



PRELIMINARY PHYTOCHEMICAL ANALYSIS AND LARVICIDAL ACTIVITY OF EDIBLE FERN (*Diplazium esculentum* (Retz.) Sw.) EXTRACT AGAINST *Culex*

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Abstract. Mosquito is one of insects spreading the vector of serious diseases to human being through its bite such as *Culex* which spreads the vector of filariasis and brain inflammatory diseases. Some people have used plants as insecticides traditionally, such as fern (*Diplazium esculentum* (Retz.) Sw.). *D. esculentum* (Retz.) Sw. popularly known as edible fern which almost all Asian people, especially Indonesian consumed it as vegetable, and some people also used it for medicinal purposes. This study was performed to investigate the secondary metabolites from *D. esculentum* leaves and its larvicidal activity against *Culex*. The phytochemical screening was undertaken on the leaves and methanol extract of *D. esculentum*, the results showed the presence of alkaloids, steroids, phenols, and tannins on leaves, while methanol extract exhibited existence of steroids, saponins, phenols and tannins. Investigation of larvicidal activity of methanol extract was carried out against *Culex* larvae for various concentrations which observed for 48 hours. Percentage of mortality was analyzed statistically using Bill Test method that showed active larvicidal activity with a LC₅₀ value of 149.279 ppm. This study indicates that fern plant have the potential as larvicidal against *Culex* and need to perform the further research to develop the new natural insecticides.

Keywords: Phytochemical, larvicidal, insecticide, *Diplazium esculentum*, *Culex*

I INTRODUCTION

Mosquitoes are among the insects that pose a serious threat to public health by spreading vector diseases in humans, including malaria, yellow fever, dengue fever and chikungunya [1]. *Aedes aegypti* L., *Anopheles gambiae*, *Culex quinquefasciatus* Say and *Culex pipens* are some of the types of mosquitoes responsible for the transmission of some human diseases caused by protozoa (malaria), nematodes (lymphatic filariasis), meningitis, urticaria and virus (West Nile encephalitis, dengue fever, yellow fever and chikungunya) [2,3]. Today more than 2 billion people in the tropics are the victims of mosquito-borne diseases. Mosquito prevention efforts have been carried out, among others, based on chemical insecticide-based intervention measures [4] and biological control. Mosquito prevention is still focused on the use of chemical insecticides, such as *N*, *N*-diethyl-meta-toluamide (DEET) which commonly found repellent of insects [5]. DEET has remarkable safety profiles after 40 years of widespread use of the world, but toxic reactions can occur, usually when the wrong use of the product [6]. In addition, mosquito spraying also

contains DEET which can cause problems to human health, due to direct contact between humans and sprinklers. Besides DEET, organophosphate compound is also used as mosquito prevention formulated in abate. The use of abate as larvicidal is very limited, it is given when an epidemic has occurred. In addition, abate is an inorganic compound that is difficult to decompose in nature, and can cause pests to become immune (pest resurgence). The research of natural materials as mosquito prevention which is easy to obtain, cheap and easy to decompose in nature continues to be developed. Traditionally, some Acehnese people have used plants to overcome mosquitoes, one of the plants used is fern (*Diplazium esculentum* (Retz.) Sw). Ferns are one of the oldest land plant groups on the surface of our earth. Compared to the other groups, fern plants are usually ignored by researchers, but those are important for the beauty and the economic uses [7]. *D. esculentum* (Retz.) Sw. belonging to Athyriaceae family known as edible fern is a forest plant that has been used by many people since the past as a vegetable. In addition, this plant also has potential of economic, cultural,

ecological and health roles [8]. *D. esculentum* is commonly found growing in humid places such as along the riverbank and also in the forest [9, 10]. Taxonomy of *D. esculentum* are: Kingdom Plantae, Division Tracheophyta, Class Polypodiopsida, Order Polypodiales, Family Athyriaceae, Genus *Diplazium* Sw, and Species *D. esculentum* (Retz.) Sw. [11,12] (Figure 1). This plant consists of creeping and branched rhizome; scales brown, lanceolate and up to 7 to 15 mm in length, long fern leaves up to 100 cm and a width up to 20 cm, rather dimorphic, higher fertile leaves, erect, sterile curved. The stalk is green and slightly smooth, with a length of 20 - 50 cm. In leaf 2 - 3 pinnate, 5 - 3 pinnate, and length up to 50 - 80 cm. The pinnules are lanceolate shaped with a length up to 2 - 5 cm and are somewhat coarsely serrated. The erect stems appear to be fleshy and thick with a length of up to 1.2 m more. Sorus is linear, has a width of 1.5 mm located along the leaf veins, and spores dark brown [13].



Figure 1 Leaves of *D. esculentum* (Retz.) Sw.

D. esculentum (Retz.) Sw. is widespread in Indonesia such as in Blangkejeren which is one of the sub-districts in Aceh Province. Traditionally, several Blangkejeren's society used it as a natural insecticide by putting the leaves of fern in the corners of the room or in the chicken coop to repel mosquito, flies, and other insects. This plant is very easy to find in Aceh also almost in all regions in Indonesia, both found in the forest and on the edge of rice fields. Several studies reported the traditional use of fern plants as insecticides and repellent of insects and pests [10,13]. Plants are the extraordinary source for the discovery of new medicinal products in drug development [14]. This activity is related to secondary metabolite compounds contained in the plants or also known as natural products [15,16]. Secondary metabolite is a common term used for more than 30.000 different substances which their compounds are usually classified according to

their biosynthetic pathways. Three large molecule families are generally considered: phenolics, terpenoids and steroids, and alkaloids [17]. Several studies have reported the secondary metabolite contents in *D. esculentum* [10,18,19,20,21], but fern plants from Indonesia have not been widely reported, even the secondary metabolite contents of Aceh fern has not been reported. Besides, larvicidal activity of *D. esculentum* also has not been reported. Based on those literatures, we report the secondary metabolite content of Aceh fern plant and its larvicidal activity against *Culex*.

II METHODOLOGY

Plant Material and Bioindicator

D. esculentum (Retz.) Sw. leaves were collected from Blangkejeren, Aceh (Indonesia) in February 2018. The bioindicator used in this research is the larva of *Culex*.

Extraction

The air-dried leaves (1 kg) of *D. esculentum* (Retz.) Sw. were ground and extracted with methanol by maceration method for 3 x 24 h, the maceration was repeated until the filtrate is clear. The extract solution was filtered and evaporated by rotary evaporator to give methanol extract.

Phytochemical Screening

Alkaloid: About 2 g of plant materials were crushed then added 1 mL of ammonia. Furthermore, 10 mL of chloroform was added, then crushed and filtered. The filtrate was added 10 mL of sulfuric acid 2N, shaken vigorously, left for a minute until the sulfuric acid solution and chloroform separated. The sulfuric acid layer is taken and divided into three test tubes and each test tube is tested by Meyer, Dragendorff, and Wagner reagents to determine the presence of alkaloids. The addition of Meyer reagent established white precipitate, Dragendorff reagent caused reddish precipitate, and Wagner reagent raised yellow precipitate. Those results indicate the presence of alkaloids [22].

Terpenoid, Steroid, and Saponin: Ten grams of fern leaves were finely ground, then extracted with hot methanol. The obtained filtrate was concentrated with rotary evaporator to yield methanol extract. The methanol extract was partitioned with hexane. The soluble extract in hexane was tested with the Liberman-Bourchard reagent. The blue or green color exhibits the presence of steroids and red color for terpenoids. The insoluble residue in hexane is added water

and shaken vigorously. The presence of the stable foam for 30 min indicates the existence of saponins, if positive for saponins, the solution was hydrolyzed with HCl and tested with the Liberman-Bourchard reagent. The green or blue color indicates the presence of steroidal saponins and the purple or red color shows the existence of terpenoid saponins [23].

Flavonoid: Leaves of *D. esculentum* (10 g) was extracted with methanol and concentrated. The concentrated methanol extract was partitioned with hexane. The residue was extracted with 10 mL of 80% ethanol, subsequently added 0.5 mg of magnesium and HCl 0.5 M. The pink or purple color shows the presence of flavonoids [16].

Phenol: Methanol extract tested by Ferric Chloride. Add 3 – 4 drops of FeCl₃ solution into extract, the formation of bluish black color exhibits the phenol compound [24].

Tannin: About 0.5 g of methanol extract was boiled in 10 ml of water in the test tube and then filtered. Add a few drops of FeCl₃ 0.1%. Forming of a brownish green or bluish black color indicates tannins [25].

Larvicidal Activity Test

The mosquito larvae were collected from ditch and identified then incorporated into the cage (50 x 50 x 50 cm) by giving the yeast as food. The larvae were identified before being cultured. The larvicidal test was performed with three repetitions. Methanol extract prepared in test cups for various concentrations of 31.25; 62.50; 125; 250; 500; 1000 and 2000 ppm in water. An abate solution used as positive control and water as a negative control. If the extract is insoluble in water, added dimethyl sulfoxide (DMSO) 0.2% (maximum limit for no effect on the compound activity), the same treatment for positive and negative controls (DMSO amount is converted according to the needs) was performed, stirred until solution is homogeneous. In each test cup, inserted 10 mosquito larvae and incubated for 48 h. After 48 h, the live and dead larvae were counted and analyzed statistically using Bill Test method. The concentration plots with percent mortality to obtain a curve. The obtained curve used to determine the LC₅₀ value.

III RESULT AND DISCUSSION

Phytochemical Screening

The phytochemical screening was carried out on leaves and methanol extract of *D. esculentum*

(Retz.) Sw. using various phytochemical reagents. Examination on leaves showed the active phytochemical classes as alkaloids, steroids, phenols, and tannins, while methanol extract established the presence of steroids, flavonoids, saponins, and tannins as showed in Table 1.

Table 1 Preliminary Phytochemical Analysis of *D. esculentum* (Retz.) Sw.

Secondary Metabolites	Leaves of <i>D. esculentum</i> (Retz.) Sw.	Methanol Extract of <i>D. esculentum</i> (Retz.) Sw. Leaves
Alkaloid	+	-
Terpenoid	-	-
Steroid	+	+
Saponin	-	+
Flavonoid	-	-
Phenol	+	+
Tannin	+	+

Generally, secondary metabolites contained in this plant are polar compounds, those are caused by the solvent used is polar. Although methanol tends to attract almost all different types of polarity compounds, but in this plant material, almost all detected compounds were polar compounds. Analysis on fern leaves indicated the presence of alkaloid compounds which is detected by Meyer reagent that yielded white precipitate, while Dragendorff and Wagner reagents did not show the presence of alkaloids. Even in methanol extract did not exhibited the presence of alkaloids by those three reagents. Examination of terpenoid on leaves and methanol extract showed negative results, but it exhibited the presence of steroids. In the other hand, the presence of saponin compounds was detected in methanol extract but it did not indicate in leaves. The existence of phenols and tannins were showed in both of leaves and methanol extract. Secondary metabolite compounds containing in this plant in general is the phenolic class, namely phenols, and tannins. These compounds contain OH groups which generally have good activity as antioxidant [26,27,28,29]. Several studies have reported the activities of *D. esculentum* (Retz.) Sw. extract such as antibacterial [30,31], antioxidant [10,32,33,34], analgesic [20], hepatoprotective [35], anti-inflammatory [35], and anthelmintic [13]. Traditionally, some of its uses as a medicinal plant were as insecticides and repellent of insects and pests [10,13,20], and to treat measles fever. Compounds that play a role in all those bioactivities are secondary metabolite compounds that contained in the plant of *D. esculentum* (Retz.) Sw.

Table 2 Bill Test Analysis of Larvicidal Activity

Dose	Log Dose	Number of Dead Mosquitoes	Number of Living Mosquitoes	Number of Accumulated Deaths	Number of Accumulated Life	Rasio Death : Total	Mortality (%)
2000	3.301	10	0	43	0	1	100.00
1000	3.000	9	1	33	1	0.9706	97.06
500	2.699	6	4	24	5	0.8276	82.76
250	2.398	6	4	18	9	0.6667	66.67
125	2.097	4	6	12	15	0.4444	44.44
62.5	1.796	4	6	8	21	0.2759	27.59
31.25	1.495	4	6	4	27	0.1290	12.90

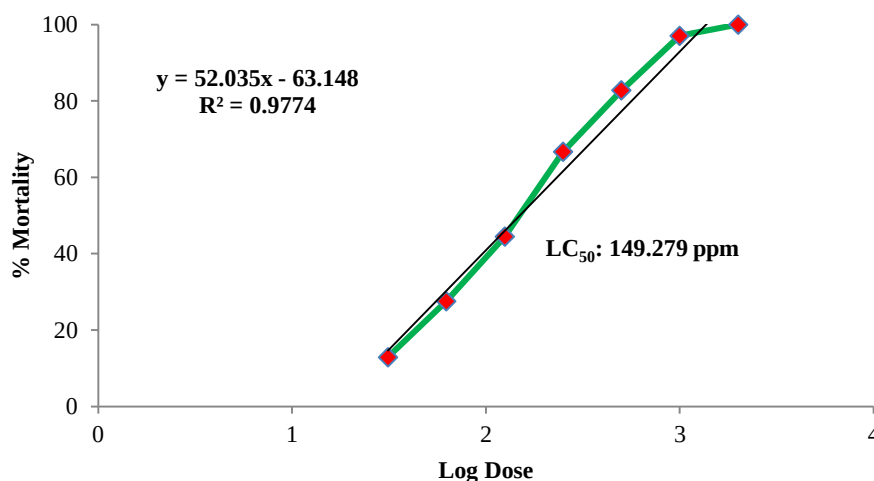


Figure 2 Curve of % Mortality vs. Log Dose for Methanol Extract of *Diplazium esculentum* (Retz.) Sw.) Leaves

Larvicidal Activity

In this study, mosquito larvae were taken randomly in nature. Once identified, the mosquito is declared as *Culex*. *Culex* larvae instar 3 or 4 used as bioindicator for larvicidal test. Investigation of larvicidal activity of methanol extract against *Culex* larvae was performed to find out the larvicidal activity of polar compounds from *D. esculentum* leaves against *Culex* mosquito larvae. Larvicidal activity test on methanol extract was carried out at various concentrations of 31.25; 62.5; 125; 250; 500; 1000 and 2000 ppm, by doing three repetitions at each concentration. The results were tabulated in Table 2. The data in table were analyzed statistically using Bill Test method to obtain percent of mortality. Then mortality data is plotted with log dose to determine LC_{50} (Figure 2). The results showed a strong activity with LC_{50} value of 149.279 ppm. It showed that methanol extract of *D. esculentum* leaves has good potential as a larvicidal agent. There are several studies that reported the larvicidal activity of some plants on mosquito larvae, such as *Monstera adansonii* plant extracts against *Culex quinquefasciatus* [36], *Nerium oleander* L. flower extracts against *C. quinquefasciatus* Say [37], Xerophytic plants extract against *Culex pipiens* and *Aedes caspius* [38], and pure

compounds of *Stereum* plant against *Aedes aegypti* (Linn) [39]. However, the larvicidal activity of *D. esculentum* extract in this study is the first report. Bioactivity in plants is contributed by the secondary metabolite compounds contained in the plants [40].

CONCLUSION

The phytochemical screening was performed on the leaves and methanol extract of *D. esculentum*, the leaves showed the presence of alkaloids, steroids, phenols, and tannins, while methanol extract exhibited the existence of steroids, saponins, phenols, and tannins. This plant showed potential larvicidal activity against *Culex* with LC_{50} value of 149.279 ppm. This is the first report about secondary metabolites and larvicidal activity of *D. esculentum* (Retz.) Sw. leaves. It is expected to carry out further research for biological activity of this plant to develop the new natural insecticides.

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REFERENCE

- González, M., López, S. & Elbal, P. M. A. (2015). Blood-Feeding Diptera (Culicidae and Ceratopogonidae) in an Urban Park of The City of Vitoria-Gasteiz (Basque Country, Spain). *Journal of The European Mosquito Control Association*, **33**, 10-14.
- Aguiar, R. W. S., Santos, S. F. D., Morgado, F. D. S., Ascencio, S. D., Lopes, M. D. M., Viana, K. F., Didonet, J., Ribeiro, B. M. (2015). Insecticidal and Repellent Activity of *Siparuna guianensis* Aubl. (Negramina) against *Aedes aegypti* and *Culex quinquefasciatus*. *PLoS ONE*. **10**(2): e0116765. doi:10.1371/journal.pone.0116765.
- Ramya, B. S. & Ganesh, P. (2013). Effect of Microbial Metabolite (Spinosaad) against Larval Stages of *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi*. *International Journal of Current Microbiology and Applied Sciences*. **2**(12), 376-383.
- Kumar, S., Wahab, N. & Warikoo, R. (2011). Bioefficacy of *Mentha piperita* essential oil against dengue fever mosquito *Aedes aegypti* L. *Asian Pacific Journal of Tropical Biomedicine*. **1**(2): 85-88.
- Widiyanti, N. L. P. M. dan Muyadihardja, S. (2004). Uji Toksisitas Jamur *Metarhizium anisopliae* Terhadap Larva Nyamuk *Aedes aegypti*. *Media Litbang Kesehatan*. XIV (3) :1.
- Fradin, M. S., MD. (1998). Mosquitoes and Mosquito Repellents: A clinician's Guide. *Annals of Internal Medicine*. **128**:931.
- Bandyopadhyay, S. & Mukherjee, S. K. (2014). A Contribution to The Fern Flora of Howrah District in West Bengal, India. *International Journal of Pharmacological Screening Methods*. **4** (1), 1-3.
- Zakiyyah (2016). Sikap Masyarakat dan Stimulus Konservasi Pakis Sayur (*Diplazium esculentum* (Retz.) Sw.) di Desa Gunung Bunder II, Taman Nasional Gunung Halimun Salak. *Tesis*. Institut Pertanian Bogor, Bogor.
- Chandra, S. & Srivastava, M. (2003). *Pteridology in the New Millennium: NBRI Golden Jubilee Volume*. (Springer Science + Business Media Dordrecht, India) p 174.
- Das, B., Paul, T., Apte, K. G., Chauhan, R., Saxena, R. C. (2013). Evaluation of Antioxidant Potential & Quantification of Polyphenols of *Diplazium esculentum* Retz. with Emphasis on Its HPTLC Chromatography. *Journal of Pharmacy Research*. **6**, 93-100.
- Diplazium esculentum* (Retz.) Sw. in GBIF Secretariat (2017). GBIF Backbone Taxonomy. Checklist dataset https://doi.org/10.15468/39_mei accessed via GBIF.org on 2018-07-13.
- United States Department of Agriculture (USDA): Natural Resources Conservation Service. Accessed via <https://plants.usda.gov/java/ClassificationServlet?source=profile&symbol=DIES&display=31> on 2018-07-14.
- Amit, S. & Singh, F. M. (2012). In-Vitro Anthelmintic Activity of *Diplazium esculentum* (Retz.) Swiss Rhizome Extract. *Journal of Pharmacognosy and Phytochemistry*. **1** (4), 84-87.
- Halimatussakdiah, Amna, U., Tan, S. P., Awang, K., Ali, A. M., Nafiah, M. A., dan Ahmad, K. (2015). *In Vitro* Cytotoxic Effect of Indole Alkaloids from The Roots of *Kopsia singapurensis* Ridl. against The Human Promyelocytic Leukemia (HL-60) and the Human Cervical Cancer (HeLa) Cells. *International Journal of Pharmaceutical Science Review and Research*, **16**, 31(2), 89-95.
- Dias, D. A., Urban, S., & Roessner, U. (2012). A Historical Overview of Natural Products in Drug Discovery. *Metabolites*, **2**(2), 303-336.
- Halimatussakdiah and Amna, U. (2016). Isolasi Senyawa Alkaloid Indol dari Ekstrak Akar *Kopsia singapurensis* Ridl. (Apocynaceae). *Jurnal Ilmiah JURUTERA*, **(3)**1, 032-037.
- Ramawat, K. G. & Mérillon, J. M. (2008). *Bioactive Molecules and Medicinal Plants*. (Berlin: Springer).
- Dash, G. K., Khadidi, S. K. J. and Shamsuddin, A. F. (2017). Pharmacognostic Studies On *Diplazium Esculentum* (Retz.) Sw. *Der Pharmacia Lettre*, **9** (3), 113-120.
- Tongco, J. V. V., Villaber, R. A. P., Aguda, R. M. and Razal, R. A. (2014). Nutritional and Phytochemical Screening, and Total Phenolic and Flavonoid Content of *Diplazium esculentum* (Retz.) Sw. from Philippines. *Journal of Chemical and Pharmaceutical Research*, **6**(8), 238-242.
- Chawla, S., Chawla, S., Ram, V., Semwal, A. and Singh, R. (2015). Analgesic Activity of Medicinally Important Leaf of *Diplazium esculentum*. *African Journal of Pharmacy and Pharmacology*, **9**(25), 628-632.
- Junejo, J. A., Ghoshal, A., Mondal, P., Nainwal, L., Zaman, K., Singh, K. D., and

- Chakraborty, T. (2015). In-vivo Toxicity Evaluation and Phytochemical, Physicochemical Analysis of *Diplazium esculentum* (Retz.) Sw. Leaves a Traditionally used North-Eastern Indian Vegetable. *Advances in Bioresearch*, **6** (5), 175-181.
22. Miles, D. H. (1985). *A Guide to Biologically Active Plants Constituent*. Departement of Chemistry, University of Florida, USA.
23. Lajis, S. M. K. (1994). *The Phytochemical Survey, Proceeding of Workshop*. Department Of Chemistry University Of Malaysia, Malaysia. Litbang Kehutanan, Jakarta.
24. Tiwari, P., Kumar, B., Kaur, M., Kaur, G., and Kaur, H. (2011). Phytochemical screening and Extraction: A Review. *Internationale Pharmaceutica Sciencia* **1**(1), 98-106.
25. Ayoola, G. A., Coker, H. A. B., Adesegun, S. A., Adepoju-Bello, A. A., Obaweya, K., Ezennia, E. C., and Atangbayila, T. O. (2008). Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants Used for Malaria Therapy in Southwestern Nigeria. *Tropical Journal of Pharmaceutical Research*, **7**(3), 1019-1024.
26. Augusto, T. R., Scheuermann Salinas, E. S., Alencar, S. M., D'Arce, M. A. B. R., Costa de Camargo, A., and Vieira, T. M. F. S. (2014). Phenolic Compounds and Antioxidant Activity of Hydroalcoholic Extracts of Wild and Cultivated Murtilla (*Ugni molinae* Turcz.). *Food Science and Technology*, **34**(4), 667-673. DOI: <http://dx.doi.org/10.1590/1678-457X.6393>
27. Zhao, H. X., Zhang, H. S., and Yang, S. F. (2014). Phenolic Compounds and Its Antioxidant Activities in Ethanolic Extracts from Seven Cultivars of Chinese Jujube. *Food Science and Human Wellness*, **3**, 183-190. DOI: <http://dx.doi.org/10.1016/j.fshw.2014.12.005>
28. Michalak, A. (2006). Review: Phenolic Compounds and Their Antioxidant Activity in Plants Growing under Heavy Metal Stress. *Polish Journal of Environmental Studies*, **15**(4), 523-530.
29. Wojdyło, A., Nowicka, P., Carbonell-Barrachina, A. A., and Hernández, F. (2016). Phenolic Compounds, Antioxidant and Antidiabetic Activity of Different Cultivars of *Ficus carica* L. Fruits. *Journal of Functional Foods*, **25**, 421-432. DOI: <http://dx.doi.org/10.1016/j.jff.2016.06.015>
30. Ullah, M. O., Haque, M., Urmi, K. M., Zulfiker, A. H. M., Anita, E. S., Begum, M., and Hamid, K. (2013). Anti-Bacterial Activity and Brine Shrimp Lethality Bioassay of Methanolic Extracts of Fourteen Different Edible Vegetables from Bangladesh. *Asian Pacific Journal of Tropical Biomedicine*, **3**(1): 1-7.
31. Amit, S., Sunil, K., Bhatt, S. P., and Arvind, N. (2011). Antibacterial Activity of *Diplazium esculentum* (Retz.) Sw. *Pharmacognosy Journal*. **3** (21), 77.
32. Choudhury, J., Majumdar, S., Roy, S., and Chakraborty, U. (2017). Antioxidant Activity and Phytochemical Screening of Two Edible Wetland Pteridophytes *Diplazium esculentum* (Retz) Sw and *Marsilea minuta* L. – A Comparative Study. *World Journal of Pharmaceutical and Medical Research*, **3**(9), 195-203.
33. Roy, S., Hazra, B., Mandal, N., and Chaudhuri, T. K. (2013). Assessment of the Antioxidant and Free Radical Scavenging Activities of Methanolic Extract of *Diplazium esculentum*. *International Journal of Food Properties*, **16**, 1351-1370. DOI: 10.1080/10942912.2011.587382
34. Sarkar, P. and Dalal, A. (2016). Evaluation of Antioxidant Activity of *Diplazium esculentum* and *Enhydra fluctuans* of West Bengal. *IOSR Journal of Pharmacy and Biological Sciences*, **11**(6), 45-50. DOI: 10.9790/3008-1106014550
35. Nair, A. G., Nikhila G. S., Sangeetha G. and Swapna T. S. (2015). In Vitro Hepatoprotective and Anti-Inflammatory Activities of *Diplazium esculentum* (Retz) Sw. – A Wild Fern from Western Ghats. *International Journal of Institutional Pharmacy and Life Sciences*, **5**(2), 341-348.
36. Gomathi, R., Indrakumar, I., and Karpagam, S. (2014). Larvicidal Activity of *Monstera adansonii* Plant Extracts against *Culex quinequefaciatus*. *Journal of Pharmacognosy and Phytochemistry*, **3**(3): 160-162.
37. Raveen, R., Kamakshi, K.T., Deepa, M., Arivoli, S., and Tennyson, S. (2014). Larvicidal Activity of *Nerium oleander* L. (Apocynaceae) Flower Extracts against *Culex quinquefasciatus* Say (Diptera: Culicidae). *International Journal of Mosquito Research*, **1** (1): 38-42.
38. Al-Mekhlafi, F. A., Taha, N. A., Mashaly, A. M. A., and Wadaan, M. A. (2013). Larvicidal Activity of Selected Xerophytic Plants Against *Culex pipiens* and *Aedes caspius* (Diptera: Culicidae). *Pakistan Journal of Zoology*, **45**(1), 241-246.
39. Chirchir, D. K., Ouma, R. B. O., Cheplogoi, P. K., and Omolo, J. O. (2013). Larvicidal

- activity of Extracellular Secondary Metabolites from A Stereum Species Hill ex Pers. (JO5289) against The Dengue Fever Mosquito, *Aedes aegypti* (Linn) (Diptera: Culicidae). *African Journal of Chemistry*, **1**(1), 001-008.
40. Rathy, M.C., Sajith, U., and Harilal, C. C, (2015). Larvicidal Efficacy of Medicinal Plant Extracts against The Vector Mosquito *Aedes albopictus*. *International Journal of Mosquito Research*, **2** (2): 80-82.