Science World Journal Vol 13(No 4) 2018
www.scienceworldjournal.org
ISSN 1597-6343
Published by Faculty of Science, Kaduna State University

Full Length Research Article

PHYTOCHEMICAL, PROXIMATE AND SEDATIVE PROPERTIES OF HENNA (*LAWSONIA INERMIS*) ON THE OPERCULA VENTILATION RATE OF *TILAPIA ZILLI* FINGERLINGS

Audu, B.S[1]., Yusuf, S[1]., Malachy, N.O.A[2]., Jamiu,O[3]. & Wade, J. W[1]

[1]Department of Zoology, Faculty of Natural Sciences, University of Jos. Nigeria
[2]Department of Fisheries and Aquaculture Technology, Federal University of Technology, Owerri, Nigeria.
[3]Department of Anatomy, Faculty of Veterinary Medicine, University of Jos. Nigeria

*Corresponding Author Email Address: audusambo@yahoo.com

ABSTRACT
The high cost of conventional anesthetics and the deleterious effects of prevailing chemical sedatives used in aquaculture have justified the search for natural plant alternative that is cosmopolitan, biodegradable, less toxic with short withdrawal period, and economically affordable. The Phytochemical, proximate and sedative properties of the aqueous crude leaf extract of *Lawsonia inermis* and its effect on the opercula ventilation rate in *Tilapia zilli* fingerlings were conducted using standard procedures. Mixed sexed fingerlings of *T. zilli* of mean weight and length of 42.64 ± 0.82 g and 11.14 ± 0.22 cm respectively were randomly distributed in batches of five fish/per experimental tank. The tank with no test material (0.00g/L) served as the control; while, tanks with test materials at concentrations of 1.50, 2.00, 2.50, 3.00 and 3.50g/L served as test tanks. The qualitative phytochemical analyses of *L. inermis* revealed the presence of varying proportions of alkaloid, tannin, saponins, cardiac glycosides phenolic and resins, while, the proximate composition includes moisture content (33.2%), crude lipid (12.0%), ash (29.9%), crude fibre (21%), crude protein (3.38%) and nitrogen free extracts (0.52%). There was significant ($p<0.05$) dose- dependent increase in the induction of sedation and recovery time of *T. zilli* exposed to *L. inermis* aqueous crude Leaf extract as well as marked dose-related decrease in the opercula ventilation rate compared to the control group. No mortality was recorded at low concentrations of 1.50-2.50g/L of the plant extract; in contrast with 50 and 100% mortalities in concentrations of 3.00 and 3.50g/L respectively. In conclusion, the present investigation revealed that *L. inermis* aqueous crude leaf extract seemed to contain diverse phytochemical constituents that caused sedation with adverse consequences on fish opercula ventilation.

Keywords: Qualitative analyses, Crude content, Henna, Respiratory rate, Freshwater Fish

INTRODUCTION

Modern aquaculture operations involve the processes of transporting, handling, tagging and weighing of fishes. These pose a lot of stress resulting to decreased performance, immune suppression, increased susceptibility to diseases and mortality (Gabriel & Akinrotimi, 2011). Opercula ventilation rate is an index of gaseous exchange and a quick pointer of stress conditions thereby acting as an overall pointer of the state of wellbeing in fishes. To alleviate stress, sedatives which are substances that have calming and relaxing effects are used (Alnamer *et al*., 2011; Trushenski *et al*., 2013). Although, conventional sedatives are expensive and scarce in countries like Nigeria (Akinrotimi *et al*., 2013), several efforts are ongoing to find suitable natural alternatives that would replace the synthetic chemical sedatives which are known to be toxic, limited in supply and expensive (Audu *et al*., 2013). The leaf extract of *L. inermis* could be a potential natural replacement to the chemical sedatives in vogue (Reference). The plant commonly known as *Lalle* and *Laaliin* in Hausa and Yoruba tribes of Nigeria respectively (Zumrutdal & Ozaslan, 2012) is a perennial shrub that belongs to the family Lythracae (Manikandan *et al*., 2011). It has been traditionally used to cure headache, hemicranias, lumbago, bronchitis, boils, ophthalmic and syphilis (Borade *et al*., 2011). According to Kaur *et al.* (2014), the leaf extract of *L. inermis* were observed to have sedative effects on albino rats and have also been reported to exhibit immunostimulatory and disease resistance effects against *Aphanomyces invadans* infection in *Channa striatus* (Uthayakumar *et al.,* 2014) with concomitant antihelminthic and antibacterial activities (Sarojini *et al*., 2012; Abulyazid *et al*., 2013). This study investigated the phytochemical, proximate and sedative properties of the aqueous crude leaf extract of *L. inermis* and attendant effects on the opercula ventilation rate in *T. zilli* fingerlings.

MATERIALS AND METHODS

Fish source and acclimatization

Mixed sexes of *T. zilli* fingerlings (mean weight and length of 42.64 ± 0.82 g and 11.14 ± 0.22 cm respectively) were purchased from Panyam Fish Farm in Mangu Local Government Area, Plateau State, Nigeria. The fingerlings were transported to the Hydrobiology and Fisheries Laboratory Unit of University of Jos, Jos Nigeria in oxygenated polythene bag containing water from the fish pond. On arrival, fish were transferred into 35L capacity aquarium containing 25 liters of borehole water and allowed to acclimatize to laboratory conditions for a period of one week. During this period, the fingerlings were fed to satiation with a commercial fish feed (Vital feed®) twice daily at 0800 and 1600 hours. Three quarter (3/4) of water in the tanks were siphoned out daily to remove leftover food and faecal matter and replaced with clean borehole water. Dissolved oxygen was supplied through the use of a giant aerator (Resun® air-pump (AC-9906), China) throughout acclimatization period.

Plant source and experimental preparation
Lawsonia inermis plant leaves were collected from Dull village in Tafawa Balewa Local Government Area, Bauchi State, Nigeria. The plant was identified and authenticated by a curator in the Herbarium of Plant Science and Biotechnology of University of Jos, Nigeria, where the voucher specimen (UJH17000278) was kept. The leaves were kept off the plant, cleaned and air dried to a constant weight (500 g) in the Laboratory. The aqueous leaf extract of *L. inermis* was prepared by pulverizing the dried leaves (500 g) using a laboratory mortar and pestle into powder and sieved using 30µm mesh size sieve.

Phytochemical analyses of *L. inermis* Leaves
The macerated aqueous leaf extract of *L. inermis* was used for the phytochemical screening of alkaloids, flavonoid, tannin, saponin, trepenes and steroids, phenols, resins and cardiac glycosides, using standard qualitative procedures (Sofowora, 1982; Trease & Evans, 1989).

Proximate compositions of *L. inermis* Leaves
The moisture, crude lipid, ash, crude protein and crude fibre contents and the nitrogen free extract of the aqueous leaf extract of the *L. inermis* were determined as described by AOAC (1990). The values obtained are percentages of dry weight.

Preparation of Stock Solution
Stock solution of the crude leaf extract was prepared according to the method described by Audu *et al.* (2013). Thereafter, definitive concentrations of 1.50 2.00 2.50, 3.00, and 3.50g/L were obtained and used for the sedation study.

Experimental design
The fingerlings of *T. zilli* were randomly divided into six groups of five fish/per experimental tank each duplicate replicated described as follows:

Group I (Control; 0.00mg/L): *T. zilli* fingerlings in this group were not exposed to the aqueous crude leaf extract of *L. inermis.*

Groups II-VI (treatment): These groups contained *T. zilli* fingerlings that were exposed to varied concentrations (1.50, 2.00, 2.50, 3.00 and 3.50 g/L) of *L. inermis*. Thereafter, the times taken for the induction of sedation, change in opercula ventilation rate and the recovery of *T zilli* from the sedative effects of *L. inermis* in clean and aerated tanks were monitored.

Induction of sedation, recovery and opercular ventilation
Induction, recovery and change in opercula ventilation rate during the sedation experiment were measured as described by Agokei & Adebisi, (2010) and Audu *et al*. (2013) respectively. The induction time was determined as the period between the introduction of fish to the varied concentrations of the aqueous leaf extract of *L inermis* and the time taken by the fish not to respond to external stimuli from probe of a glass rod. Also, the recovery time from sedation was evaluated to be time taken for the fish to regain external stimuli when removed from the tanks with graded concentrations of the plant extract to clean water (Agokei & Adebisi, 2010). Opercula ventilation rate (OVR/min) in all the treatments and control was measured using a stop watch as the fish progresses in the various sedative concentrations of *L inermis* leaf extract (Audu *et al*.,2013).

Mortality rate
The mortality rate of *T. zilli* fingerlings treated with graded levels of aqueous crude leaf extract of *L inermis* was determined by close examination of the fingerlings after 24hours in well aerated tanks. Specifically, fish were considered dead when they failed to respond to probe from a glass rod and by having the fingerlings floated or sink to the bottom of the tank.

Physicochemical parameters
Water quality parameters of the experimental tanks namely, temperature, pH, alkalinity, dissolved oxygen and free carbon (IV) oxide were monitored following the procedures described in APHA (1998).

Date Analysis
The results obtained for the water quality parameters and opercula ventilation rate before and after sedation were subjected to Analysis of Variance (ANOVA) (Single classification) at 5% level of probability using SPSS version 16.0.

RESULTS

Phytochemical Composition
The phytochemical screening of the aqueous crude leaf extract of *L. inermis* indicated strong (+++) presence of bioactive substances such as tannins, phenols and balsam while, components such as alkaloid, flavonoids, saponins, cardiac glycosides and resins were weakly (+) present as shown in Table 1.

Table I: Qualitative Phytochemical Composition of Aqueous Crude Leaf Extract of *L. inermis* Obtained from Dull Village, Tafawa Balewa Local Government Area, Bauchi State Nigeria

Bioactive substances	Colour	Remarks
Alkanoid	Light orange	+
Flavanoids	Light yellow	+
Tannins	Greenish blue	+++
Saponins	Froth	+
Cardiac glycoside	Reddish brown	+
Balsam	Dark green	+++
Phenol	Deep blue	+++
Resins	Light violet	+

Key: +++ Strong, ++ Moderate, +Mild

Proximate values
The proximate value of the aqueous crude leaf extract of *L. inermis* showed that the plant extract had moisture content of 33.2%, crude lipid (12%), ash (29.9%), fibre (21%) and crude protein content of 3.38% while the calculated value of nitrogen free extract was found to be 0.52% (Table 2).

Table 2: Proximate Composition (mg/100g) of the Leaf of *L. inermis*

Constituent	Mean % composition of *L. inermis*
Moisture content	33.2
Crude lipid	12.0
Ash content	29.9
Fibre	21.0
Protein	0.99
NFE	0.52

NFE: Nitrogen Free Extract

Induction, recovery and mortality rate

The induction, recovery and mortality rate of *T. zilli* exposed to sedative concentrations of the crude aqueous Leaf extract of *L. inermis* are represented in Table 3.

Table 3: Mean Induction and Recovery Time of *T. zillii* Fingerlings Exposed to Aqueous Crude Leaf Extract of *L. inermis*

Concentration 24hours (g/L)	Induction Time (mins)	Recovery Time (mins)	Percentage Mortality After Sedation (%)
1.5	18.80 ± 0.98^{a}	5.00 ± 0.65^{a}	0
2.0	17.20 ± 0.35^{a}	6.10 ± 0.52^{a}	0
2.5	11.60 ± 1.02^{b}	8.00 ± 0.37^{b}	0
3.0	8.10 ± 0.86^{c}	10.10 ± 0.69^{c}	50
3.5	6.30 ± 0.39^{d}	12.40 ± 0.52^{d}	100

Values tagged with different alphabet superscripts are significantly different at P=0.05

The induction time decreased significantly ($p<0.05$) with increased concentrations of the leaf extract. The shortest induction time (6.3±0.39mins) was observed at the highest concentration (3.50g/L) of *L. inermis* aqueous crude leaf extract, while the longest induction time (18.8±0.98mins) was recorded at the lowest concentration (1.50g/L). The recovery time of the *T. zilli* fingerlings increased significantly with increased concentration of the crude leaf extract, with the evidence of longest recovery time (12.4±0.52mins) at 3.50 g/L concentration and a corresponding shortest (5.0±0.65mins) recovery period at concentration of 1.50g/L. *T. zilli* fingerlings given low concentrations (1.50-2.50 g/L) of the experimental plant extract did not record mortality. However, fishes exposed to higher concentrations of 3.00 and 3.50mg/L had corresponding mortalities of 50 and 100% respectively.

Change in opercular ventilation rate

The mean changes in opercular ventilation rates before and after sedation (OVRBS and OVRAS) are presented in Table 4.

Table 4: Mean Opercular Ventilation Rate (Beat /Mint) before and After Sedation of *T. zillii* Fingerlings Exposed to Crude Leaf Extract of *L. inermis*.

Concentration 24hours (g/L)	OVRBS (Beat/Min)	O V R A S (Beat /Min)	C O V R	% COVR
0.00	82.40 ± 0.50	82.20 ± 1.30^{c}	0.2	0.33
1.50	82.60 ± 0.81	76.80 ± 1.36^{bc}	5.8	9.57
2.00	83.20 ± 1.02	71.80 ± 1.36^{bc}	11.4	18.81
2.50	82.20 ± 0.86	71.00 ± 4.09^{ab}	11.2	18.48
3.00	82.00 ± 0.89	66.80 ± 2.03^{a}	15.2	25.08
3.50	83.20 ± 0.80	66.40 ± 2.22^{a}	16.8	27.72

Values tagged with different alphabet superscripts are significantly different at P=0.05

O V R B S: Opercular Ventilation Rate Before Sedation (Beat/mins); O V R B S: Opercular Ventilation Rate After Sedation (Beat/mins); C O V R: Change in Opercular Ventilation Rate (Beat/mins)

There was no significant difference ($p>0.05$) in opercular ventilation rate before sedation OVRBS (beat/mins) in the fish exposed to graded concentrations of the aqueous crude leaf extract of *L. inermis* when compared to the control group. Contrastingly, OVRAS values in fish exposed to graded concentrations of the extract showed a significant ($p<0.05$) dose-dependent decrease relative to the control group.

Physico-chemical parameters

The results of water physico-chemical parameters (Table 5) of the experimental tanks recorded during the study indicated that water in the tanks treated with aqueous crude leaf extract of *L. inermis* showed a significant ($p<0.05$) dose-dependent increase in temperature, most especially in tanks containing high concentrations (3.0 and 3.5 g/L) of the plant extract relative to the others.

Table 5: Mean Physico-Chemical Parameters of Holding Tanks for the Sedation of *T. zilli* Fingerlings with Aqueous Crude Leaf Extract of *L. inermis*

Concentration (g/L)	Temperature (0°C)	pH (mg/L)	O_2 (mg/L)	CO_2 (mg/L)	Alkalinity
0.00	22.0±1.00^{d}	25.05±0.05	7.15±0.05^{a}	3.85±0.15^{a}	8.150±1.50^{b}
1.50	28.0±0.10^{c}	25.05±0.05	5.15±0.05^{c}	2.05±0.06^{b}	5.250±2.50^{c}
2.00	35.5±1.50^{b}	25.05±0.15	5.25±0.05^{c}	1.85±0.05^{b}	6.25±2.508^{c}
2.50	36.0±1.50^{b}	25.15±0.25	4.75±0.05^{d}	1.65±0.05^{b}	6.40±1.0^{c}
3.00	56.5±0.50^{a}	25.25±0.10	4.65±0.05^{d}	1.15±0.15^{c}	8.250±2.50^{b}
3.50	56.5±0.50^{a}	25.20±0.01	6.50±0.50^{b}	0.0±0.0.0^{d}	10.25±2.5^{a}

Values tagged with different alphabet superscripts are significantly different at P=0.05

There was no significant difference ($p>0.05$) in the pH levels of all the test concentrations relative to the control. The dissolved oxygen levels significantly decreased ($p<0.05$) with increasing concentrations of the extract. However, the dissolved oxygen content in the 3.5 g/L tank markedly increased relative to other test concentrations. Free carbon dioxide (Free CO_2) levels showed a progressive dose-dependent significant decrease ($p<0.05$) relative to the control. Similarly, alkalinity levels

significantly ($p<0.05$) decreased with increased concentrations of the extract relative to the control group.

DISCUSSION

The physico-chemical properties of the water in various experimental tanks were similar to the report of Manikandan *et al.* (2011) while induction of sedation of sedation and recovery and opercular ventilation rates of the fish exposed to the graded concentrations of the extract exhibited dose-dependent alterations which were similarly reported by Agokei and Adebisi (2010) using *Nicotina tabacum*; Becker (2013) using *Condolia buxifolia* and Anju *et al.* (2015) with *Tephrosia vogeliasa*.

The presence of bioactive compounds (tannins and phenol) in appreciable quantities in the *L. inermis* leaves could be responsible for the observed deranged opercular ventilation rate elicited by the graded levels of the leaf extract. Studies (Ufodike & Omoregie, 1994; Van Andel, 2000; Singh & Singh, 2002) have shown that the excessive presence of bioactive substances in animal's body can be poisonous or precipitate sedation. This finding on bioactive substances corroborate the reports of Audu *et al*. (2013) and Wagini *et al*. (2014) on the closely related phytochemicals incriminated in precipitating similar impaired respiration rates and responsible for the induction of sedation in the test fish.

The percentage composition of protein (3.38%), observed in this study appeared to be slightly lower relative to the standard recommendation of 25 – 35 % (Jauncey & Ross, 1982). The functional implication is that the plant may not be suitable for inclusion in fish feed. On the contrary, Chaudhary *et al*. (2010) reported on the proximate analysis of seed of *L. inermis* and established that the seed had considerable higher values of protein (5.00%) and fibre (33.5%) with lower value (10-11%) of fatty acid, which contrast the leaf component, with protein as 3.38%, and fibre content of 21.01% while higher crude lipid content of 12.00% was recorded in this study.

Generally, comparative evaluation of the proximate analysis of *L. inermis* with few other shrubs and plants of economic importance revealed that *L. inermis* has higher moisture content (33.2%) as against *Datura innoxia* (7.5%) (Ayuba, 2004); *Cannabis sativa* (6.87%) Audu *et al*. (2014) and *Manihot esculenta* (11.97%) *Ipoma batata* (10.89%) and *Colopia esculenta* (7.08%) (Mzengereza *et al.,* 2014). This comparative edge on moisture content could be an advantage because *L. inermis* leaves can easily be digested and absorbed by body system and also enhances elimination of digestive waste from the body (Ngaha *et al*., 2016).

The high ash content (29.9%) value suggests that the leaves of *L. inermis* are rich in mineral elements. The value obtained in this study was higher compared to those reported for the leaves of *C. sativa* (11.8 %) (Audu *et al*., 2014) Cassava (13.6 %) and Cocoa yam (14.84 %) but lower than the ash content in sweet potato (85.75 %) leaves reported by Mzengereza *et al*. (2014). In addition, the high value of fibre (21.0%) recorded is indicative of the leaves richness in structural carbohydrate. Comparatively, this value is higher when compared to the fibre contents reported for *C. sativa* (18.96%) (Audu *et al*., 2014); Cassava (16.35%) and Cocoa yam leaves (Mzengereza *et al*., 2014). The nitrogen free extract (0.52%) in *L. inermis* crude leaf extract obtained is comparatively lower to the value documented for the leaves of *C. sativa* (19.25%) (Audu *et al*., 2014).

The physico-chemical parameters including temperature, pH level, dissolved oxygen content, alkalinity and free carbon dioxide are crucial for estimating water quality, as they provide important information on the suitability of water for optimum growth of aquatic animals (Djukic *et al.,* 1994; Ehiagbonare & Ogunrinde, 2010; Bhavimani & Puttaiah, 2014). The observed progressive decrease in dissolved oxygen and alkalinity levels and the accompanied rise in free carbon dioxide content could be due to overload of the plant bioactive substances in the test tanks.

The progressive dose-dependent decrease in the induction and increase in the recovery time from sedation in fish exposed to water treated with *L. inermis* is probably due to diverse factors that include the chemical nature of the plant, species of fish and exposure route, as earlier reported (Ferreira *et al*., 1984; Ross, 2001; Harms, 2003). The effects of the crude leaf extract of *L. inermis* on the sedation and opercula ventilation rate of *T. zilli* fingerlings, were similar with those reported by Akinrotimi *et al.* (2013); Audu *et al*. (2013) and Cheikyula *et al*. (2015) when they exposed fish to crude leaf extracts of *T. vogelii* and *C. sativa* and the seed extracts of *L. falcipinus* respectively.

The significant dose-dependent decrease in opercula ventilation rate observed in this study implies that the operculum ventilation beat is prone to alteration with increasing dose of the plant leaf extract, which could precipitate a respiratory distress condition. This finding agrees with the report of Audu *et al*. (2013) when they similarly observed dose-dependent induced respiratory stress in fish intoxicated with aqueous crude leaf extract of *C. sativa*. It is plausible that mortality rate in fish could be influenced by reduced opercula ventilation rate. Thus, the varying mortality rates observed in the fish exposed to the higher concentrations of *L. inermis* could be attributable to the decreased opercular ventilation rate recorded. This finding collaborates with mortality rate recorded when *Oreochromis niloticus* fingerlings were anaesthetized with aqueous extract of tobacco (Agokei & Adebisi, 2010).

Conclusion: This study has demonstrated that the aqueous crude leaf extract of *L. inermis* contained appreciable levels of moisture, crude lipid, ash and fibre contents, with accompanied reduction in the levels of protein and nitrogen free extract and bioactive compounds with great sedative efficacy that markedly influence the opercula ventilation rate of *T. zilli* fingerlings.

REFERENCES

Abulyazid, I., Mahdy, E. M. and Ahmed, R. M. (2013). Biochemical study for the effect ofhenna (*Lawsonia inermis*) on *Escherichia coli*. *Arabian Journal of Chemistry*, *6*(3): 265 – 273.

Agokei, O. E. and Adebisi, A. A. (2010). Tobacco as an anesthetic for fish handling procedures. *Journal of Medicinal Plants Research*, 4(14): 1396 – 1399.

Akinrotimi, O.A., Edun, O.M and Dan, M.E (2013). "Effects of Clove Seed as Anaesthetic Agents in Two Species of Grey Mullets *Liza falcipinnis* and *Liza grandisquamis*." *Journal of Aquatic Science* 1.1 (2013): 7-10.

Alnamer, R., Alaoui, K., Bouidida, E. H., Benjouad, A. and Cherrah, Y. (2012). Sedative and hypnotic activities of the methanolic and aqueous extracts of *Lavandula officinalis* from Morocco. *Advances in Pharmacological Sciences*, 2012*:* doi:10.1155/2012/270824.

Anju, T. D., Solomon, S. G. and Cheikyula, J. O. (2015). Effects of aqueous leaf extract of *Tephropsia vogeli* as a tranquilizer on the African catfish *Heterobranchus longifilis* Val. (Pisces 1840).

American Journal of Research Communication, 3(6): 45 – 59.

AOAC. (1990). *Official Methods of Analysis.* Association of Official Analytical Chemists, Arlington, VA, USA.

APHA. (1998). *Standards Methods for Examination of Water and Wastewater*. American Public Health Association (APHA), Washington DC, USA.

Audu, B. S., Adamu, K. M. and Ufodike, E. B. C. (2013). Behavioural response and opercular ventilation rate of Nile tilapia (*Oreochromis niloticus*) fingerlings after anesthesia with crude leaf extract of marijuana (*Cannabis sativa*). *Applied Science Research Journal*, 2(2): 67 – 78.

Audu, B.S., Ofojekwu, A., Ujah, A and Ajima, M.N.O (2014). Phytochemical, proximate composition, amino acid profile and characterization of Marijuana (*Cannabis sativa* L). *Journal of Phytopharmacology,* 3(1): 35-43.

Ayuba, V. O. (2004). *Toxicities and Sedative Effects of Toloache, Datura innoxia Miller, on the African Catfish Clarias gariepinus Burchell, Fingerlings*. Ph.D Thesis, Department of Zoology, University of Jos, Jos, Nigeria.

Becker, A. G., Cunha, M. A. D., Garcia, L. D. O., Zeppenfeld, C. C., Parodi, T. V., Maldaner, G., Morel, A. F. and Baldisserotto, B. (2013). Efficacy of eugenol and the methanolic extract of *Condalia buxifolia* during the transport of the silver catfish *Rhamdia quelen*. *Neotropical Ichthyology*, 11(3): 675 – 681.

Bhavimani, H. and Puttaiah, E. T. (2014). Fish culture and physico-chemical characteristics of Madikoppa pond, Dharwad Tq/Dist, Karnatak. *Hydrology: Current Research*, *5*(1): 162.doi: 10.4172/2157-7587.1000162.

Borade, A. S., Kale, B. N. and Shete, R. V. (2011). A phytopharmacological review on *Lawsonia inermis* (Linn *International Journal of Pharmacy and Life Sciences*, 2(1): 536 – 541.

Chaudhary, G., Goyal, S. and Poonia, P. (2010). *Lawsonia inermis* Linnaeus: a phytopharmacological review. *International Journal of Pharmaceutical Sciences and Drug Research*, *2*(2): 91 – 98.

Cheikyula, J. O., Solomon, S. G. and Anju, T. D. (2015). Evaluation of the anaesthetic properties of freeze-dried leaf extract of *Tephrosia vogelii* on *Heterobranchus longifilis* Val. (Pisces: 1846). *American Journal of Research Communication*, 3(6): 127 – 141.

Djukic, N., Maletin, S., Pujin, V., Ivanc, A. and Milajonovic, B. (1994). Ecological assessment of water quality of Tisze by physico-chemical and biological parameters. *Tisca Szeged*, 28(1): 37 – 40.

Ehiagbonare, J. E. and Ogunrinde, Y. O. (2010). Physico-chemical analysis of fish pond water in Okada and its environs, Nigeria. *African Journal of Biotechnology*, 9(36): 5922 – 5928.

Ferreira, J. T., Schoonbee, H. J. and Smit, G. L. (1984). The uptake of the anaesthetic benzocaine hydrochloride by the gills and the skin of three freshwater fish species. *Journal of Fish Biology*, 25(1): 35 – 41.

Gabriel, U. U. and Akinrotimi, O. A (2011). Management of stress in fish for sustainable aquaculture development. *Researcher*, *3*(4): 28 - 38.

Harms, C. A. (2003). Fish. Pages 2 – 20. *In:* Fowler, M. E. and Miller, R. E. (Eds.). *Zoo and Wild Animal Medicine*. Fifth Edition, Saunders, St. Louis, USA.

Jauncey, K. and Ross, B. (1982). *A Guide to Tilapia Feeds and Feeding.* Institute of Aquaculture, University of Sterling, Sterling, Scotland.

Kaur, M., Dangi, C.B., Singhai, A., Singh, M., Kosta, S., Singh, H., Peter, J. and Jain, S. (2014). Toxicity profile of ethanolic extract of Lawsonia inermis leaves in albino Wistar rats. *World Journal of Pharmacy and Pharmaceutical Sciences*, 3(5): 835 – 848.

Manikandan. A., Revathi. R., Merrine R., Geethanjali. S. and Poorni, K. E. (2011). Acute toxicity study of hydroethanolic leaf extracts of *Lawsonia inermis* and *Mangifera indica* L. on *Mugil cephalus* L. *International Journal of Pharmacy and Technology*, 3(1): 1768-1776.

Mzengereza, K., Singini, W., Msiska, O. V., Kang'ombe, J. and Kaputе, F. (2014). Proximate composition of potential plant feedstuffs for production of *Tilapia rendalli* in northern Malawi. Pages 415 – 418. *In: RUFORUM Fourth Biennial Conference,* Maputo, Mozambique, 19 – 25 July 2014.

Ngaha, N.M.I., Dahlan, I., Massoma, L.D., Mandengue, S., H and Yusuf, A.A. (2016). Comparative Proximate Analysis Of Leaves And Bark Of Alchornea Cordifolia (Euphorbiaceae). *Journal of Agriculture and Environmental Sciences.* 5(1): 84 -90.

ROSS, L. G. (2001). Restraint, anaesthesia and euthanasia. Pages 75 – 83. *In: BSAVA Manual of Ornamental Fish.* 2nd Edition, BSAVA, Gloucester.

Sarojini, N., Kanti, C. C., Priyanka, J. and Kumari, S. U. (2012). Correlation between phytochemical constituents and anthelminthic activity of *Lawsonia inermis* leaf extract. *International Journal of Research in Ayurveda and Pharmacy,* 3(4): 559 – 562.

Singh, D. and Singh, A. (2002). Piscicidal effect of some common plants of India commonly used in freshwater bodies against target animals. *Chemosphere*, *49*(1): 45 – 49.

Sofowora, E. A. (1982). *Medicinal Plants and Traditional Medicine in Africa.* John Wiley and Sons, New York.

Trease, G. E. and Evans, W. C. (1989). *Trease and Evans Pharmacognosy*. 13th Edition, Bailliere Tindall, London.

Trushenski, J. T., Bowker, J. D., Cooke, S. J., Erdahl, D., Bell, T., Macmillan, J. R., Yanong, R. P., Hill, J. E., Fabrizio, M. C., Garvey, J. E. and Sharon, S. (2013). Issues regarding the use of sedatives in fisheries and the need for immediate-release options. *Transactions of the American Fisheries Society*, 142(1): 156 – 170.

Ufodike, E. B. C. and Omoregie, E. (1994). Acute toxicity of water extracts of barks of *Balanites aegyptiaca* and *Kigelia africana* to *Oreochromis niloticus* (L.). *Aquaculture Research*, 25(9): 873 – 879.

Uthayakumar, V., Chandirasekar, R., Sreedevi, P. R., Senthilkumar, D., Jayakumar, R. and Ramasubramanian, V. (2014). Immunostimulatory effect and disease resistance induced by *Lawsonia inermis* against *Aphanomyces invadans* in striped murrels (*Channa striatus*). *Malaya Journal of Biosciences,* 1(4): 231 – 241.

Van Andel, T. (2000). The diverse uses of fish-poison plants in Northwest Guyana. *Economic Botany*, 54(4): 500 – 512.

Wagini, N. H., Soliman, A.S., Abbas, M. S., Hanafy, Y. A. and Badawy, E. M. (2014). Phytochemical analysis of Nigerian and Egyptian henna (Lawsonia inermis L.) leaves using TLC, FTIR and GCMS. *Plant*, *2*(3), 27 – 32.

Zumrutdal, E. and Ozaslan, M. (2012). Miracle Plant for the Herbal Pharmacy; Henna (*Lawsonia inermis*). *International Journal of Pharmacology*. 8 (6): 483-489.