IJBPAS, August, 2018, 7(8): 1591-1600

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

TOXICITY AND TERATOGENICITY OF *Dieffenbachia amoena* LEAF EXTRACT

ALIZZA YVETTE B. MEMITA, SHEENA D. MACALINAO, LEA ESPERANZA G. REYES, ERNESTO E. DAMIAN JR. AND RICH MILTON R. DULAY*

Bioassay Laboratory, Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija, 3120 Philippines

*Corresponding Author, E-mail:richmiltondulay@clsu.edu.ph

Received 15th Feb. 2018; Revised 25th March. 2018; Accepted 29th April 2018; Available online 1st August 2018

https://doi.org/10.31032/IJBPAS/2018/7.8.4516

ABSTRACT

This paper demonstrated the toxic and teratogenic effects of *Dieffenbachia amoena* water extract in zebrafish embryo (*Danio rerio*) and its cytotoxic effect in brine shrimp (*Artemia salina*) nauplii. A 100 % mortality was recorded with the embryos exposed at 1000 µg/ml and higher concentrations while those at 500 µg/ml had 66.67% in all periods of observation. No mortality was observed to embryos at 100 µg/ml and embryo water. Exposed embryos to latter treatments showed 100% hatchability while no hatched was observed at 500 µg/ml and higher concentrations. Although normal, significantly lower heartbeat rate was noted with embryos at 100 µg/ml. Only growth retardation was observed as the most distinct teratogenic effect of the water extract. In cytotoxicity assay, the percentage mortality of nauplii increased as the concentration of the extract increases. The computed LC_{50} value of the extract is 1190 µg/ml, which therefore considered that the extract is non-toxic. However, other bioactivities of this plant must be studied.

Keywords:*D. amoena*, hot water extract, embryo-toxic, brine shrimp nauplii, zebrafish

INTRODUCTION

Dieffenbachia amoena (Araceae family) is a tropical ornamental house plant with thick, waxy, broad and white spotted leaves that can grow up to 4-8 feet **[1, 2]**. This plant has the capacity to significantly reduce benzene on indoor air **[3]**, however, it increases the amount of carbon dioxide in the absence of

enough light **[4]**. Aside from being a decorative plant, *Dieffenbachia* species have been also used as food, medicine, stimulants and to inflict punishment **[5]**. Although this plant is used for decorative and medicinal purposes, wrong utilization such as ingestion may result to unnecessary morbidity or mortality **[2]**. Oxalate crystals and proteolytic enzyme found in *D. amoena* are reported to be the cause of toxicity of the plant **[6, 7]**.

Previously, extracts of some plants have been reported for their toxic and teratogenic effects in zebrafish (*Danio rerio*) embryo model, these plants include *Moringa olifeira, Tinospora cordifolia, Ocimum sanctum, Tamarindus indica L,* and a toxic plant, *Derris elliptica***[8-11]**. Coagulation of embryo was the most toxic effect while growth retardation and morphological abnormalities were the most teratogenic effects of these plants. Zebrafish has been widely used as model for developmental biology and screening of toxic substances for they are very sensitive to chemicals during early development **[12-14]**. They lay 50 to 100 non-adherent transparent eggs a day with ease of maintenance, small size, high fecundity, rapid development, amenability to genetic and chemical screens, and an extensive literature base **[15, 16]**. Transparency of the zebrafish embryos facilitates easy identification of craniofacial, cardiac, and skeletal deformities **[17]**. Zebrafish embryo exhibits similarity to higher form of vertebrates which makes them a standard model for toxicity testing **[18]**. On the other hand, brine shrimp (*Artemia salina*) nauplii are animal model used in cytotoxicity assay, which is a simple test to determine the toxic effects of substances or compounds and is extensively used in the evaluation of chemicals and natural plant extracts **[19]**.

This study determined the toxic and teratogenic effects of the different concentrations of hot water extracts of *D. amoena* in zebrafish embryos and brine shrimp nauplii in order to establish its functional activities.

MATERIALS AND METHODS

Sourceand extraction of plant

The leaves of *D. amoena* were collected from Brgy. San Roque, Lupao, Nueva Ecija, Philippines. The leaves were washed, cut into smaller pieces and air dried at room temperature under shaded conditions for 10 days. The dried leaves were pulverized using a blender and 10 g was added to 300 mL of distilled water and subjected in a double boiler at 80-90 ˚C for 2 hours **[20]**. The extract was filtered and stored in a flask covered with foil and refrigerated prior to evaluation.

Maintenance and spawning of zebrafish

Mature 7 female and 14 male zebrafish were maintained in a glass aquarium containing non-treated and clean tap water with continuous flow of oxygen. They were fed three times with high protein commercial fry dry flakes, brine shrimp and pellets. The remaining foods were removed daily to ensure most favourable water quality **[21]**. They were confined in a plastic mesh inside the aquarium and allowed to spawn following the procedure of Dulay *et al*., **[22]**. After spawning and fertilization, the fertilized eggs were siphoned using a hose, rinsed three times and examined under the microscope for their uniformity. Coagulated eggs were discarded.

Teratogenicity test

The different concentrations of the extracts were prepared by diluting it to embryo water**[23]**. Three ml of each concentration were dispensed into each vial. Triplicate test was done. Four embryos were exposed to each replicate vial and placed at room temperature. The percentage mortality was recorded after 12, 12, 36 and 48 hours. The hatchability and heartbeat rate were also determined. Morphological endpoint evaluation of the zebrafish embryo was based on the parameters established by Nagel **[24]** where: lethal (coagulation, tail not detached, no somites, and no heart beat); teratogenic (malformation of head, tail and heart, scoliosis, deformity of yolk, and growth retardation); and normal.

Cytotoxicity assay

The cytotoxicity test was done based on the method described by Olowa and Nuneza **[25]**. One gram of *A. salina* were allowed to hatch in amodified hatchery using clean 1 liter plastic bottle with artificial seawater**[26]**,proper lighting and aeration for 24 hours. The hatchery is covered with thin gauze to protect the eggs against undesirable animal or insects that may invade the hatchery. The nauplii were collected and prepared for the assay. Ten nauplii were placed into each vial containing the diluted extract in artificial seawater following the different concentrations. The vials were placed under lighted condition. Mortality was monitored after 12, 24, 36 and 48 hours. The LC_{50} value was computed using probit analysis and toxicity rating established by Clarckson *et al.,* **[27]** was used.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) and compared using Duncan Multiple Range Test (DMRT) at 5% level of significance. The SAS statistical software was used in the analysis.

RESULTS AND DISCUSSION

Embryotoxic effect in zebrafish

Mortality of the embryos is defined by coagulation and no visible heartbeat. The percentage mortality of the embryos as affected by different concentrations of *D. amoena* extract was determined (Table 1). Apparently, the mortality of embryos is significantly influenced by the different concentrations and not by the time of exposure. In all periods of observation, a 100% mortality was significantly recorded to the embryos exposed at 1000 µg/ml and higher concentrations. However, embryos at 500 µg/ml had 66.67% mortality. Coagulated embryo (Figure 1A) is the most marked toxic effect of the extract.No mortality was noted to embryos at 100 µg/ml and embryo water. These results strongly indicate the embryo-toxic effect of *D. amoena* extract at higher concentrations. Similarly, other plant extracts also showed embryo toxic effects. *Garcinia mangostana* extract exhibited 100% mortality at 0.5% or higher concentrations of leafextract after 12 hours **[28]**. Also, 100% mortality of embryos was found at 1000 µg/ml and higher concentrations of *Persea americana* and at 5000 µg/ml and higher concentrations of *Syzygium cumini* after 48 hours of exposure**[29]**. *Morus alba* L. (Alfonso variety) leaves extract also exhibited 100% mortality at 0.5% and higher concentrations but in a time dependent manner **[30]**. Moreover, in the study of Kivçak e*t al.***[31]**, Fractions of n-hexane, ethanol, methanol, ethyl acetate and water extracts of leaves of *Ceratonia siliqua* showed toxic effect on zebrafish embryos while ethyl acetate extract showed no cytotoxic activity. Embryo-toxicity of *D. amoena* extract can be attributed to its contraceptive activity as studied by De Pasquale *et al.* **[32]** where doses of aqueous extract of *D. amoena* reversibly interrupted the estrous cycle and temporarily inhibited ovulation of the female Wistar rats.

Hatchability and heartbeat rate

Hatching is an indicative of successful developmental processes of the embryos. It takes place between 48-72 h depending on the thickness of the chorion. Hatchability also indicates the viability of the offspring inside the chorion. In this study, the percentage hatchability of zebrafish was also determined. Based on the results, no hatched was observed to those at 500 µg/ml and higher concentrations (Table 2). However, all embryos at 100 µg/ml and control hatched after 36 hours of exposure. Similarly, hatchability is also observed in the lowest concentration of the *Momordica charantia* leaf extract **[33]**.

Moreover, the heartbeat of the hatched zebrafish at 100 µg/ml was also determined (Table 2). Hatched at this concentration significantly recorded lower heartbeat rate of 113.67 beats per minute (bpm) when compared to hatched in control (150.00 bpm). No heartbeat was noted to those at 500 µg/ml and higher concentrations. The same observation is reported by Romagosa *et al.,* **[34]**. The lower heartbeat rate in treated embryos compared to normal embryos indicates cardiotoxicity that may be caused by no blood flow **[35]**. This could probably due to the phytochemicals present in the extract, which needs further study in order to elucidate the responsible bioactives.

Teratogenic effects of the extract

The assessment of teratogenic effects of the extract was based on the established parameters by Nagel **[24]**. It was found out in the present study that delayed growth was the most distinct teratogenic effect of *D. amoena* extract. The morphological endpoints of the zebrafish exposed to 500µg/ml, 100 µg/ml and embryo water are shown in Figure 1. Delayed growth is very obvious to survived 72 hpta-embryo (Figure 1B), which still remain unhatch. Growth retardation may be caused by high number of cardiac apoptotic cells resulting in underdevelopment of heart and pericardium, which might produce abnormal heart beat and circulation failure, subsequently resulting in body growth retardation due to insufficient nutrients **[36]**.Cafirma *et al.,* **[37]** reported that delayed growth is one of the most distinct teratogenic effect of *Origanum vulgare*, however, it was observed in 1000 and 10000 µg/ml concentrations of the extract. Moreover, *Morus* sp.S54 variety extract also caused growth retardation as one of the most common teratogenic effect in zebrafish embryo **[38]**. On the other hand, no morphological abnormality was observed in this study. In the study of Tan *et al.,* **[39]** the aqueous extracts of *Ocimum suave* also did not reveal any foetal abnormalities in rats.

Cytotoxic effects of the *D. amoena* extract

The cytotoxic effect of *D. amoena* extract was investigated using *A. salina*nauplii. Table 3 shows the percentage mortality and the LC_{50} value. A 100% mortality was recorded at 5000 µg/ml and higher concentrations while low percentage mortality was noted at lower concentrations. The computed LC_{50} value of the extract was 1190 µg/ml, and based on toxicity rating of Clarckson *et al*. **[27]** for the toxicity assessment, extracts is considered non-toxic. In contrast, essential oils of the leaves *Dieffenbachia picta* showed many active compounds and proved to be toxic **[40]**.

These suggest the toxic effect of *Dieffenbachia* group is dependent on the species, extraction process, and solvent. Several aqueous extracts of plants are also considered non-toxic such as *Cinnamomum porrectum* **[41]**, *Salvia sclareoides***[42]**, and *Thaumatococcus daniellii* **[43]**.

Table 1: Mortality of embryos exposed to the different concentrations of *D. amoena* extract at 4 observation periods

Extract	Concentration (µg/ml)	Mortality (%)			
		12 hours	24 hours	36 hours	48 hours
*D. amoena*leaves	10000	100.00[a]	100.00[a]	100.00[a]	100.00[a]
	5000	100.00[a]	100.00[a]	100.00[a]	100.00[a]
	1000	100.00[a]	100.00[a]	100.00[a]	100.00[a]
	500	66.67[a]	66.67[a]	66.67[a]	66.67[a]
	100	0.00[b]	0.00[b]	0.00[b]	0.00[b]
Control	0	0.00[b]	0.00[b]	0.00[b]	0.00[b]

Means with the same letter of superscript are not significantly different with each other at 5% level of significance using DMRT

Table 2: Hatchability and heartbeat rate of zebrafish exposed to different concentrations of *D. amoena* extract

Extract	Concentration (µg/ml)	Hatchability (%)	Heartbeat rate (per minute)
*D. amoena*leaves	10000	0.00[b]	0.00[c]
	5000	0.00[b]	0.00[c]
	1000	0.00[b]	0.00[c]
	500	0.00[b]	0.00[c]
	100	100.00[a]	113.67[b]
Control	0	100.00[a]	150.00[a]

Means with the same letter of superscript are not significantly different with each other at 5% level of significance using DMRT

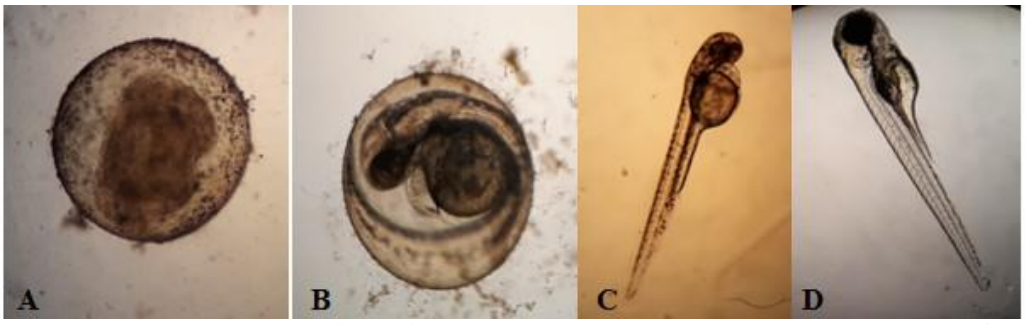

Figure 1: Toxic and growth retardation effects of *D. amoena* leaf extract: (A) coagulated embryo at 500 µg/ml 12 hour post treatment application (hpta); (B) Unhatched embryo at 500 µg/ml 72 hpta; (C)normal hatched zebrafish at 100 µg/ml 48 hpta, and(D)control normal hatched zebrafish 48 hpta

Table 3: Mortality of brine shrimp nauplii after 24 hours and the LC_{50} of the extract

Extract	Concentration(µg/ml)	Mortality(%)	LC_{50} (µg/ml)
D. amoena leaves	10000	100.00[a]	1190
	5000	100.00[a]	
	1000	26.67[b]	
	500	13.33[c]	
	100	6.67[c]	
Control	0	0.00[d]	

Means with the same letter of superscript are not significantly different with each other at 5% level of significance using DMRT

CONCLUSION

In conclusion, *D.amoena* leaf water exhibits embryo-toxic and teratogenic effects (delayed growth) in zebrafish. However, this extract is considered non-toxic in brine shrimp nauplii based on the toxicity rating.

REFERENCES

[1]Pohl R. 1961. Poisoning by *Dieffenbachia*. Journal of the American Medical Association, 177, 812–813.

[2]Drach G, Maloney W. 1963. Toxicity of the common houseplant *Dieffenbachia* report of a case. Journal of the American Medical Association, 183, 1047-1048.

[3] Sevik H, Cetin, M,Guney K, Belkayali N. 2018. The effect of some indoor ornamental plants on CO2 levels during the day. Pol. J. Environ. Stud., 27, 839-844.

[4] Liu YJ, Mu YJ, Zhu YG, Ding H, Arens NC. 2007. Which ornamental plant species effectively remove benzene from indoor air? Atmospheric Environment, 41, 650–654.

[5]Arditti J, Rodriguez E. 1982. *Dieffenbachia*: uses, abuses and toxic constituents. Journal of Ethnopharmacology, 5, 293-302.

[6] Bruni I, De Mattia F, Galimberti A, Galasso G, Banfi E, Casiraghi M, Labra M. 2010. Identification of poisonous plants by DNA barcoding approach. Int. J. Legal Med., 124, 595–603.

[7] Walter WG, Khanna PN. 1972. Chemistry of the aroids I. *Dieffenbachia* *seguine*, *amoena*, and *picta*. Economic Botany, 26, 364-372.

[8]David C, Angeles A, Angoluan R, Santos J, David E, Dulay R. 2016. *Moringa oleifera* (malunggay) water extracts exhibit embryo-toxic and teratogenic activity in zebrafish (*Danio rerio*) embryo model . Der Pharmacia Lettre, 8(7), 163-168.

[9]Romagosa C, David E, Dulay R. (2016). Embryo-toxic and teratogenic effects of *Tinospora cordifolia* leaves and bark extracts in zebrafish (*Danio rerio*) embryos. Asian Journal of Plant Science and Research, 6(2), 37-41.

[10]De Vera JS, De Castro MEG, Dulay RMR. 2016. Phytochemical screening and teratogenic effect of lyophilized water extracts from *Ocimum sanctum* L. (Holy Basil) and *Tamarindus indica* L. (Tamarind) leaves in *Danio rerio* embryos. Der Pharma Chemica, 8(8), 86-91.

[11]Tolentino J, Undan J. 2016. Embryo-toxicity and teratogenicity of *Derris elliptica* leaf extract on zebrafish (*Danio rerio*) embryos. International Journal of Pure and Applied Bioscience, 4(3), 16-20.

[12] Ali N. 2007. Teratology in zebrafish embryos: a tool for risk assessment. Swedish University of Agricultural Sciences, Uppsala.

[13]Selderlaghs I, Rompany A, De Coen W, Witters H. 2009. Development of screening assay to identify teratogenic and embryotoxic chemical using zebrafish embryo. Reproductive toxicology, 28, 309-328.

[14]Laale H. 1977. The biology and use of zebrafish, *Brachydanio rerio*, in fisheries research. Jounal of Fish Biology, 10, 121-173.

[15]Chen J, Fishman M. 1996. Zebrafish tinman homolog demarcates the heart field and initiates myocardial differentiation. Journal of Development and Advances in Developmental Biology as stem cells, 122, 3809-3816.

[16]Strähle a U. 2012. Zebrafish embryos as an alternative to animal experiments- a commentary on the definition of the onset of protected life stages in animal walfare regulations. Reproductive Toxicology, 33(2), 128-132.

[17]Cheng SH, Wai AWK, So CH, Wu RSS. 2000. Cellular and molecular basis of cadmium- induced deformities in zebrafish embryos. Environmental Toxicology and Chemistry, 19(12), 3024-3031.

[18] De Castro MEG, Dulay RMR. 2015. Toxic and teratogenic effects of *Lentinus sajor-caju* and *Pleurotus ostreatus* ethanol ethanolic extracts in *Danio rerio* embryo model. International Journal of Biology, Pharmacy and Allied Sciences. 4(4), 2261-2269.

[19]Wu C. 2014. An important player in brine shrimp lethality bioassay: the solvent. Journal of Advance Pharmaceutical Technology and Research, 5(1), 51-58.

[20] Eguchi F, Watanabe Y, Zhang J, Miyamoto K, Yoshimoto H, Fukuhara T, Higaki M. 1999. Inhibitory effects of hot water extract from *Agaricus blazei* fruiting bodies (CJ-01) on hypertension development in spontaneously hypertensive rats. Journal for Traditional Medicine, 16, 201-207.

[21]Jose BV. 2016. Toxicity and teratogenicity of *Grania mangostana* extracts on zebrafish (*Danio rerio*) developing embryos. Unpublished Undergraduate Thesis. Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines.

[22]Dulay RMR, Kalaw SP, Reyes RG, Cabrera EC. 2014. Embryo-toxic and teratogenic effects of Philippine strain of *Lentinus tigrinus* (tiger sawgill basidiomytes) extract on zebrafish (*Danio rerio*) embryos. Annals of Biological Research, 5(6), 9-14.

[23] Thomas J. 2000. The Zebrafish Book, 4th edition, University of Oregon. USA.

[24]Nagel R. 2002. DarT: the embryo test with the zebrafish (*Danio rerio*) and general model in exoticology and toxicology. ALTEX, 19, 38-48.

[25]Olowa L, Nuñeza O. 2013. Brine shrimp lethality assay of the ethanolic extracts of three selected species of medicinal plants from Iligan City, Philippines. International Research Journal of Biological Sciences, 2(11), 74-77.

[26]McLaughlin J, Chang C, Smith D. 1991. Bench top" bioassay for the discovery of bioactive natural products: an update. Studies in Natural Products Chemistry, 9, 383-409.

[27]Clarkson C, Maharaj VJ, Crouch N, Grace O, Pillay P, Matsabisa MG, Bhagwandin N, Smith PJ, Folb PI. 2004. In vitro antiplasmodial activity of medicinal plants native to or naturalised in South Africa. Journal Ethnopharmacology, 92, 177-191.

[28]Jose B, Dulay RMR, David ES. 2016. Toxic and teratogenic assessment of Mangosteen (*Garcinia mangostana* L.) leaves and stem-bark lyophilized water extracts in zebrafish (*Danio rerio*) embryos. Advances in Environmental Biology, 10(5), 96-101.

[29] Dulay RMR, De Castro MAG. 2017. Embryo-toxic and teratogenic activities of lyophilized water extracts of *Persea americana* Mill. and *Syzygium cumini* (L.) skeels leaves in *Danio rerio*. International Journal of Biology, Pharmacy and Allied Sciences, 6(3), 504-513.

[30] Viloria MJT, Dulay RMR, Waing KGD. 2017. Biological activities of *Morus alba* L. leaves (Alfonso variety). International Journal of Biology, Pharmacy and Allied Sciences, 6(9), 1629-1636.

[31]Kivçak B, Mert T, Öztürk H. 2001. Antimicrobial and cytotoxic activities of *Ceratonia siliqua* L. extracts. Turk J Biol, 26, 197-200.

[32] De Pasquale RC, Ragusa S, Circosta C, Forestieri AM. 1984. Investigations on *Diffenbachia amoena* Gentil. I: endocrine effects and contraceptive activity. Journal of Ethnopharmacology, 12(3), 293-303.

[33]Santos MMR, Agpaoa AR, Sayson AD, De Castro MAG, Dulay RMR. 2017. Toxic and teratogenic effects of water leaf extract of *Momordica charantia* in zebrafish (*Danio rerio*) embryos. Der Pharma Chemica, 9(6), 119-122.

[34]Romagosa C, David E, Dulay RMR. 2016. Embryo-toxic and teratogenic effects of *Tinospora cordifolia* leaves and bark extracts in zebrafish (*Danio rerio*) embryos . Asian Journal of Plant Science and Research, 6(2), 37-41.

[35] Wang S, Liu K, Wang X, He Q, Chen X. 2010. Toxic effects of celastrol on embryonic development of zebrafish (*Danio rerio*). Drug and Chemical Toxicology, 34(1), 61-65.

[36]Yumnamcha T, Roy D, Damanyati Devi M, Nongthomba U. 2015. Evaluation of developmental toxicity and apoptotic induction of the aqueous extract of *Milettia pachycarpa* using zebrafish as model organism. Toxicological & Environmental Chemistry, 97(10), 1363-1381.

[37] Cafirma EP, De Leon GA, Garcia RAMV, Miguel CM, Milandres JJB, Waing KGD, Dulay RMR. 2018. Phytochemical compositions and biological activities of leaf extract of oregano (*Origanum vulgare*). International Journal of Biology, Pharmacy and Allied Sciences, 7(5), 792-801.

[38] Serrano JLD, Dulay RMR, Waing KGD. 2018. Biological activities of leaf extracts of *Morus* sp. S54 variety. International Journal of Biology, Pharmacy and Allied Sciences, 7(3), 370-377.

[39]Tan P, Mezui C, Orock G, Njikam N, Dimo T, Bitolog P. 2008. Teratogenic effects, acute and sub chronic toxocity of the leaf aqueous extract of *Ocimun suave* wild (Lamiaceae) in rats. Journal of Ethnopharmacology, 115, 232-237.

[40]Oloyede G. 2011. Chemical composition, toxicity, antimicrobial and antioxidant activities of leaf and stem essential oils of *Dieffenbachia picta* (Araceae). European Journal of Scientific Research, 49(4), 567-580.

[41]Siti Farah H, Nazlina I, Yaacob WA. 2013. Biological activities of aqueous extract from *Cinnamomeum porrectum*. AIP Conference Proceedings, 1571, 250-253.

[42] Batista D, Falé PL, Serralheiro ML, Araújo ME, Dias C, Branco I, Grosso C, Coelho J, Palavra A, Madeira PJA, Martins A, Rauter AP. 2017. Phytochemical characterization and biological evaluation of the aqueous and supercritical fluid extracts from *Salvia sclareoides* Broth. Open Chemistry, 15, 82-91.

[43]Adeogun O, Adekunle A, Ashafa A. 2016. Chemical composition, lethality and antifungal activities of the extracts of leaf of *Thaumatococcus daniellii* against foodborne fungi. Beni-Suef University Journal of Basic and Applied Sciences, 5(4), 356-368.