In vitro study of anthelmintic effect of butterfly pea (Clitoria ternatea) flower aqueous extract on Tubifex tubifex

A R M Alnaz, R Ridha, R F G Nasution, A H Nasution, M Ichwan*

Department of Pharmacology, Faculty of Medicine, Universitas Sumatera Utara, Medan, North Sumatra 20155, Indonesia

*E-mail: mhd.ichwan@gmail.com

Abstract. Clitoria ternatea (CT) or butterfly pea was one herbs used for Ayurvedic and other traditional medicines. Utilization of its roots, flowers, and leaves proposed several medical advantages with anthelmintic was one of it. Among current anthelmintic burden of resistance, this study aimed to examine the anthelmintic profile from CT flower aqueous extract on Tubifex tubifex. The study was done by conducting a pilot experimental study by extracting CT flowers to water and piping the extracts of different level of dilution to several group of pots containing Tubifex tubifex. Anthelmintic activity was determined with its paralyzing effect and was compared to negative and positive controls with levamisole. Phytochemicals substances were detected with alkaloids, flavonoids, glycosides, saponins, tannins, and triterpenoids detected. The extract exerts anthelmintic activity in 1:4, 1:40, and 1:400 dilutions and was comparable to 1mg/mL and 0.1mg/mL levamisole. Time to paralysis observed suggested a dose-response relationship of the extract on its anthelmintic activities. It was understood the phytochemicals influents the anthelmintic activity by paralysis worms and leads to death. It was concluded that CT flower aqueous extract presents anthelmintic activity, with further experimental study will needed to be conducted.

1. Introduction

Clitoria ternatea (CT) or known as butterfly pea is a herbaceous plant from Fabaceae family characterized by blue flower petals and white-colored center[1]. This plant was originated from tropical regions of Asia and spread worldwide to South America, China, and India [2]. The blue color of its flower petals is commonly utilized as natural food colorant, especially in Southeast Asia countries such as Malaysia and Thailand [1]. In India, C. ternatea has been used since ancient times as herbs for the traditional Ayurvedic medicine [2,3]. Brazilians commonly called this plant, cunhã, and is heavily used as fodder plants in some regions in Brazil [4].

Butterfly pea is utilized for its leaves, flowers, and roots. The blue flowers were most common for food industries and traditional medications [5]. There were reports on flavonol, anthocyanins, and several other metabolites isolated from the blue flower [1]. In Ayurvedic medicines, CT was used for its nootropic, anticonvulsant, analgesic, antipyretic, anti-inflammatory, antioxidant, and anthelmintic activities. Yet there were still lack of studies prevails scientific evidence of those medical use of CT but remains potential to test as future herbal medicines [6].

Current anthelmintic therapies with common drugs encountered problems with burden of emerging anthelmintic resistance. Albendazole and mebendazole were reported with significant reduced efficacies in last two decades [7]. This requires a development of novel anthelmintic therapies to overcome the ineffective treatment of the Neglected Tropical Disease. CT exhibits promising potentials as...
anthelmintic profiles provided from its leaf extract, but no current studies reported the utilization of CT flower extract [6]. This study aimed to examine the anthelmintic effect of CT flower aqueous extract on *Tubifex tubifex*.

2. Methods
This study is a pilot experimental study with controlled time series design. The study was conducted in May 2021 in Pharmacology Laboratory, Department of Pharmacology, Faculty of Medicine, University of Sumatera Utara, Medan, Indonesia. The study was conducted to observe the anthelmintic profile by examining response of *Tubifex tubifex* after exposed to several concentrations of CT flower aqueous extract and compared to Levamisole as controlled group.

Other paragraphs are indented (BodytextIndented style).

2.1. Collection of Material
The extract used in this study was made from the flower of *Clitoria ternatea* obtained from the gardens in University of Sumatera Utara on April 2021. The collected flower was selected only for fresh flowers. The anthelmintic activity was evaluated by using *Tubifex tubifex* worms collected from Mentawai Marine Biology Center, Medan, Indonesia.

2.2. Preparation of Extract
100g of CT flower was soaked in 5L of water in a container for 24 hour. The aqueous extract then heated 60-70°C on a wide surface to evaporate amount of water and stopped when viscous. Heated extract then diluted to 1:2 ratio to aquadest. Treatment were separated to negative and positive control with Levamisole. Treated group were divided to 4 different groups (A-D) of CT extract concentrations, with 10 times dilution each.

2.3. Phytochemical Testing
The prepared extract was screened with qualitative tests of phytochemical constituents in the Laboratory of Biology, Faculty of Pharmacy, University of Sumatera Utara.

2.4. Treatment Model
The study was conducted with 7 groups of treatment. All groups were consisted of 2 pots, prepared with 1cc of aquadest and 2mg of *Tubifex tubifex*. First group was considered as negative control aquadest. Two positive control groups was made with 1mg/cc and 0.1mg/cc of Levamisole. Each prepared and diluted extract of CT were piped 1cc into each pots, resulting to another 2 times dilution as group A-D were labeled as 1:4, 1:40, 1:400, and 1:4,000 of CT extract.

2.5. Evaluation of Anthelmintic Activity
Evaluation of anthelmintic activity was done by observing *Tubifex tubifex* movement every 15 minutes lasting at 75 minutes. Time recording was started as extract was piped into the pots. Anthelmintic activity was considered with paralysis of the *Tubifex tubifex* after a vigorous shake. Any movement of *Tubifex tubifex* were evidence of alive, graded to active movement (+) in colony and weak movement as separated from colony and inability to curl. Observation of *Tubifex tubifex* movement in solid color of extracts would require illumination through the pots.
3. Result

![Image of Clitoria ternatea flower aqueous extract]

**Figure 1.** *Clitoria ternatea* flower aqueous extract

| Metabolite          | Reagent                                      | Result |
|---------------------|----------------------------------------------|--------|
| Alkaloids           | Dragendorff, Bouchardat, Meyer               | +      |
| Flavonoids          | Magnesium powder + amyl alcohol + hydrogen chloride | +      |
| Glycosides          | Molish + H₂SO₄                              | +      |
| Saponins            | Stirred hot water                            | +      |
| Tanins              | FeCl₃                                        | +      |
| Triterpenoids/Steroids | Lieberman-Bourchat                       | +      |

The study was using CT aqueous extract with mentioned process. The extract used for the phytochemical screening test was shown in Figure 1. The phytochemical constituents of the CT flower extract was identified as Table 1. Several important group of phytochemical constituents such as … were identified through the phytochemical screening.
The anthelmintic activity was measured by groups of experiments conducted with controls as presented in Figure 2. The evaluation of anthelmintic activity was recorded in time series observations of *Tubifex tubifex* movements as mentioned in Table 2.

From the table, the survival of the *Tubifex tubifex* was validated as all were active and alive in C(-) groups. The treatment with C(+)<sub>A</sub> with 1g/cc of levamisole remains the most effective in anthelmintic activity with paralysis was noticed since 3<sup>rd</sup> minutes of observation, while C(+)<sub>B</sub> in both pots manifested anthelmintic activity with gradual decrease of *Tubifex tubifex* activity level but stayed with weak movement by the end of the study.

The treatment in Group A demonstrated an anthelmintic activity with paralysis was remarked since the 5<sup>th</sup> minutes of study, and was comparable to C(+)<sub>A</sub>. The movement of *Tubifex tubifex* in Group B became weak starting in the 15<sup>th</sup> minutes, and was stated paralyzed in 30<sup>th</sup> minutes observation. Both Group A and B were in contrast to C(+)<sub>B</sub> in duration for paralysis, while Group C with 1:400 dilution presented less response, but was still better than C(+)<sub>B</sub> as one of 2 pots were found to be paralyzed in...
last observation in 75th minutes. Less response in Group C and no movement alteration observed up to the end of the study suggested a dose-response relationship in anthelmintic activity of CT flower aqueous extract.

4. Discussion

The potencies of CT on anthelmintic activities and also its antimicrobial, memory enhancing, anticonvulsant, and other medicinal benefits were related to phytochemical compounds in it. It was also detected with phytochemical substances such as alkaloid, tannins, glycoside, resin, and flavonoids of the flowers. Phytochemical substances were reportedly most abundant in its root, but flower do provide major compounds. Flowers contained second most flavonoid in the plant after root with 42±1 mg/mL.[8]

Flavonoids and tannins was mostly detected in phytochemical studies. Flavonoid metabolites such as kaemperol, quercetin, and myricetin 3-glucoside were isolated from the blue flower. Other flavonoids such as epicatechin and rutin may also extracted. Other substances isolated generally form all parts of plant were pentacyclic triterpenoids such as taraxerol and glucoside-clitorin. Peptide compounds was also remarkable with cyclotides peptide which structured cyclical and poses three disulfide bonds preventing degradation in contrast to other peptides. These phytochemical constituents were also related to increase acetylcholine and cholinergic activities in animal model which potentially affecting helminth activities.[8,9]

Special constituents origins from the CT flowers was the acylated anthocyanins, which was derivatives of delphindin 3’,3’,5’-triglucoside. This compound was then refered as ternatins, responsible for its blue color. Several ternatins then found such as ternatin A1-3, B1-4, and C1-5. Currently it is known for protective antiinflammation role with further study will be required to be conducted for many medical potentials.[9,10]

Anthelmintic number of trials for CT was still limited, but studies reported proposed a promising potency the profile.[11,12] Major phytochemicals such as glycoside and tannins known taken role in this abilities. Tannins bind free proteins of the GI tract of the host, and reported to bind to the glycoprotein in the cuticle of the parasites.[13] Other substances such as phenols were reported interfering energy generation and leaving worms paralyzed due lack of energy. Alkaloids also impaired worms’ central nervous system activities and disturbing sucrose metabolism in worms’ gastrointestinal tracts. These mechanism leads those parasites to death.[14]

Phytochemical profiles of CT and its activity was related to types of extraction and depends on solvent used. Different solvent may alter the substances, while other may induce. Aqueous extract was noted to present one of most numbers of constituents and less altering than methanol, ethyl acetate, and benzene extracts.[15,16] Although it was mentioned by Salhan et al that there was no differences between CT leaves aqueous extract compared to ethanolic extract in its constituents, but aqueous extract took more time to paralysis. However, both extract still exerts significant anthelmintic activity compared to control, and no extract comparison of trial for flower extract conducted previously.[14]

A study from Nirmal et al reported similar time profiles of CT flower extract anthelmintic activity with this study. All parts of CT was tested with positive anthelmintic activity, but flower had the longest time to paralysis in dose of 20mg/mL for 10.1±0.32 minutes while roots had the strongest activity. However, flower extract has second earliest time to death amounng various CT extracts, only less than roots. Hence this remarked a strong potency of CT flower aqueous extract as an anthelmintic. It was also mentioned in which non polar solvents generated better anthelmintic activities, yet the study didn’t conducted aqueous extract.[17]

5. Conclusion

It was concluded that the CT flower aqueous extract proposed an anthelmintic activity on Tubifex tubifex from this in vitro study. Phytochemical constituents of the extract was responsible in its anthelmintic activity. Yet this is a pilot study, it was suggested further research to determine anthelmintic activity of CT flower aqueous extract with quantitative phytochemical study with precise dose to determine the dose-response relationship and a trial to a true human parasite among helminthes.
References

[1] Lijon M B, Meghla N S, Jahedi E, Rahman M A and 2017 *J Nat Soc Sci* 4 1
[2] Gupta G K, Chahal J and Bhatia M 2015 *J Pharm Res* 3 2610
[3] Mukherjee P K, Kumar V, Kumar N S and Heinrich M 2008 *J Ethnopharmacol* 120 291
[4] Escher G B, Marques M B, do Carmo M A V, Azevedo L, Furtado M M, Sant’Ana A S, da Silva M C, Genovese M I, Wen M, Zhang L, Oh W Y, Shahidi F, Rosso N D and Granato D 2020 *Food research international* 128 108763
[5] Muhammad Ezzudin R and Rabeta M S 2018 *Food Research* 2 415
[6] Gollen B, Mehla J and Gupta P 2018 *J Pharmacol Reports* 3 1
[7] Moser W, Schindler C and Keiser J 2019 *Advances in parasitology* 103 91
[8] Manjula P, Mohan C H, Sreekanth D, Keerthi B and Devi B P 2013 *J Indian bot Soc* 92 173
[9] Oguis, G K, Gilding, E K, Jackson, M A and Craik, D J 2019 *Frontiers in plant science* 10 645
[10] Nair V, Bang W Y, Schreckinger E, Andarwulan N and Cisneros-Zevallos L 2015 *J Agric Food Chem* 63 63
[11] Al-snafi A E 2016 *J Pharm* 6 68
[12] Purba E C 2020 *EduMatSains* 4 111
[13] Nahar K, Rahman M A, Parvin M N and Sarwar S *J Pharm Sci* 3 46
[14] Salhan M, Kumar B, Tiwari P, Sharma P, Sandhar H K and Gautam M 2011 *J Drug Dev Res* 3 68
[15] Jain R A and Shukla S H 2011 *Pharmacognosy Journal* 3 62
[16] Taur D J, Taware S B, Patil R N, Patil R Y and Kharya M D 2010 *Pharmacognosy Journal* 2 260
[17] Nirmal SA, Bhalke RD, Jadhav RS, Tambe VD 2018 *Pharmacologyonline* 1 114