group there was no difference in final body weight of animals compared to negative control untreated Group. In vancomycin treated group mortality was 16% (n=1/6) compared to *C. erectus*

extract treated group was 0%. Hematological parameters effect shown in Table 2.

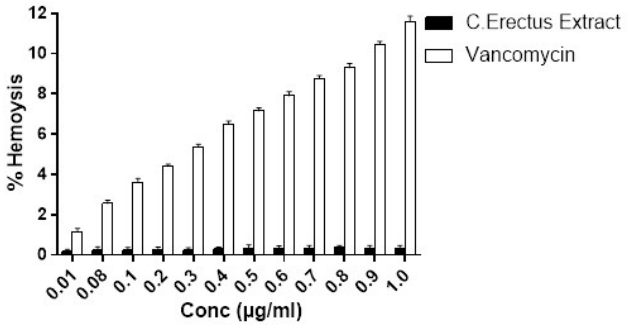

Fig. 5: % Hemolysis of rat RBCs after treatment with ethyl acetate *C. erectus* extract and vancomycin at different concentrations (*p<0.01*).

DISCUSSION

The treatment of drug resistant *S. aureus* infections is a challenge for clinicians all over the world. Bacteria acquire resistance against conventional antibiotics through various mechanisms (Sultan *et al.*, 2018). *S. aureus* resistance mechanism against vancomycin may be due to cell wall thickening. In addition; prior exposure to vancomycin increases chances of isolation of strains of *S. aureus* with reduced susceptibility and reason due to haphazard use of antibiotic (Amatya *et al.*, 2014). (Shariati *et al.*, 2020). At present when infections due to MRSA have become serious public health concern; development and rapid spread of resistance of *S. aureus* to reserve drug (vancomycin) is very fearsome and immediate actions should to be taken to halt it (Amatya *et al.*, 2014). To prevent situation of drug resistance from worsening; use of herbal origin treatment for patients should be preferred. These rapidly emerging drug resistant pathogens impede development process of novel antibiotics, as these learn to resist effect of antibiotics in no time. To counter pan-drug resistant bacteria, plant extracts offer promising direction (Cruz *et al.*, 2014); (Ruddaraju *et al.*, 2020). In present study, ethyl acetate *C. erectus* extract was evaluated which effectively targeted MDR *S. aureus* clinical isolates, both *in-vitro* and *in-vivo.*

Antibiogram of clinical isolates from burn patients revealed *S. aureus* was resistant to all antibiotic applied and was little bit sensitive to vancomycin. Resistance of local bacterial isolates may be due to production of beta-lactamase enzymes such as ESBL enzymes which degrade penicillin's and cephalosporin's (Salman and Ghaima, 2018). The issue regarding increased sensitivity of subjected drug molecules is higher dosage requirement, which further create drug resistance toxicity. The drug toxicity outcomes represent existing research gap and need to explore natural substances, to replace toxic and resistant drugs for treatment of *S. aureus* resistant strains (Othman *et al.*, 2019).

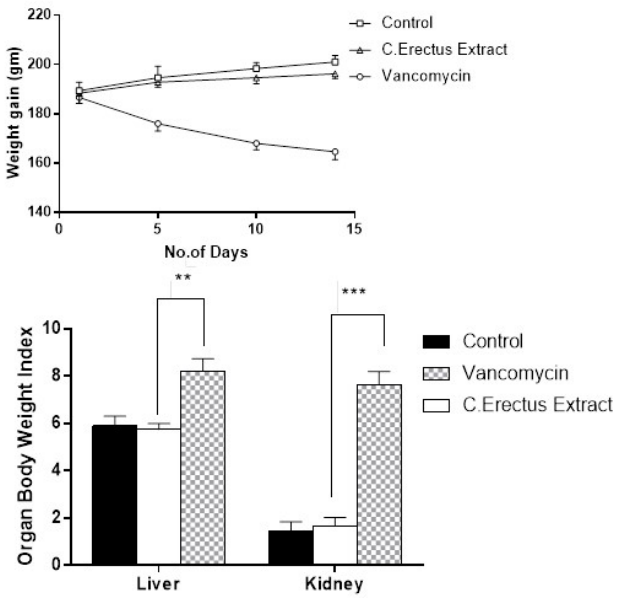

Fig. 6: Effect of vancomycin and ethyl acetate *C. erectus* extract in rat (a) Change in body weight (b) Organ body weight index after 14 days (***P<0.001*, ****P<0.0001*).

Phytochemical findings agreed with previous who reported presence of flavonoids, phenols, tannins, glycoside and saponin as active compounds in *C. erectus* aqueous extracts (Nascimento *et al.*, 2016). Current isolation and chemical purification methods used include solvent extraction processes that utilize solvent polarity as major separation technique. These methods frequently include use of ethyl acetate, phenol/chloroform, aqueous, and several other approaches (Tohidi *et al.*, 2017). Ethyl acetate extract of *C. erectus* exhibited positive therapeutic produced zones of inhibitions comparable to vancomycin. Vancomycin MIC against MRSA isolates demonstrated susceptibility to vancomycin MIC ranging from 0.01-1.0 µg/ml consistent with earlier reported results (Kshetry *et al.*, 2016). However, ethyl acetate *C. erectus* extract showed comparable zone of inhibition to positive control. LC-MS has been found to enable accurate identification of compounds in complex extracts with co-eluting peaks and compounds were characterized by comparing obtained molecular (precursor) ions and fragmentation patterns (i.e., product ions) from our LC-MS/MS data with data from NIST database libraries for standard compounds. Ethyl acetate extracts of *C. erectus* leaf showed highest antiradical activity toward DPPH at IC 0.5µg/ml. These results elucidate presence of polyphenols in *C. erectus* extract attributing to antioxidant capacity consistent with earlier results.

Hemolytic activity of vancomycin was increasing as concentration was increased fig. 6. Safety and toxicity of plant extracts must be ensured using scientific validations methods before their recommendation for human use. For injectable formulations erythrocyte % hemolysis served as toxicity indicator (Greco *et al.*, 2020).

Table 1: Effect of inoculum treatments on hematological parameters of rats. Results are represented as mean ± S.D.

Blood Parameter	Effect (Mean ± SD) on hematological parameters			
	-ve Control	3×10^8 CFU/ml	5×10^8 CFU/ ml	6×10^8 CFU/ml
RBC (10^6/L)	6.26 ± 0.9	6.9 ± 0.4	8.01± 0.67	9.93 ± 0.77
Hb (g/dL)	12.63 ± 1.4	13.0 ± 0.45	16.86 ± 1.00	18.16 ± 1.01
MCV(fL)	76.04 ± 20.1	78.26 ± 2.66	80.46 ± 1.96	97.46 ± 2.31
MCH (pg)	25.53 ± 4.8	26.26 ± 0.60	30.83 ± 1.35	48.40 ± 0.81
MCHC (g/dL)	35.69 ± 12.5	37.86 ± 1.89	39.45 ± 1.86	48.50 ± 1.95
WBC (/mm)	9042 ± 2773	1046 ± 2004	1200 ± 2076	1533.33 ± 2516
Platelets 10^9/L	364000 ± 20000	369000 ± 20000	534000 ± 20000	566333.30 ± 25166.11
Neutrophils (%)	21.64 ± 6.4	22.40± 0.45	25.43 ± 0.60	36.43 ± 0.70
Basophils (%)	0.36 ± 0.5	0.43 ± 0.49	0.600 0.50	1.66± 0.55
Eosinophils (%)	2.77 ± 1.5	2.9 ± 0.55	3.43 ± 0.60	5.63 ± 0.41
Monocyte (%)	0.20 ± 0.4	0.71 ± 1.60	0.90 ± 2.00	1.43 ± 2.35
Lymphocyte (%)	74.17 ± 6.6	76.36 ± 1.90	64.63 ± 2.79	75.73 ± 2.50

Table 1: Effect of treatments on hematological parameters of rats. Results are presented as mean ± S.D.

Blood Parameter	Effect (Mean ± SD) on hematological parameters		
	-ve Control	+ve control	*C. erectus*
RBC (10^6/L)	6.26 ± 0.9	8.9 ± 0.4	7.01± 0.67
Hb (g/dL)	12.63 ± 1.4	16.0 ± 0.45	13.86 ± 1.00
MCV(fL)	76.04 ± 20.1	88.26 ± 2.66	77.46 ± 1.96
MCH (pg)	25.53 ± 4.8	36.26 ± 0.60	27.83 ± 1.35
MCHC (g/dL)	35.69 ± 12.5	47.86 ± 1.89	36.45 ± 1.86
WBC (/mm)	9042 ± 2773	1446 ± 2004	1020 ± 2076
Platelets 10^9/L	364000 ± 20000	369000 ± 20000	534000 ± 20000
Neutrophils (%)	21.64 ± 6.4	26.40± 0.45	22.43 ± 0.60
Basophils (%)	0.36 ± 0.5	0.43 ± 0.49	0.400 ± 0.50
Eosinophils (%)	2.77 ± 1.5	1.9 ± 0.55	2.9 ± 0.60
Monocyte (%)	0.20 ± 0.4	0.31 ± 1.60	0.30 ± 2.00
Lymphocyte (%)	74.17 ± 6.6	70.36 ± 1.90	76.63 ± 2.79

Hemolysis resulted from rupturing of lipid bilayer membrane leading to red blood cells destruction. *C. erectus* extracts revealed 0.35% hemolytic activity at its highest concentration showing biocompatibility with RBCs. While vancomycin represented hemolytic activity at all concentrations consistent with previously reported result (Cafiso *et al.*, 2012).

Sepsis model was created and optimized using MDR *S. aureus* strains in rats to evaluate efficacy of plant extract (Harris *et al.*, 2017). Blood samples were prone to hematological assessment to observe changes in blood parameters of *S. aureus* infected rat model organisms treated with vancomycin and *C. erectus* respectively. Significant impact of *C. erectus* treatment over blood cells count was observed declaring validity of *C. erectus* extract to treat drug resistant infections. While vancomycin treated animals showed insignificant change in hematological parameters compared to control. It can be attributed to its hemolytic activity as mentioned in this study in also consistent with previously reported results. *C. erectus* being biocompatible *in-vivo* showing compatibility with RBCs as compared to vancomycin make it favorable therapeutic choice in future against MDR infections. Results obtained in rat sepsis models may not manifest all pathological mechanisms occurring in human body. Despite intrinsic limitation of models, these studies provide basic understanding for this critical human pathogen and helps to establish mechanism of action of novel extracts. Further work is necessary to evaluate pharmacokinetics behavior of suggested plant extract.

CONCLUSION

The study enables to explore therapeutic potential of *C. erectus* extract both *in-vivo* and *in-vitro* from cellular level to animal study. *C. erectus* extract demonstrated biocompatibility with RBC, compared to vancomycin. Organ weights remained unaffected with *C. erectus* extract. The ability of extract to treat sepsis model against *S. aureus* may make it efficient future drug delivery system for clinical applications. However, detailed biodistribution studies are required to substantiate the above.

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