

## Anti-leech activity of *Scutellaria baicalensis* and *Morinda citrifolia* extracts against *Piscicola geometra*

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**Abstract.** *Piscicola geometra* leeches are naturally infecting cobia juvenile. The leeches attach to cobia by sucking and biting its surface and provide the gate of second infection. Water extracts of *Scutellaria baicalensis* root and *Morinda citrifolia* leaves were used to be tested through *In Vitro* method to look for the anti-leeches activity against *Piscicola geometra*. In this study, a total number of 800 leeches from infected cobia were prepared. The anti-leech activity from water extract of *S. baicalensis* root and *M. citrifolia* leaves were compared in different dilutions of plant extracts for 96 hours. Significant anti-leech activity was observed with *M. citrifolia* leaves with 80% mortality of leeches. *S. baicalensis* root showed higher anti-leech activity with 100% mortality of leeches. The average time was needed for *S. baicalensis* root to paralyzing and kill the leeches were 8h, 40h, 48h, 72h, and 96h in various dilutions of *S. baicalensis* root. This study indicated that *S. baicalensis* water extract had a potent for new anti-leeches agent.

### 1. Introduction

Cobia (*Rachycentron canadum*) culture has become popular in more than 23 countries in the Asia-Pacific region [1]. However, rapid expansion and intensification of cobia culture has resulted in increasing incidences of disease outbreaks [2]. Parasitic diseases are particularly common to infect the gastrointestinal tract, gills, and skin of cobia, which have caused economic losses [3, 4]. Moreover, leeches infestation are considered as the portal for other pathogens that develop secondary infection and lead to death [5].

It has been demonstrated that the impact of leeches are correlated with the level of infestation [6]. *P. geometra* is a member of Piscicolidae family which is considered as blood-sucking marine leeches [7]. It has a cylindrical body shape and an anterior bell-shaped sucker [8]. Moreover, it has much higher rate of oxygen consumption, it prefers running water and it is oligotrophic with pH range varies from 7.0 to 9.0 [9]. Since the leeches are found in wide variety of habitats, it is conceivable that the leeches can tolerate much broader ranges of conditions [10]. *P. geometra* was previously reported to be found in *Pleuronectes platessa* and *Myoxocephalus* [11], *Rutilus rutilus*, *Scardinius erythrophthalmus*, *Blica bjoerkna*, *Tinca tinca* [12], *Esox lucius*, *Barbus rajanorum*, *Salmo salar*, *Salmo girdneri*, *Rutilus rutilus* [13], *Gasterosteus aculeatus*, *Cottus scorpius* and *Platichthys flesus* [14].



Medicinal plants contain bioactive compounds and shows impact in controlling fish diseases as a low-cost and eco-friendly method and it has a broad spectrum of activity [15, 16]. *Scutellaria baicalensis* and *Morinda citrifolia* have been widely used as medicinal plant [17, 18]. unique compounds contained in those plants are for example baicalein, wogonin, and oroxylin that are highly present in the root of *S. Baicalensis* and they can directly bind with the active site of enzymes to increase the stimulation of protein synthesis [19]. *S. baicalensis* root has been reported to be useful to treat liver problems [20], diarrhea, hypertension, hemorrhaging, inflammations, and respiratory infections, as well as to act as antimicrobial, , anti toxoplasm [21] and anti trypanosomal activities [22]. The leaf of *M. citrifolia* has been studied for its antimicrobial, anticancer [23], antioxidant, and larvicidal activities [24, 25]. Moreover, the plant extract has also been used as anti-helminthic and anti-parasitic agent [26-29]. The aim of this study was to determine the anti-leech activity of water extracts of *S. baicalensis* root and *M. citrifolia* leaves against *Piscicola geometra*.

## 2. Methodology

### 2.1. Sample collection

Eight hundred of leeches ( $1.27 \pm 0.44$  cm in length) were collected from infected cobia in Tungkang Marine Biotechnology Research Station, Taiwan. The bell shape of anterior sucker, cylindrical body shape, and clear segments with longitudinal white spots on the body were the main signs for detection of *Piscicola geometra* species (figure 1).



**Figure 1.** *Piscicola geometra*.

### 2.2. Plant extract preparation

The dried roots of *S. Baicalensis* were purchased in the local market of Neipu, Pingtung, Taiwan. Fresh *M. citrifolia* leaves were collected from noni farm, Pingtung, Taiwan. The leaves were chopped into small pieces and rinsed with distilled water before put into the oven for 12 h. The dried plant was grounded separately in a mechanical grinder and stored in an airtight container. Water extract was prepared by boiling 150 mL distilled water with 10 g of plant powder at 95 °C. After extraction, the supernatant was centrifuged at 4 °C, 1000 g for 10 min. The clear supernatant was filtered and stored at -20 °C.

### 2.3. Anti-leech assay

The clear supernatant of *S. baicalensis* roots and *M. citrifolia* leaves was diluted with sea water to obtain concentrations of 50x, 100x, 200x, 400x, 500x, 700 x, and 1000x dilutions.

Five leeches per petri dish were used in three replicates involving eight petri dish of seven different concentrations and a control (sea water). Their effects were screened for 96 h with 8 h interval for observation of mortality. The behavior of the leeches and the time used to paralyze and kill them were recorded. The dilutions were renewed every 12 h. The efficacy of the plant extract that were able to kill the leeches within 1–96 h reflects the anti-leech activities and would be used to treat infected cobia

in the future. The differences between control and treated groups were analyzed by one-way ANOVA.

### 3. Results

The anti-leech activities of *S. baicalensis* root and *M. citrifolia* leave water extracts against *P. geometra* infestation are shown in table 1 and table 2, respectively. Introduction of plant extract caused the reduction of swimming activity of *P. geometra*. Total mortality (100 %) of the leeches was observed in *S. baicalensis* root water extracts (table 1). During the observation, leeches gradually became weak and their bodies turned to be wrinkled. They moved weakly and finally died. On the other hand, *M. citrifolia* leaves extract was able to cause about 60 % of mortality in leeches. According to the statistical analysis, 50x and 100x dilutions exhibited the strongest effect of both plant extract, while 700x and 1000x dilutions showed the lowest. At 500x, 700x and 1000x dilutions, all the leeches immersed in *S. baicalensis* root died while few leeches could survive in *M. citrifolia* extract (table 2). Control sample showed no anti-leech effect in both studies. It was obvious that the water extract of *S. baicalensis* root showed higher anti-leech activity than *M. citrifolia* leaves extract, which was characterized by the paralysis and death of leeches in a short time.

**Table 1.** Mortality of leeches for 96 h immersion in different serial dilution of *S. baicalensis* root extract (N=5 leeches per treatment in 3 replicate). a, b, c, d, e character show that there are no significant differences among treatments.

Time observed (hour)	Serial dilution							
	Control <sup>e</sup>	1/50x <sup>a</sup>	1/100x <sup>a</sup>	1/200x <sup>b</sup>	1/400x <sup>bc</sup>	1/500x <sup>c</sup>	1/700x <sup>cd</sup>	1/1000x <sup>d</sup>
1	0	5	5	0	0	0	0	0
8	0	5	5	0	0	0	0	0
16	0	-	-	2	0	0	0	0
24	0	-	-	1	1	1	1	0
32	0	-	-	0	0	0	0	0
40	0	-	-	2	0	1	1	0
48	0	-	-	-	4	0	0	0
56	0	-	-	-	-	1	1	1
64	0	-	-	-	-	1	1	1
72	0	-	-	-	-	1	1	0
80	0	-	-	-	-	-	-	1
88	0	-	-	-	-	-	-	0
96	0	-	-	-	-	-	-	2
<b>Total</b>	0	5	5	5	5	5	5	5

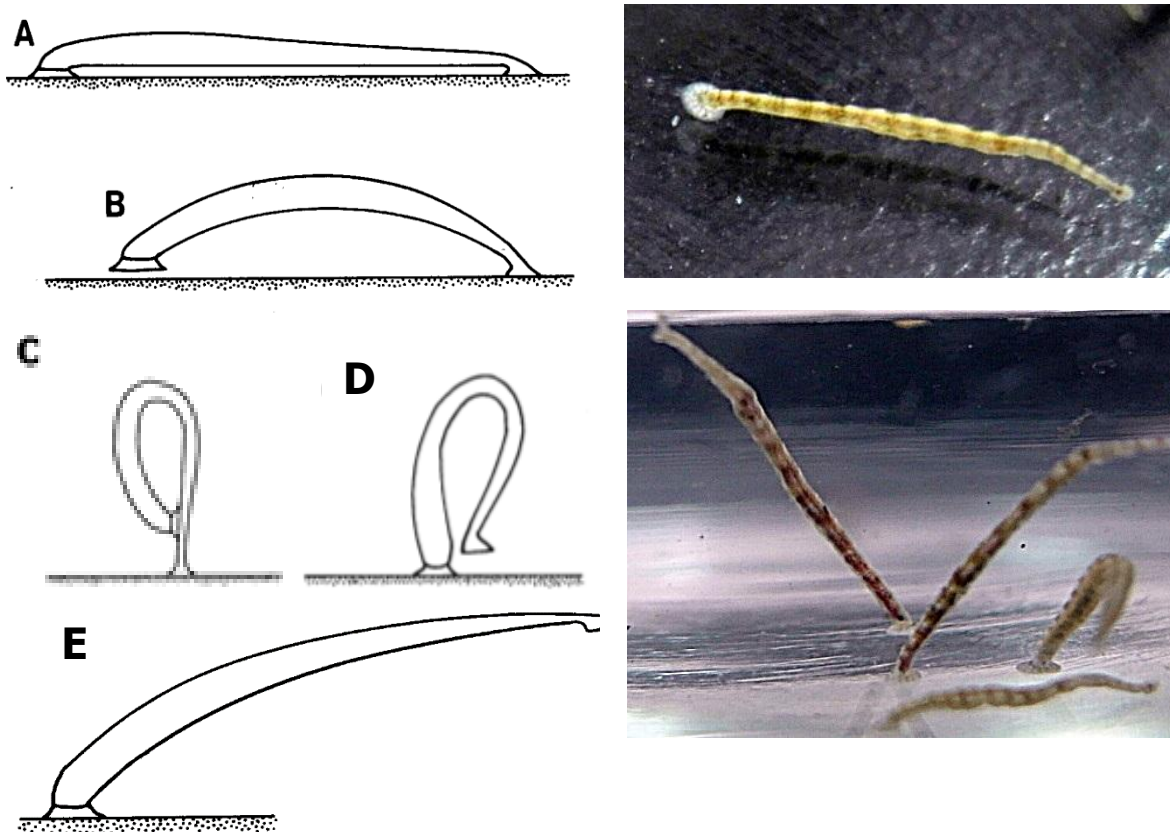
**Table 2.** Mortality of leeches for 96 h immersion in different serial dilution of *M.citrifolia* leaves extract (N=5 leeches per treatment in 3 replicate). a, b, c, d, e character show that there are no significant differences among treatments.

Time observed (hour)	Serial dilution							
	Control <sup>e</sup>	1/50x <sup>a</sup>	1/100x <sup>ab</sup>	1/200x <sup>b</sup>	1/400x <sup>c</sup>	1/500x <sup>d</sup>	1/700x <sup>d</sup>	1/1000x <sup>de</sup>
1	0	0	0	0	0	0	0	0
8	0	2	0	0	0	0	0	0
16	0	0	2	0	0	0	0	0
24	0	2	0	1	0	1	0	0
32	0	1	2	0	0	0	0	0
40	0	-	0	2	1	2	1	0
48	0	-	1	1	1	0	0	0
56	0	-	-	1	0	0	0	0
64	0	-	-	-	0	0	1	0
72	0	-	-	-	0	1	1	0
80	0	-	-	-	1	0	1	1
88	0	-	-	-	1	0	0	0
96	0	-	-	-	1	0	0	2
<b>Total</b>	0	5	5	5	5	4	4	3

### 3.1. Behavior of leeches

Leeches (*Piscicola geometra*) use their sucker for locomotion; after the posterior sucker attached, they extend their bodies and the oral sucker will be attached (figure 2A). The posterior sucker is drawn forward, it reattaches near the anterior sucker and it will be released (Figure 2B). This movement is rapid and efficient. After detachment, the posterior sucker is applied high up on the ventral surface,

producing an aggregate loop (figure 2C-D) before the body reaches its usual position (figure 2E). When the leeches were immersed in several dilutions of extract, they swayed in random movement until the posterior sucker reached the bottom of the petri dish. The leeches gradually became weak and their bodies turned to be wrinkled over the increasing time of immersion.



**Figure 2.** Behavior of *Piscicola geometra*

#### 4. Discussion

Infestation of *P. geometra* has caused extensive damage characterized by small circular lesions with the presence of blood on the skin, eyes, and gill of cobia. The damaged scale is considered as the portal of bacteria and fungi infections that can lead to second infections [30]. Leeches are rarely pathogens to their host and only become a serious problem when the infestations are high [31].

No standard medicine has been registered to treat leeches infestation. Anti-histamine, calcium hydroxide, lidocaine, hypertonic saline, bicarbonate, and citrus extract are traditional treatments to treat leeches infection and have been noted as ineffective treatments [32]. Pharmaceutical plants can be used as a source for a wide variety of natural antioxidants and they have been proven as safe therapeutic effects to treat fish disease [33, 34]. Flowering plant *Scutellaria baicalensis* and *Morinda citrifolia* have been listed in a traditional Chinese medicine (TCM) to treat many diseases [35]. The dried root of *S. Baicalensis* and dried leaves of *M. citrifolia* produce large amounts of bioactive flavones and provide a variety of specific health benefits [36, 37].

*S. baicalensis* and *M. citrifolia* have been reported to support immunostimulatory activity, antitumor, antibacterial, antiseptic, anti-fungal, anti-inflammatory, and anti-helminthic activity with no

side effects [38, 39]. As an effective drug with no side effects, this study has offered the use of *S. baicalensis* root and *M. citrifolia* leaves against leeches' infestation. The result of this study shows that high rate of mortality was observed within 96 h of immersion of leeches with *S. baicalensis* root water extract indicating that the herb was effective to control leeches in cobia culture. *S. baicalensis* root was able to kill the leeches in 8 h, 40 h, 48 h, 72 h, and 96 h, respectively. The average time for leeches to die with *M. citrifolia* were 32 h, 48 h, 56 h, and 96 h with 50x, 100x, 200 x, and 400x dilutions, respectively. Meanwhile, few leeches could still survived at 500x, 700x, and 1000x dilutions of *M. citrifolia* leaf after 96 h of immersion.

A preliminary investigation on the toxicity of herbal extract on *Piscicola geometra* was reported by. It was shown to reduce swimming activity of leeches in the 42 h, 30 h, 18 h, and 12 h for 5 %, 10 %, 15 %, and 20 % concentration of *Tetracer alnifolia*. Toxic effect of *Raphia vinifera* on *Piscicola geometra* has been demonstrated previously in a study by [40]. Terrestrial blood sucking leeches (Haemadipsidae) population can be reduced by an aqueous extract of *C. spathulifolia* (62.6%), *V. elaeagnifolia* (63.0%), and *S. rarak* (82.6%) [6]. The root of *S. baicalensis* has been effective as anti-parasitic agent against *Toxoplasma gondii* and *Trypanosoma cruzi* [41]. However, there is no report of the anti-parasitic or anti-leech activity of *S. baicalensis* and *M. citrifolia* on *P. geometra*.

## 5. Conclusions

This study has demonstrated that water extract of *S. baicalensis* dried root has stronger anti-leech activity than the water extract of *M. citrifolia* dried leaves and it has ability to inhibit the swimming activity of *P. geometra*. *S. baicalensis* water extract demonstrated an effective anti-leech activity against *P. geometra* infection. Further study on bath treatment of infected cobia and analysis of active compounds of *S. baicalensis* root extract will be required in the future.

## 6. References

- [1] Lee D-H, Lim S-R, Han J-J, Lee S-W, Ra C-S, et al 2014 *J. Anim. Sci.* **27** 1303-1310
- [2] Chu KB, Abdullah A, Abdullah SZ and Bakar RA 2013 *Trop. Life Sci. Res.* **24** 77-84
- [3] Shaffer RV and Nakamura EL 1989 Synopsis of biological data on the cobia *rachycentron canadum* (pisces: Rachycentridae)
- [4] Megharajan S, Ranjan R, Xavier B, Muktha M, Edward L, et al 2017 *J. Asia Pac.* **13** 12-16.
- [5] Vongsombath C, De Boer HJ and Pålsson K 2011 *Acta trop.* **119** 178-182
- [6] McLean E, Salze GandCraig SR 2008 *Ribarstvo* **66** 1-16
- [7] Mann KH 1962 *Chapter 1 - introduction* Book title: Pergamon; p 1-4
- [8] Kearn GC 2004 *Leeches, Lice and Lampreys a Natural History of Skin and Gill Parasites of Fishes* (Netherland : Dordrecht-Springer)
- [9] Mann KH 1962 *Chapter 2 - The Medicinal Leech, Hirudo Medicinalis* Book title: Pergamon; p. 5-21
- [10] Landsberg JH 1982 *Infection Strategies of Fish Ectoparasites* [Thesis type]
- [11] Mann KH 1962 *Chapter 3 - A Survey of The Group: A More Detailed Systematic Survey is Given in the Appendixes* Book title: Pergamon p 22-35
- [12] Demirtas M 2011 *Turkiye Parazitol Derg* **35** 159-163
- [13] Ceylan M, Boyaci YO, Meke T, Inceoglu H and Kara A 2011 *Turkiye Parazitol Derg* **35** 207-209
- [14] Kendzierska H, Dąbrowska A, Cichocka J, Janas U and Bielecki A 2014 The first record of *piscicola pojmanskae bielecki*, 1994 in the gulf of gdańsk (the southern baltic sea) with species characteristics distinguishing it from *Piscicola geometra* (linnaeus, 1758) *Secondary title* p 324
- [15] Madhuri S, Mandloi A, Govind P and Sahni Y 2012 *IOSR J Pharm* **3** 28-30
- [16] Mehana EE, Rahmani AH and Aly SM 2015 *Annu Res Rev Biol* **5** 477
- [17] Abou Assi R, Darwis Y, Abdulbaqi IM, Khan AA, Vuanghao L, et al 2017 *Arab. J. Chem.* **10** (5): 691-707



- [18] Zhao Q, Chen X-Y and Martin C 2016 *Sci. Bull.* **61** (18): 1391-1398
- [19] Mahmoud M, El-Lamie M, Dessouki A and Yusuf M 2014 *Glob. Res J. Fis. Sci.* **1** 26-33
- [20] Liu A, Huang L, Fan H, Fang H, Yang Y, et al 2015 *Int. Immunopharmacol* **24** 72-79
- [21] Yang X, Huang B, Chen J, Huang S, Zheng H, et al 2012 *J. Parasitol. Res.* **110** 2221-2227
- [22] Mamadalieva NZ, Herrmann F, El-Readi MZ, Tahrani A, Hamoud R, et al 2011 *Afr. J. Pharm. Pharmacol.* **63** 1346-1357
- [23] Sang S, Wang M, He K, Liu G, Dong Z, et al 2002 Chemical components in noni fruits and leaves (*morinda citrifolia* l.) Book title: *ACS Publications* p 33
- [24] Kovendan K, Murugan K, Shanthakumar SP and Vincent S 2012 *Asian Pac. J. Trop. Dis.* **2** S362-S369
- [25] Kovendan K, Shanthakumar SP, Praseeja C, Kumar PM, Murugan K, et al 2014 *Asian Pac. J. Trop. Dis.* **4** S173-S180
- [26] Militz TA, Southgate PC, Carton AG and Hutson KS 2014 *J. Fish. Dis.* **37** 451-461
- [27] Chitmanat C, Tongdonmuan K, Khanom P, Pachontis P and Nunsong W 2003 *Antiparasitic, antibacterial, and antifungal activities derived from a terminalia catappa solution against some tilapia (*oreochromis niloticus*) pathogens*. Secondary title p 179-182.
- [28] Huang A-G, Yi Y-L, Ling F, Lu L, Zhang Q-Z, et al 2013 *J. Parasitol. Res.* **112** 4065-4072
- [29] Ling F, Wang J-G, Lu C, Wang G-X, Lui Y-H, et al 2012 *J. Parasitol. Res.* **111** 841-848
- [30] Nwabueze AA 2013 *J. Agric. Sci.* **1** 1-9
- [31] Bielecki A, Cichocka J, Terlecki J and Witkowski A 2011 *Biologia* **66** 294-298
- [32] Bahmani Ma nd Rafieian-Kopaei M 2014 *Asian. Pac. J. Trop. Dis.* **4** 315-316
- [33] Rafieian-Kopaei M 2013 *J. Herb. Med.* **1** 1-2
- [34] Shirzad H and Nasri H 2014 *J. Herb. Med.* **2** 21-22
- [35] Calabrò S 2015 *Plant secondary metabolites* Book title: Springer p 153-159
- [36] Zhao Q, Zhang Y, Wang G, Hill L, Weng J-K, et al A specialized flavone biosynthetic pathway has evolved in the medicinal plant, *scutellaria baicalensis* *Sci. Adv.* **2** e1501780
- [37] Jeyabalan S, Subramanian K, Cheekala UMR and Krishnan C 2017 *J. Appl. Pharm. Sci.* **7** 089-095
- [38] Torres MAO, Fátima Braga Magalhães I, Mondêgo-Oliveira R, Sá JC, Rocha AL, et al 2017 *Phytother. Res.* **7** 5817 - 5821
- [39] Muluye RA, Bian Y and Alemu PN 2014 *J. Tradit. Complement Md.* **4** 93-98
- [40] Fafioye O and Adebisi A 2001 Toxic effect of *raphia vinifera* on fish leech (*piscicola geometra*)
- [41] Schinella GR, Tournier HA, Prieto JM, Ríos JL, Buschiazzi H, et al 2002 *Fitoterapia* **73** 569-575

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