View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by CURVE/open

Thrombolytic and cytotoxic activity of different bioactive extracts of E. coli

Munaza Maqsood, Zahid Mushtaq, Tahir Rasheed, Zaka Un Nisa, and Farooq Sher

Published Version of Record deposited by Coventry University's Repository

Original citation & hyperlink:
Maqsood, M., Mushtaq, Z., Rasheed, T., Nisa, Z.U. and Sher, F., 2021. Thrombolytic and cytotoxic activity of different bioactive extracts of E. coli. *Case Studies in Chemical and Environmental Engineering*, Vol 3, 100080.
http://dx.doi.org/10.1016/j.cscee.2021.100080

DOI 10.1016/j.cscee.2021.100080
ISSN 2666-0164

Publisher: Elsevier

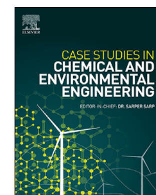
© 2021, Elsevier. Licensed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International
http://creativecommons.org/licenses/by-nc-nd/4.0/

Case Studies in Chemical and Environmental Engineering 3 (2021) 100080

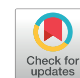
Contents lists available at ScienceDirect

Case Studies in Chemical and Environmental Engineering

journal homepage: www.editorialmanager.com/cscee/default.aspx



Thrombolytic and cytotoxic activity of different bioactive extracts of *E. coli*



Munaza Maqsood [a], Zahid Mushtaq [a,**], Tahir Rasheed [b], Zaka Un Nisa [c,d], Farooq Sher [e,*]

[a] Bioactive Molecules Research Lab (BMRL), Department of Biochemistry, University of Agriculture, Faisalabad, 38040, Pakistan
[b] School of Chemistry and Chemical Engineering, Shanghai Jiao Tong University, Shanghai, 200240, China
[c] Faculty of Medicine, Quaid-i-Azam University, Islamabad, 45320, Pakistan
[d] International Society of Engineering Science and Technology, Coventry University, Coventry, United Kingdom
[e] School of Mechanical, Aerospace and Automotive Engineering, Faculty of Engineering, Environmental and Computing, Coventry University, Coventry CV1 5FB, United Kingdom

ARTICLE INFO

Keywords:
Escherchia coli
Cytotoxic activity
Thrombolytic activity
Clot lysis
Methanol extract and eco-friendly thrombolytic agent

ABSTRACT

Novel therapeutic agents discovery have become crucial because humankind is experiencing issues regarding health and the environment. The present study is focusing on thrombolytic agents, which are therapeutics for cardiovascular diseases, eco-friendly, safe and can be used in comparison to synthetic agents that are associated with some side effects and their production is not environment friendly. The main objective of the present research was to evaluate the thrombolytic and cytotoxic potential of different extracts of *Escherchia coli*-ATCC35218. Soxhlet extraction method with ethanol was used for extraction from cells. Crude protein was extracted from the cell mass with protein extraction buffer. Crude isoprenoid quinone and polar lipids were extracted by using petroleum ether with methanol and chloroform as extraction solvent. In another method, the extracts were prepared using methanol as well as *n*-hexane, chloroform, ethyl acetate and water in serial extraction. These organic and aqueous extracts of *E. coli* were dried, weighed, dissolved in DMSO and tested for their hemolytic and thrombolytic potential. Mild to moderate percentage of thrombolytic activity was shown by these extracts and compared with citric acid (standard). Chloroform, ethyl acetate and methanol soluble extracts showed significant thrombolytic activities that were 43.5% ± 0.06, 41.8% ± 0.16 and 40.5% ± 0.07 respectively, while citric acid showed 73.7% ± 0.05. Maximum hemolytic activities were shown by polar lipids, crude methanol extract and *n*-hexane that are 87.20% ± 0.08, 82.5% ± 0.06 and 73.46%± 0.08 respectively as compared with 0.1% Triton X-100 (positive control) with 94.75% ± 0.15 hemolysis. Finally, results were analyzed statistically through analysis of variance (ANOVA). Further studies on isolation and identification of bioactive thrombolytic agents from each bioactive thrombolytic extract will help us in reporting natural thrombolytic agents from *E.coli* that could benefit human medically. This is one of the few reports of thrombolytics available from natural sources.

1. Introduction

Since decades, environmental and health problems are the main challenges for men. As pathogens have developed resistance against a number of antibiotics therefore, treatment of infections caused by them is a great challenge [1]. A variety of problems regarding health causes due to the toxins in the food and due to thrombolytic disorders e.g., intravascular thrombosis, myocardial infarction and acute ischemic strokes. From the World Health Organization (WHO) records these thrombolytic disorders causes the death of about 117 people [2,3]. Different toxins naturally present in foods such as aflatoxin M1 (AFM1) and Aflatoxin B1 (AFB1) can cause risks to human health regarding the impairment of them in the intestine [4]. *Clostridium perfringens* produce four major toxins that causes different types of food poisoning issues that may be fetal [5].

Therefore, there is a need for new therapeutic agents due to these health and environmental issues. To overcome these hazardous challenges, new therapeutic technologies for the discovery of novel therapeutics is the main focus of scientists [6]. Compounds that have interaction with living cells and imposes important health effects are the bioactive compounds. These bioactive compounds with important health effects such as least cytotoxicity and thrombolytic effect are produced by

* Corresponding author.
** Corresponding author.
E-mail addresses: zahidmushtaquaf@uaf.edu.pk (Z. Mushtaq), Farooq.Sher@coventry.ac.uk, Farooq.Sher@gmail.com (F. Sher).

https://doi.org/10.1016/j.cscee.2021.100080
Received 20 November 2020; Received in revised form 6 January 2021; Accepted 9 January 2021
2666-0164/© 2021 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

all living organisms [7]. Bioactive compounds are mostly secondary metabolites like alkaloids, terpenoids, isoprenoids and proteins like defensins which possess hemolytic and thrombolytic potential [8]. These bioactive compounds have broad range applications in industries and medicine [6].

Variety of enzymes with thrombolytic potential have been identified in different microorganisms like staphylokinase produced by *Streptococcus aureus* and Streptokinase produced by *Streptococcus hemolyticus* with significant thrombolytic potential. Nattokinase produced by *Bacillus subtilis* [2]. These enzymes from various biological sources usually acted by catalyzing or activating the plasminogen to plasmin which in response catalysis the degradation of thrombus or fibrin clots whereas sometimes they directly degrade the clot [9]. *Escherichia coli* was first demonstrated for the presence MEP (methyl-D-erythritol-4-phosphate) pathway which is involved in plastidic isoprenoids biosynthesis [10]. These isoprenoids are reported for their thrombolytic potential [11]. The *E. coli* strain is also studied for the production of recombinant streptokinase which later on used for the thrombolytic analysis and found active [12].

From the literature survey, *E. coli* is not much studied for its thrombolytic potential [13]. Kiwi-fruits ethanol fractions are ascorbic acid, flavonoids (Quercetin) and phenols rich that possess considerable bioactive potentials like cytotoxicity and antioxidant activities [14]. *E.coli* can play a positive role in the development of novel thrombolytic therapeutic agents and could be of good scope to study this strain in more detail [12]. Moreover, natural products are much more eco-friendly to be synthesized as compared to the synthetic therapeutics with least side effects too. Ethanol extract of the microalgae like *Synechocystis sp*. and algae *Himanthalia elongate* possess compounds with bioactive potential like palmitoleic acid, palmitic or neophytadiene, fucosterol, oleic acids and phytol were screened. These extracts exhibit antimicrobial and antioxidant activities [15]. Chloroform, methanol, chloroform: alcohol (1:1), ethyl acetate and hexane fractions of different algae contain pigments like chlorophylls, vitamin C and amino acids with cytotoxic, antimicrobial and antioxidant activities [16]. Bioactive compounds of ethyl acetate fractions of *C. humicola* are saponins, alkaloids, fatty acids, flavanoids and amino acids with antimicrobial potential [17].

There is a need for novel bioactive agents and other techniques for screening of these compounds from microorganisms (i.e. natural sources). Therefore, the present research work is conducted for the evaluation of more bioactive compounds from *E. coli* to address the thrombolytic and cytotoxic potential of each extract. The main purpose of the study is to explore novel bioactive compounds from microbial resources of *E.coli* ATCC35218 for therapeutic and cytotoxic potentials. Furthermore, the research is quite worthy in environmental perspectives and could be considered an eco-friendly therapeutic approach of discovery.

2. Material and methods

2.1. Culturing and cell harvesting of bacterial strain

E. coli (ATCC 35218) strain was used in our study. The strain of *Escherichia coli* specie was confirmed from the Institute of Microbiology, University of Agriculture Faisalabad, Pakistan. Nutrient agar (NA) and nutrient broth (NB) prepared by MERCK Company was used as a basal medium to grow bacterial cultures [18]. Then the broth culture was centrifuged at 10,000 rpm for 10 min at 4 °C to obtain bacterial cell mass that was dried, weighed and used for the extraction procedure. Fig. 1 represents the methodology and main findings of current research.

2.2. Extraction and fractionation

2.2.1. Protein extraction (PE)

Bacterial protein was extracted by following the procedure described by Ref. [19], with little modifications. 1 g of dry cell mass of organism was resuspended and sonicated for 10 min in 5 mL of protein extraction buffer (10 mM Na_2HPO_4, 15 mM NaH_2PO_4, 100 mM KCl, 2 mM EDTA, 1.5% polyvinylpoly pyrrolidone (PVPP), 1 mM phenyl methyl sulfonylfloride (PMSF) and 2 mM thiourea) [20]. This mixture was then centrifuged at 10,000 rpm for 10 min stored at 4 °C and filtered. The crude protein contents of the sample were determined spectrophotometrically by the Bradford method [21], using bovine serum albumin (BSA) as standard.

2.2.2. Extraction through different organic and aqueous solvents

Method for solvent extraction was described by Emran and his coworkers [22]. 5 g dry cell mass of *E. coli* was used for extraction. Extraction was done by resuspending the dry cell mass in methanol followed by stirring for 48 h and centrifuged to separate the cellular debris. Then this crude methanol extract (CM) was subjected to fractionation with different aqueous and organic solvents [water, n-hexane (NH), chloroform (CL), ethyl acetate (EA) and methanol (ME) respectively]. Depending upon polarity of respective solvent different layers were formed each time and carefully separated. These layers were then dried, weighed, dissolved in DMSO (Dimethyl sulfoxide) and stored for further bioactivity based studies.

2.2.3. Soxhlet extraction (SE)

The Soxhlet extraction (SE) was implemented with 5 g of dry cell mass of *E. coli*, a solvent was used for extraction was ethanol (b.p. 78.37 °C). 12 h of extraction process at 100 °C, followed by solvent evaporation. The extract was dried, weighed, dissolved in Dimethyl sulfoxide (DMSO) and used to evaluate their bioactive potential [23].

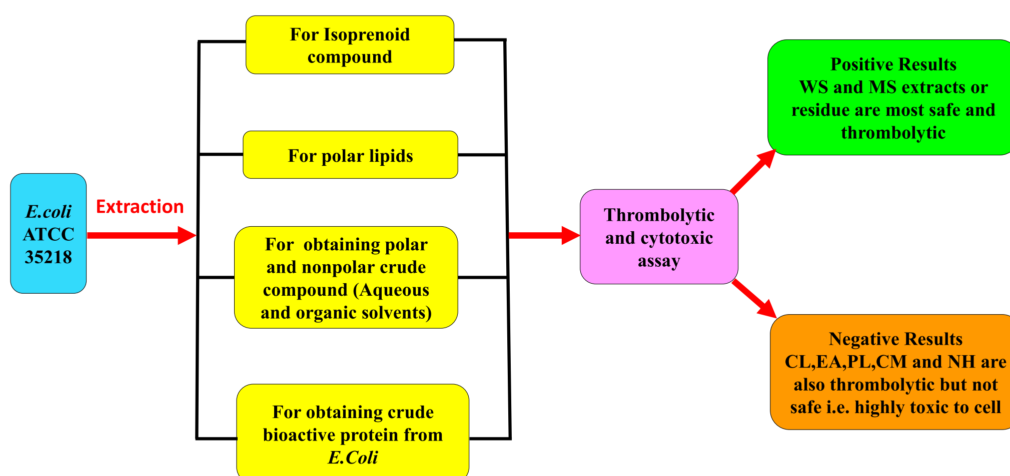


Fig. 1. Overview of working strategy adopted for using *E.Coli* extracts as thrombolytics and significant findings of the present study.

2.2.4. Isoprenoid quinone (IQ) and polar lipids (PL) extraction

Mixtures containing chloroform and methanol have been shown to provide efficient extraction of isoprenoid quinine (IQ) and polar lipids (PL), and the established procedure of Bligh and Dyer has been conveniently used for the extraction of bacterial lipids [24].

2.3. Thrombolytic activity

Experiments for clot lysis were carried out using standard procedure reported earlier [25]. Each pre weighed tube containing clot was properly labelled and 100 μL of *E. coli* extracts were added to the tubes. As a positive control, 100 μL of citric acid and as a negative control, 100 μL of distilled water were separately added. All the tubes were then incubated overnight (O/N) at 37 °C and observed for clot lysis. The clot lysis calculations were performed using Eq. (1).

$$\text{Clot lysis } (\%) = \frac{\text{Wt. of released clot after drug}}{\text{Sample application } \times \text{ Clot weight}} \times 100 \quad (1)$$

where clot weight = weight of clot containing tube – weight of an empty tube.

2.4. Cytotoxicity studies by hemolytic activity

The cytotoxic studies of the *E. coli* extracts were analyzed by hemolytic activity [26], 3 mL of freshly obtained blood was added in heparinized tubes to avoid coagulation and gently mixed, poured into a sterile 15 mL falcon tube and centrifuged for 5 min at 850 x g. RBCs were washed three times with 5 mL of chilled (4 °C) sterile isotonic phosphate buffer saline (PBS) solution, pH 7.4. The washed RBCs were counted on hemocytometer. The RBCs count was maintained to 7.068 x 10^8 cells per mL for each assay. The 20 μL of *E. coli* extracts were taken in 2 mL microcentrifuge tube then added 180 μL diluted blood cell suspension. The samples were incubated for 35 min at 37 °C and then centrifuged for 5 min at 1310 x g. After centrifugation 100 μL supernatant was taken from the tubes and diluted with 900 μL ice-cold PBS. After this 200 μL mixture from each microcentrifuge tube was added into 96 well plates. For each assay, 0.1% triton X-100 was taken as a positive control for 100% lysis and phosphate buffer saline (PBS) was taken for each assay as a negative control. The absorbance was noted at 576 nm with a Bio TeK, μ Quant (BioTek, Winooski, VT, USA) [26]. The experiment was performed in triplicate and average results were calculated. The percentage Hemolysis was calculated using Eq. (2).

$$\text{Lysis of RBCs } (\%) = \frac{\text{Absorbance of sample } - \text{Absorbance of Negative control}}{\text{Absorbance of positive control}} \times 100 \quad (2)$$

2.5. Statistical analysis

Results were analyzed statistically in which the significance of mean zone of inhibition between standards and *E. coli* extracts were tested by the paired t-test (Tucky's test) analysis using software Statistix, version 8.1. Data were expressed as mean ± S.E.

3. Results and discussion

3.1. Extraction of bioactive fractions from E. coli

An integrated procedure was used [24] for the extraction of isoprenoid quinines (IQ) and polar lipids (PL) in which 500 mg of dry cell mass of *E. coli* was used. Crude IQ and PL yields were 20 and 26.6 mg/mL of DMSO respectively. Isoprenoid quinines (IQ) are derivative of quinine and active biological motif. The extraction of quinone an organic motif from different bacterial strains have been reported well in literature [27, 28]. The bacterial strains which were used for the extraction of quinine are AS-S20-I [28] and Bacillus clausii [27]. One of these strains has shown fibrinolytic activity, while both (AS-S20-I and Bacillus clausii) of these exhibited a great potential of the thrombolytic activity. Whereas different E.coli strains have also been used for the extraction of lipid molecules active against the thrombosis a severe medical condition. In addition to thrombolytic activity hemolytic potentials are also reported [29]. In literature, a detergent octyl-β-D-glucopyranoside is considered as a lipid forming chemical which showed the above-mentioned activities [29].

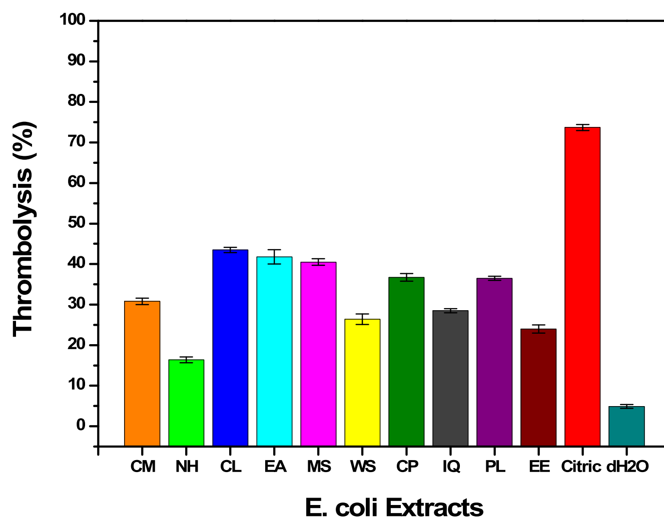


Fig. 2. Percentage thrombolytic activity of crude aqueous and organic extracts of *E. coli*. The thrombolytic activity of *E. coli* extracts with different concentrations were evaluated. Citric acid was used as a control for thrombolytic agent in this experiment. Each bar shows the data from three experiments with error bar representing standard deviations on each. Chloroform (CL), ethyl acetate (EA), and methanol soluble (MS), fractions showed maximum thrombolytic activity. Other extracts also showed significant thrombolytic activity.

In previous research work, the strain (ATCC 35218) was also used for the evaluation of antimicrobial and antioxidant potentials and found good results [30]. However, in the current study, the strain (ATCC 35218) of *E. coli* was employed for the extraction of polar lipids and showed high thrombolytic and cytotoxic activities, proved by our experimental work. Soxhlet extraction (SE) was performed by using 5 g of dry cell mass of *E. coli* and its ethanol extractable yield was 150 mg/mL of dimethyl sulfoxide (DMSO). For solvent-based extraction a method reported earlier [22] was followed. *E. coli* dry cell mass of 5 g was subjected to extraction by using different aqueous and organic solvents by following the method already reported [22]. By which extracts abbreviated as CM, NH, CL, EA, MS and WS with yields of 125, 61, 60, 100, 146 and 50 mg/mL of dimethyl sulfoxide respectively were obtained. Crude protein contents were also extracted by following reported procedure [19], using 1 g dry *E. coli* cell mass and 5 mL protein extraction buffer [20] and its yield was 163 μg/mL.

Table 1
Total Percentage thrombolytic and hemolytic activity of *E. coli* extracts.

Sample	Thrombolytic activity Mean ± S.E	Hemolytic activity Mean ± S.E
CM	$30.8^{E} \pm 0.08$	$82.57^{C} \pm 0.06$
NH	$16.4^{I} \pm 0.10$	$73.46^{D} \pm 0.08$
CL	$43.5^{B} \pm 0.06$	$46.47^{E} \pm 0.09$
EA	$41.8^{C} \pm 0.16$	$33.72^{F} \pm 0.12$
MS	$40.5^{C} \pm 0.07$	$3.2^{K} \pm 0.17$
WS	$26.4^{G} \pm 0.15$	$6.65^{J} \pm 0.21$
CP	$36.7^{D} \pm 0.09$	$23.52^{G} \pm 0.13$
IQ	$28.5^{F} \pm 0.05$	$20.46^{H} \pm 0.17$
PL	$36.5^{D} \pm 0.05$	$87.20^{B} \pm 0.08$
EE	$24^{H} \pm 0.09$	$18.63^{I} \pm 0.13$
Citric acid (+ve control)	$73.7^{A} \pm 0.05$	–
dH_2O (-ve control)	$4.9^{J} \pm 0.12$	–
Triton-X100 (+ve control)	–	$94.75^{A} \pm 0.15$
PBS (-ve control)	–	$0.57^{L} \pm 0.015$

The values are mean ± S.E (standard error) of three replicate experiment.
*Means sharing same alphabets are statistically non-significant ($P > 0.05$).

3.2. In vitro thrombolytic potential of E. coli extracts

The main objective of this thrombolytic assay evaluated the ability of extracts to disrupt already formed blood clot that may cause serious consequences like acute arterial thrombosis, pulmonary embolism, myocardial infarction and ischemic strokes etc. [31]. Fig. 2 represents percentage thrombolytic activity of crude aqueous and organic extracts of *E. coli*. All the extracts showed considerable clot lysis activity (Table 1). Citric acid and distilled water were taken as positive and negative controls that showed 73.7% ± 0.05 and 4.9% ± 0.12 clot lysis respectively. Reduction in clot weight is expressed as its percentage of thrombolytic activity. CL showed maximum (43.5% ± 0.06) clot lysis activity. This maximum clot lysis activity was due to the presence of lipids in CL [24,32]. EA and MS showed 41.8% ± 0.16 and 40.5% ± 0.07 activities respectively. This activity that was due to the EA extracts possess molecules like carbohydrates, fatty acids, amino acids and MS extracts such as phospholipids [17,24]. Maximal clot lysis activity was shown by these extracts. On the other hand, minimal clot lysis activity was shown by NH (16%).

Other extract's clot lysis activities are documented in Table 1. In a study held by Nighat *et al.* [27], the thrombolytic potential of *Trevesia palmate* methanolic fractions (EA, MS, NH, CL and WS) were evaluated. Results showed that CL showed maximum clot lysis activity (44.5%) as compared to all others like MS (43%), NH (44.21%), CL (43.2%) and AQ (40.3%) clot lysis activity. Results of these percentage clot lysis activity were quite similar to our data except WS (Fig. 2). Reason for this difference in-activity is the extraction from two different sources as activity varies from source to source. Our MS extract showed significant thrombolytic activity (40.5% ± 0.07) when compared with citric acid as a positive control. In another study [33], MS extract of Commelina benghalensis showed significant thrombolytic activity (40.94%) when compared with streptokinase (75%) as a positive control. These values are in good agreement with the present results (Table 1).

3.3. Hemolytic activity of E. coli extracts

E. coli extract's cytotoxicity was evaluated by the hemolytic assay as described by Powell et al. [26]. Hemolysis is the healthy red blood cells rupturing that is the indication of cytotoxicity to RBC's cells [34]. Fig. 3 represents percentage Hemolytic activity of *E. coli* extracts. The results showed that PL and CM extracts/samples showed maximum hemolytic activity that is 87.20% ± 1.25 and 82.57% ± 0.94 respectively. The activity of CM may be due to the presence of phospholipids in it [24]. Results are compared with 0.1% Triton X-100 (positive control) with 94.75% ± 1.22 hemolytic activity. In a study held by Warawa *et al.* [35], in which the hemolytic activity of proteins (EspA, EspB, and EspD) of Entero-pathogenic *E. coli* (EPEC) was evaluated. These three proteins

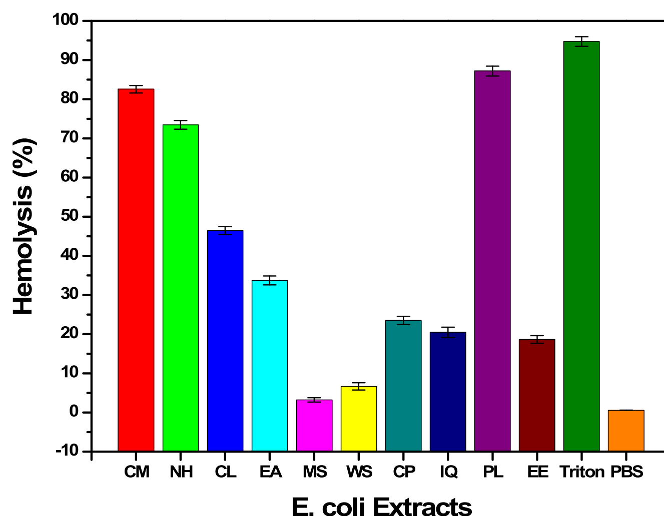


Fig. 3. Hemolytic activity of *E. coli* extracts. Hemolytic activity of *E. coli* extracts with respect to 0.1% Triton X-100 (positive control) presented as Triton in graph. Each bar showing the data from three experiments with error bar representing standard deviations. Polar lipid extract showed maximum hemolytic activity and methanol soluble fraction showed minimum activity when compared with Trion-X100.

were found to possess significant hemolytic activity. Present study of *E. coli* (ATCC 35218) crude protein extract also showed some hemolytic activity (23.527%) (Table 1).

The detailed description of the methodology adopted and its output in terms of positive and negative results from the *E. coli* strain ATCC35218 are presented in Fig. 1. The present work used *E.coli* strain (ATCC 35218) for the extraction of thrombolytic compounds such as Isoprenoid quinone, polar lipids and crude bioactive proteins, polar and nonpolar active compounds. For obtaining polar and non-polar crude compounds different organic solvents are used (Fig. 1). All these samples showed thrombolytic and cytotoxic properties. However, these extracts or samples possessed positive (WS and MS extracts and residues are most safe thrombolytics) and negative results (CL, EA, PL, CM and NH are also thrombolytic but not safe i.e. highly toxic to cell).

3.4. Environmental issues and health benefits of E. coli

E.coli is a Gram-negative bacterium from the family Enterobacteriaceae. According to the World Health Organization (WHO), the bacterium is causing food borne diseases and can transmit in humans and animals as well. However, it is just one side of the picture, there is a bright side of this bacterium; it has potential thrombolytic activity as well as antimicrobial and antioxidant activities [30]. Therefore, this study is of good scientific worth as it is designed to minimize potential environmental and health concerns. It could also be described as an approach to introduce *E. coli* as an eco-friendly source of therapeutic agents with different safety levels that are still needed to be explored.

4. Conclusions

Based on the present study, it is suggested that *E. coli* extracts like chloroform, ethyl acetate, methanol soluble fraction or residue and crude methanol extract, *n*-hexane, polar lipids can be used against thrombolytic and hemolytic disorders respectively. Still, there is a need for extensive studies in order to explore the agents present in each sample that could be an important way to report safe thrombolytic molecules for drug designing. *In vivo* studies could be helpful in determining the real potential usefulness of these *E. coli* samples for the treatment of hemolytic and thrombolytic disorders.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors are thankful for the co-operation and facilities of chemicals and equipments received from PMBL (Protein Molecular Biology Laboratory) and MBL (Molecular Biology Laboratory), Department of Biochemistry, University of Agriculture, Faisalabad, 38040, Pakistan.

References

[1] D. Ferber, From Pigs to People: the Emergence of a New Superbug, 2010. American Association for the Advancement of Science.
[2] Y. Peng, X. Yang, Y. Zhang, Microbial fibrinolytic enzymes: an overview of source, production, properties, and thrombolytic activity *in vivo*, Appl. Microbiol. Biotechnol. 69 (2) (2005) 126.
[3] S.R. Levine, E.C. Haley, P. Lyden, Pilot and Preliminary Studies of Thrombolytic Therapy for Stroke, Thrombolytic Therapy for Acute Stroke, 2005, pp. 95–110. Springer.
[4] L. Zhang, et al., Exploring novel bioactive compounds from marine microbes, Curr. Opin. Microbiol. 8 (3) (2005) 276–281.
[5] P.E. Granum, *Clostridium perfringens* toxins involved in food poisoning, Int. J. Food Microbiol. 10 (2) (1990) 101–111.
[6] B. Guo, et al., Bioactive natural products from endophytes: a review, Appl. Biochem. Microbiol. 44 (2) (2008) 136–142.
[7] T. Long, et al., Structure-bioactivity relationship for benzimidazole thiophene inhibitors of polo-like kinase 1 (PLK1), a potential drug target in Schistosoma mansoni, PLoS Neglected Trop. Dis. 10 (1) (2016), e0004356.
[8] A. Guaadaoui, et al., What is a bioactive compound? A combined definition for a preliminary consensus, Int. J. Nutr. Food Sci. 3 (3) (2014) 174–179.
[9] H. Ashraf, et al., Fibrinolytic enzyme: restoration tool for obliteration of blood clots, J. Mol. Imag. Dynam. 7 (2) (2017) 1000138.
[10] R. Rehman, et al., Biosynthesis of essential oils in aromatic plants: a review, Food Rev. Int. 32 (2) (2016) 117–160.
[11] J. Fenton, et al., Statin drugs and dietary isoprenoids downregulate protein prenylation in signal transduction and are antithrombotic and prothrombolytic agents, Biochemistry 67 (1) (2002) 85–91.
[12] S. Mahmoudi, et al., Production of recombinant streptokinase in *E. coli* and reactivity with immunized mice, Pakistan J. Biol. Sci.: PJBS 13 (8) (2010) 380.
[13] S. Rao, et al., Soluble expression and purification of the recombinant bioactive peptide precursor BPP-1 in *Escherichia coli* using a cELP-SUMO dual fusion system, Protein Expr. Purif. 118 (2016) 113–119.
[14] Y.S. Park, et al., Nutritional and pharmaceutical properties of bioactive compounds in organic and conventional growing kiwifruit, Plant Foods Hum. Nutr. 68 (1) (2013) 57–64.
[15] M. Plaza, et al., Screening for bioactive compounds from algae, J. Pharmaceut. Biomed. Anal. 51 (2) (2010) 450–455.
[16] I. Michalak, K. Chojnacka, Algae as production systems of bioactive compounds, Eng. Life Sci. 15 (2) (2015) 160–176.
[17] S. Bhagavathy, P. Sumathi, I.J.S. Bell, Green algae Chlorococcum humicola-a new source of bioactive compounds with antimicrobial activity, Asian Pacific J. Trop. Biomed. 1 (1) (2011) S1–S7.
[18] A. Müller, et al., Effect of culture medium on the extracellular synthesis of silver nanoparticles using *Klebsiella pneumoniae, Escherichia coli and Pseudomonas jessinii*, Biocatal. Agric. Biotechnol. 6 (2016) 107–115.
[19] A. Jamil, et al., Screening of some medicinal plants for isolation of antifungal proteins and peptides, Pakistan J. Bot. (2007).
[20] Z. Mushtaq, et al., Antioxidants from selected indigenous plants possessing cyclotides, Oxid. Commun. 1 (2017) 102–119.
[21] B. Okutucu, et al., Comparison of five methods for determination of total plasma protein concentration, J. Biochem. Biophys. Methods 70 (5) (2007) 709–711.
[22] T.B. Emran, et al., Effects of organic extracts and their different fractions of five Bangladeshi plants on in vitro thrombolysis, BMC Compl. Alternative Med. 15 (1) (2015) 1–8.
[23] Y. Li, et al., A comparative study: the impact of different lipid extraction methods on current microalgal lipid research, Microb. Cell Factories 13 (1) (2014) 14.
[24] D. Minnikin, et al., An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids, J. Microbiol. Methods 2 (5) (1984) 233–241.
[25] S. Prasad, et al., Development of an in vitro model to study clot lysis activity of thrombolytic drugs, Thromb. J. 4 (1) (2006) 14.
[26] W. Powell, C. Catranis, C. Maynard, Design of self-processing antimicrobial peptides for plant protection, Lett. Appl. Microbiol. 31 (2) (2000) 163–168.
[27] F. Nighat, et al., Cytotoxic, a-amylase inhibitory and thrombolytic activities of organic and aqueous extracts of *Bacillus clausii* KP10, Pak. J. Pharm. Sci. 33 (1) (2020).
[28] A.K. Mukherjee, S.K. Rai, A statistical approach for the enhanced production of alkaline protease showing fibrinolytic activity from a newly isolated Gram-negative Bacillus sp. strain AS-S20-I, N. Biotech. 28 (2) (2011) 182–189.
[29] M. Dinesh, et al., Evaluation of octyl-β-D-glucopyranoside (OGP) for cytotoxic, hemolytic, thrombolytic, and antibacterial activity, Appl. Biochem. Biotechnol. 185 (2) (2018) 450–463.
[30] M. Maqsood, et al., Active fractions from E. COLI ATCC 35218 with antimicrobial and antioxidant properties, JAPS, J. Anim. Plant Sci. 29 (5) (2019) 1433–1441.
[31] V.M. Abascal, et al., Left atrial myxoma and acute myocardial infarction: a dangerous duo in the thrombolytic agent era, Chest 109 (4) (1996) 1106–1108.
[32] S.M.A. Islam, et al., A comparative study of the antioxidant, antimicrobial, cytotoxic and thrombolytic potential of the fruits and leaves of Spondias dulcis, Asian Pacific J. Trop. Biomed. 3 (9) (2013) 682–691.
[33] T.A. Chowdhury, et al., Thrombolytic and cytotoxic activity of methanolic extract of Commelina benghalensis (Family: Commelinaceae) Leaves, J. Sci. Innov. Res. 4 (2) (2015) 100–104.
[34] G. Lakshmi, et al., Phytochemical profile, in vitro antioxidant and hemolytic activities of various leaf extract of Nymphaea Nouchali Linn: an in vitro study, Int. J. Pharm. Pharmaceut. Sci. 6 (6) (2014) 548–552.
[35] J. Warawa, B.B. Finlay, B. Kenny, Type III secretion-dependent hemolytic activity of enteropathogenic Escherichia coli, Infect. Immun. 67 (10) (1999) 5538–5540.