Evaluation of The Anaesthetic Properties of Freeze-dried Leaf extract of *Tephrosia vogelii* on *Heterobranchus longifilis* Val. (Pisces: 1846).

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ABSTRACT

The present study was carried out to evaluate the the anaesthetic properties of the freeze-dried leaf extract of *Tephrosia vogelii* on the African catfish *Heterobranchus longifilis* juveniles (Mean weight 115.00±25.00g). The experimental fish were obtained from wild caught from the Benue River and transported in jerry cans to the fish farm of the Department of Fisheries and Aquaculture, University of Agriculture, Makurdi, Nigeria. The fish were acclimatize under laboratory condition for two weeks in plastic tanks of 70 litre capacity filled with 40 litres of water prior to the experiment. Each tank containing acclimatization water was aerated to enhance dissolved oxygen, and water was changed daily to prevent metabolic waste build up in order to maintain good water quality. Experimental fish were starved for 24 hours prior to sedation to prevent regurgitation from the gastro-intestinal tract (GIT). Four healthy *H. longifilis* were selected randomly from both control and treatment groups. Each fish was weighted and injected 0.05ml of the extract at concentrations of 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06g/l using No.23 needle and a 2ml heparinized syringe. Injection was done intramuscularly (IM} at the dorsal saddle just above the lateral line behind the operculum. Fish in the control group were injected with distilled water. Injected fish were observed for behavioural responses. The result showed that *H. longifilis* injected with the freeze-dried leaf extract of *T. vogelii* passed sequentially through the first three stages of anaesthesia but could not attain total loss of equilibrium (stage 4 of anaesthsia). Behavioural responses included mucus secretion, slow and erratic swimming, excrement discharge, increase in opercular beat rate, strong retension of reflex action, partial loss of equilibrium and colour change. The induction time decreased with increasing concentration of the anaesthetic extract. The recovery time followed the reverse order. The

opercular beat rates, both before and after sedation in the treatment groups showed increase over that in the control group and it increased with increasing concentration. The percentage increase in opercular beat rate did not show a definite pattern. Recovery time ranged from 506.67 minutes (8.45 hours) to 553.67 minutes (9.23 hours) increasing with increase in concentration of the anaesthetic solution. The effective concentration was 0.06g/l with an induction time of 49.33 seconds and a recovery time of 553.67 minutes. The of this study revealed that the freeze-dried leaf extract of *T. vogelii* can be used as a tranquilizer for transporting over long distances, biopsy and morphological evaluation.

Keywords: Anaesthetics, tranquilizer, freeze-dried, leaf, induction, recovery, time

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INTRODUCTION

Chemical anaesthetics have been used in the world's aquaculture to immobilize fish for 60 years. Brown,(2011). The sedative effect of anaesthetics reduce stress on the fish during handling procedures such as weighing, measurement, tagging, transportation, capture, breeding, morphological evaluation and surgical procedures. Anaesthetics also reduce excitement and hyperactivity-related trauma that occur during handling and thus directly reduces mortality and morbidity. Cooke *et al*,(2004); Harms (1999); Ross,(2001). However, the use of chemical anaesthetics has attendant problems or disadvantages such as poor solubility in water and long reduction time (quinaldine), acute toxicological effects at high concentrations and potential health risks on humans including development of rashes (2-phenoxyethanol), and scarcity and high cost especially in developing countries (Benzocaine and MSS-222). Marking and Meyer, (1987), FAO, (1997); Yanar and Kumijj, (2001); Solomon and Amali, (2004); Agokei and Adebisi, (2010).

On the other hand the use of natural plant extracts such clave oil (extracted from *eugenia caryophyllata*) plant as an anaesthetic has been shown to be cheaper, safer, biodegradable and more effective when compared to conventional chemical anaesthetics. Soto and Burhauddin, (1995). When the shortcomings, of chemical anaesthetic agents are considered in comparison to the relative advantages offered by anaesthetic plant extracts like clove oil, there is every need to intensify investigation into crude extracts of plants with apparently high anaesthetic potentials.

Tephrosia vogelii is one of the numerous pescicidal plants in Nigeria. It has long been used by the Tiv people of Benue State in central Nigeria where it is known as "Kuhwa indyar" to kill fish in natural bodies of water. *T. Vogelii* is a perennial shrub commonly found in tropical Africa –West Africa, Central Africa and Southern African sub-regions. It grows 3- 4 meters high, branching low and ramified (Michael, 2002).

The use of *T. Vogelii* varies from one part to another. In the Nigeria the leaves are widely and indiscriminately used to harvest fish in natural waters while in Central and Southern Africa it is used in the treatment of ecto-parasites in cattle. The roots are used for the treatment of tooth decay and rheumatism while the barn is used to treat boils. Mshana *et al*, (2000).

The leaves of *T. Vogelii* contain a number of different compounds commonly referred to rotenoids, which are highly effective poison against fish and insects. These compounds include rotenone, deguelin and tephrosin. McDavid and Lesseps, (1994). The rotenone is chemical that has pesticidal property with an acute toxicity of 133-1500 mg/kg. Gadzirayi *et al*, (2009).

MATERIALS AND METHODS

Fresh samples of *T. Vogelii* leaf were collected during the rainy season between July and September, 2011. The samples were air-dried for 21 days under shade and then, Oven – dried for 3-4 hours to constant weight (Ominiyi *et al*, 2002). The dried samples were pulverized to powder using an electric kitchen blender and stored in air-tight laboratory bottles. 200g of the stored leaf samples of *T.vogelii* was weighed into a flat–bottom flask of 2.5 litre capacity and 1 litre of distilled water was added to cover the samples. The flask was covered, shaken and allowed to stand for 24 hours. The mixture was then filtered using Mushlin Cloth and sunction filtration. The filtrate was concentrated using Rotary Evaporator and then dried using Iyovat gt3 freeze-drying machine. The freeze-dried leaf extracts were weighed into samples bottle and stored. The

anaesthetic solution of the freeze-dried leaf extract of *T. vogelii* was prepared by dissolving graded series of stored freeze–dried leaf samples of *T. vogelii* (0.01, 0.02, 0.03, 0.04, 0.05 and 0.06g/l) in de-ionized water under laboratory condition for 24 hours at room temperature (27.00 $\pm$ 0.04^0C) and the mixture filtered using No. 1 Whatman filter paper.

Preliminary experimental tests were carried out using the standard methods of APHA,(1985) to determine suitable concentrations of the freeze-dried leaf extracts of *T. vogelii* that would be used for the sedation of the fish which would not result in their death. The administration of the anaesthetic solution of the freeze–dried leaf extract of *T. Vogelii* was carried out using the parenteral (injection) route of anaesthesia. Before sedation the fish were starved for 24 hours to prevent regurgitation from the gastro-intestinal tract (GIT), and observation and recovery baths provided with aeration. Four healthy *Heterobranchus longifilis* post juveniles were selected randomly from both the control and the treatment groups. Each was weighed and injected 0.5ml of the extract concentrations (0.01, 0.02, 0.03, 0.04, 0.05 and 0.06g/l) using No. 23 needle and a 2ml syringe. Injection was done intramuscularly (IM) at the dorsal saddle, just above the lateral line, behind the operculum. Neiffer and Stamper, (2009). Injected fish were observed for behavioural responses and transfered into 70 litre plastic tanks containing 40 litres of water for recovery and time taken to recover noted.

Statistical Analysis

Statistical analysis of the results obtained was carried out using Genstat Discovery Edition 4 for one–way Analysis of variance (ANOVA) to determine differences in haematological variables of experimental fish treated with the freeze–dried leaf extract of *T. Vogelii*. Graph Pad Prim 5 and SSC stat V2.18 were used to test if differences existed between the haematological variables measured. Summary statistics were obtained for the variables using Minitab 14 for windows. Significant differences were accepted if the P-value was less than 0.05.

RESULTS

The result of the behavioural responses in *Heterobranchus longifilis* exposed to the freeze-dried leaf extract of *T. vogelii* shown in Table 1 revealed that the experimental subjects attained the first three stages (tranquillity period, excitation period and light anaesthesia) of

anaesthesia. Behavioural responses included mucus secretion, slow and erratic swimming, excrement discharge, increase in opercular beat rate, strong retension of reflex action, partial loss of equilibrium and colour change. The time taken for anaesthetized fish to enter anaesthesia decreased with increasing concentration of the anaesthetic extract. The recovery time followed the reverse order where faster recovery was observed with low concentrations of the anaesthetic extract. The opercular beat rates, both before and after sedation in the treatment groups showed increase over that in the control group and it increased with increasing concentration. The percentage increase in opercular beat rate did not show a definite pattern. Recovery time ranged from 506.67 minutes (8.45 hours) to 553.67 minutes (9.23 hours) increasing with increase in concentration of the anaesthetic solution. Significant differences ($P<0.05$) were observed among treatment groups in opercular beat rates before and after injection (sedation) at anaesthetic stage 1 and in the recovery time.

Table 1: Behavioural Responses *H. longifilis* injected various concentrations of *Tephrosia vogelii* Freeze-dried leaf extract

Conc. (g/ℓ)	Weight Of fish (g)	Volume injected (ml)	Behavioural Responses: Induction Time (Seconds) in Stages of Anaesthesia I	II	III	OBR (M^{-1}) BFS	AFS	Percentage increase in OBR (%)	Recovery time (Minutes)	Mortality After 48hrs
0.01	82.3±3.71^{b}	0.5	24.67±1.76^{a}	47.67±9.56^{a}	79.3±23.10^{a}	57.00±1.53^{c}	61.33±1.67^{b}	7.60±0.54^{a}	506.67±3.33^{c}	-
0.02	89.00±5.57^{b}	0.5	23.33±0.88^{ab}	42.33±5.04^{a}	76.67±5.49^{a}	60.67±2.40^{bc}	67.00±1.53^{ab}	10.61±2.35^{a}	518.33±4.41^{bc}	-
0.03	87.33±7.22^{b}	0.5	21.00±0.58^{abc}	37.67±4.84^{a}	65.67±5.49^{a}	61.33±1.76^{bc}	66.67±3.18^{ab}	8.58±2.13^{a}	526.33±2.91^{b}	-
0.04	100.67±8.09^{ab}	0.5	20.00±1.15^{bc}	30.67±4.67^{a}	57.00±8.54^{a}	64.00±1.53^{ab}	70.33±2.96^{a}	9.83±2.72^{a}	531.33±2.91^{b}	-
0.05	92.00±4.62^{b}	0.5	17.67±0.88^{cd}	29.33±2.03^{a}	57.33±3.93^{a}	64.67±0.88^{ab}	71.00±1.00^{a}	9.84±2.38^{a}	547.33±5.21^{a}	-
0.06	115.33±7.42^{a}	0.5	15.33±0.88^{d}	26.33±2.19^{a}	49.33±4.98^{a}	67.00±0.58^{a}	71.00±1.53^{a}	5.96±1.68^{a}	553.67±4.91^{a}	-

- OBR BFS = Opercular Beat Rate Before Sedation
- OBR AFS = Opercular Beat Rate After Sedation
- Data were subjected to analysis of co-variance using weight as covariate
- means in the same column followed by different subscripts differ significantly ($P<0.05$)

Figure 1 shows the result of recovery time in *H. longifilis* treated with various concentrations of freeze-dried leaf extract of *Tephrosia vogelii*.

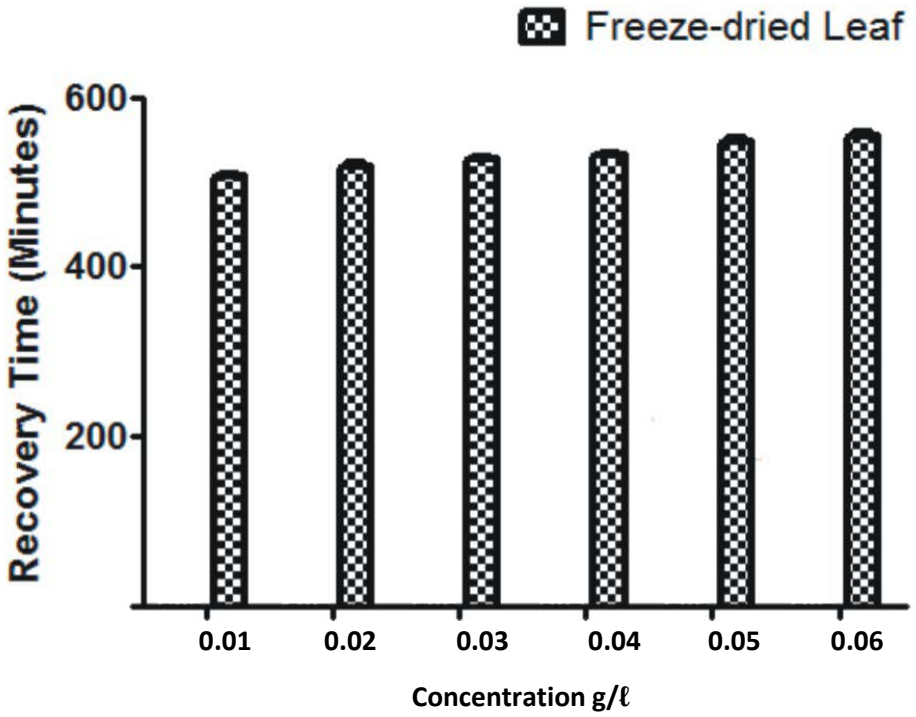

Figure 1:Mean values of recovery time in *H. Longifilis* juveniles injected various concentration of *T. vogelii* freeze-dried leaf extract.

Figure 2 shows the result of the opercular beat rate before sedation (OBR BFS) and opercular beat rate after sedation (OBR AFS) in *H. longifilis* obtained with various concentrations of freeze-dried leaf extract of *T. vogelii*.

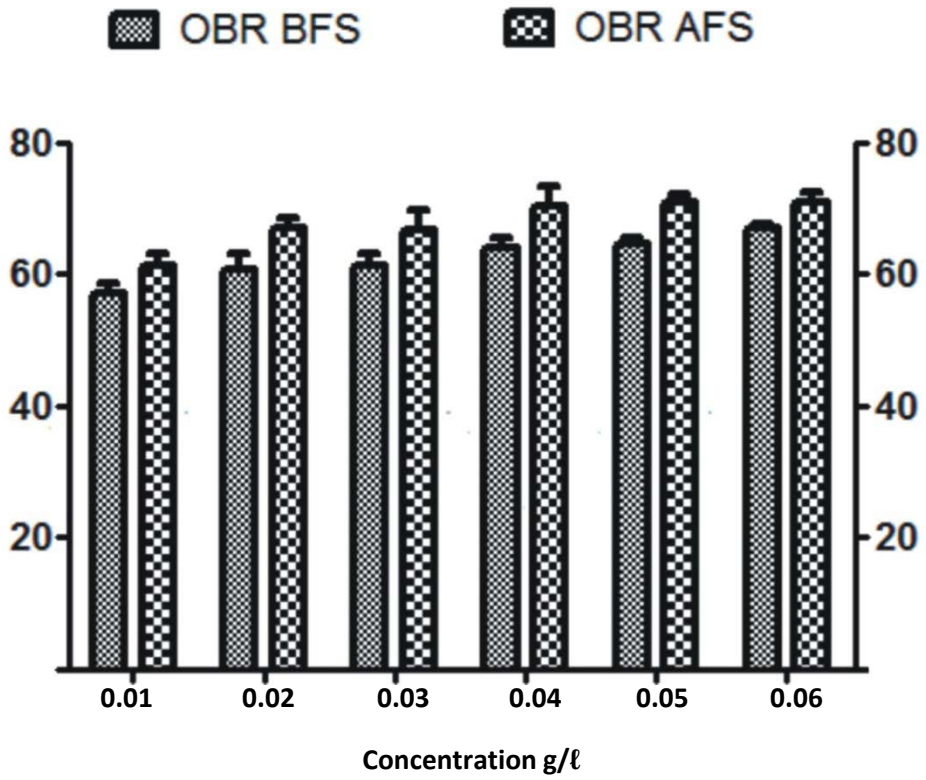

Figure 2: Comparison of Mean values of opercular beat rate before and after sedation in *H. longifilis* injected various concentrations of *T. vogelii* Freeze-dried Leaf extracts.

- OBR BFS = Opercular Beat Rate Before Sedation
- OBR AFS = Opercular Beat Rate After Sedation

Figure 3 shows the result of percentage change in opercular beat rate in *H. longifilis* treated with various concentration of freeze-dried leaf extract of *Tephrosia vogelii.*

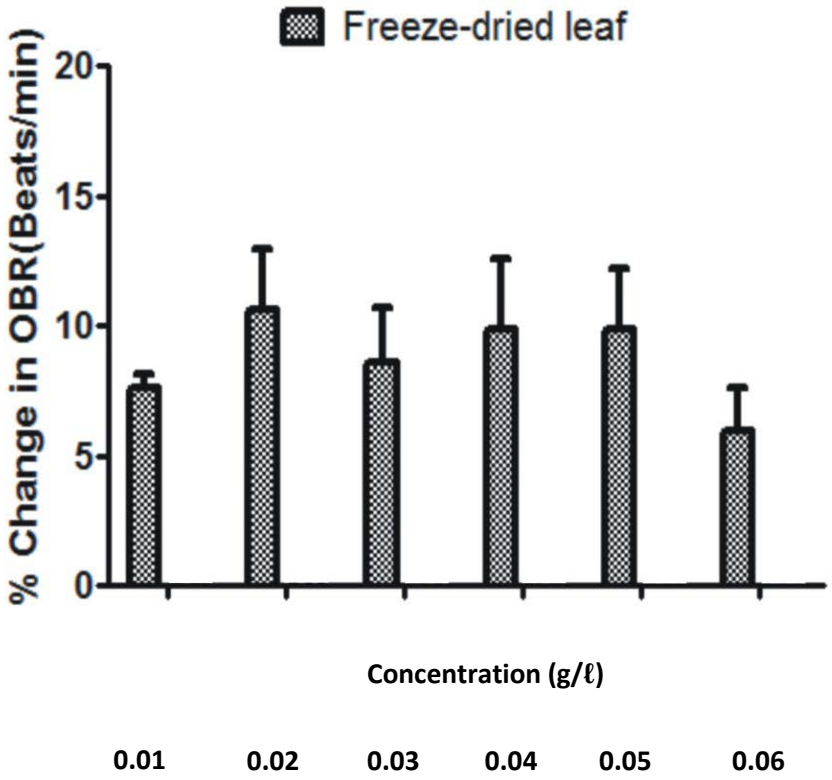

Figure 3: Mean values of percentage change in opercular beat rate and recovery time in *H. longifilis* injected various concentration of *T. vogelii* freeze-dried leaf extract.

DISCUSSION

The results obtained from the present study shows that *H. longifilis* post-juveniles injected with the freeze-dried leaf extract of *T. vogelii* sequentially progressed through the first three stages of anaesthesia and the experimental fish were successfully tranquilized at all levels of concentration, similar to the findings from the study on the effects of sodium bicarbonate on common carp (*Cyprinus Carpio*) juveniles which only reached the third stage of anaesthesia. Altun et al, (2009). The effect of the anaesthetizing extracts appeared to be concentration dependent since faster tranquilization was achieved at higher concentration of the extract as reported in other studies. Hseu,(1998); Griffiths,(2000); Solomon and Amali,(2004); Mylonas *et al*,(2005). This observation is also in agreement with Trevor and Miller,(1987) that the degree of

anaesthesia is influenced by the concentration of the anaesthetic in the central nervous system (CNS) of the organism. Therefore, in the present investigation the shorter induction time taken to tranquilize the experimental fish, *H. longifilis*, with increased concentration of the anaesthetic extract may be attributed to the accumulation of the active ingtredients, rotenoids, in the body system of the fish which impaired the activity of CNS at a much faster rate. Solomon and Amali,(2004). The failure of the anaesthetized fish to enter deep sedation (anaesthesia stage 4) could be due to the size and weight of the fish in relation to the low concentration used since larger individuals generally require a greater concentration of anaesthetic than smaller individuals (Colye *et al*, 2004). This may also be due to stage of the life cycle, age, lipid content and body condition of the fish, all of which are biological factors that influence the metabolic rate and therefore the pharmacokinetics of the anaesthetic compound. Iversen, (2003).

When the time taken for *H. longifilis* to enter anaesthesia or to be tranquilized (induction time) and recovery time are considered in the present investigation, significant differences in induction time were recorded at anaesthetic stage 1 depicting the effect of concentration on induction time at this stage of anaesthesia. The induction times of 79.30 seconds obtained with the freeze-dried leaf extracts of *T. vogelii* is compartible with the average induction time of 1-2 minutes for light sedation of common carp (*Cyprinus carpio)* juveniles exposed to sodium bicarbonate (Altun *et al*,2009) and the 1.5 minutes reported for *Acipenser persicus* exposed to clove oil. Bagheri and Imanpoor,(2001). When the rapid induction time (3-5 minutes) required of an ideal anaesthetic (Marking and Meyer,1985; Iversen,2003; Coyle *et al*,2004; Mylonas *et al*,2005; Brown,2011) is considered, the result obtained in the the experiment with the freeze-dried leaf extract of *T. vogelii* closely meet the requirement of an ideal anesthetic.

The recovery times in agreement with other researchers (Peake,1998; Griffiths,2000; Solomon and Amali,2004; Filiciotto *et al*,2012) tended to increase with increasing concentration of freeze-dried leaf anaesthic extract of *T. vogelii*. Hseu *et al*,(1998) reported that higher drug concentration or dose increase recovery time. In the case of immersion anaesthetics Griffiths,(2000) and Tort *et al*,(2002) suggested that this may be due to the fact that higher dose induced anaesthesia more rapidly thus allowing the the experimental fish to be removed from the anaesthetic bath and placed in clean water earlier than fish exposed to lower doses. Since the degree of anaesthesia is influenced by the concentration of the anaesthetic in the CNS of the

experimental fish (Trevor and Miller,1987), in the present study where the parenteral route of anaesthesia was used this may explained by the fact that more of the active ingredients of the anaesthetic extract accumulated in the CNS of the fish at higher concentrations thus suppressing the activity of the CNS to a greater degree than at lower concentrations and consequently prolonging the recovery time. The recovery time of 506.67 minutes (8.43 Hrs) obtained with the freeze-dried leaf extract is close to the 12 hour recovery time reported for *Oreochromis niloticus* anaesthetize with quinaldine. Sado,(1985).

Chemical anaesthetic agents have been used in handling and transportation of fish to reduce mortality which occurs as a result of excitement and hyperactivity. Schoettgel *et al*, (1967). It has been suggested that the long recovery time observed with clove essence could be an added advantage in activities such as morphological evaluation, biopsy and stripping which require long handling periods outside water. Anderson et al (1997); Munday and Wilson,(1997); Park *et al*,(2009). It has also been suggested that light sedation is desirable during transportation of fish. Summerfelt and Smith,(1990). This is because fish anaesthetized at deep sedation (anaesthetic stage 4) levels lose equilibrium and may sink to the botton, pile up and finally suffocate. Dupree and Huner, (1984). Since transportation of fish often involve long distances and time, the long recovery time of the freeze-dried leaf extract of *T. vogelii* which tranquilized *H. longifilis* at the light sedation level of anaesthesia could be considered as an advantage for use as a tranquilizer in the delivery of fish over long distances and other handling procedures such as morphological evaluation, biopsy and stripping.

In the present study with freeze-dried leaf extract of *T.vogelii* no mortalities were recorded in a 48 hour post-anaesthetic period similar to reports by Mohammed, (1999) and Altun and Danabas, (2006). However, three sea bass (*Dicentrarchus labrax*) were reported dead at the two highest concentrations when anaesthetized with eugenol (Filiciotto *et al*,2012), and the researchers attributed the death of experimental fish to the capacity of eugenol to markedly induce anaesthesia which could cause death. The absence mortalities in the experiments with freeze-dried leaf extract of *T. vogelii* in the present study may therefore be due to the fact that the various concentrations of the freeze-dried leaf extract used in this research lack the capacity to induce deep anaesthesia in the experimental subjects. The colour change observed with experimental fish after anaesthetization from light brown to light orange colour could be due to

the effect of the anaesthetic extract on the cells and tissues of the epidermis. This, however, is a matter for further investigation.

The opercular beats are an indicator of stress. They increase and decrease according to the type and concentration of anaesthetic. Altun and Danabas,(2006). Accordingly, it has been reported that enhancing the concentrations of quinaldine sulfate and diazapem from 2.5-20mg/ℓ increased the opercular rate in sea bream (*Sparus aurata*) while increasing the concentration of clove oil decreased opercular rate in sockeye salmon (*Oncorhyncus nerka*). Kumlu and Yanar,(1999); Woody *et al*,(2002). In the current study involving *H. longifilis* with the freeze-dried leaf extract of *T. vogelii* the opercular beat rate (0BR) after administration of the extract tended to be higher than values obtained before administration (control) of the extract at all levels of concentration at light sedation (patial loss of equilibrium or stage 3 of anaesthesia). This observation is consistent with Solomon and Amali,(2004) that the OBR of *C. gariepinus* exposed to *Datura innoxia* increased as the fish attained partial loss of equilibrium. At deep sedation level i.e stage 4 of anaesthesia, the opercular rate of sea bass (*Dicentrarchus labrax*) exposed to quinaldine sulfate decreased until it almost ceased. Yanar and Kumijj,(2001). Filiciotto *et al*,(2012) similarly reported that the opercular rate of *Dicentrarchus labrax* treated with clove oil assumed values that were generally lower after administration of the anaesthetic and attributed this to the notable power of eugenol and its capacity to induce deep anaesthesia. In the present experiment the increase in the values of opercular rate after administration may be attributed to the failure of the anaesthetizing extracts to induce deep anaesthesia in the experimental subjects since it is at deep anaesthesia level that the opercular rate after administration of an anaesthetic tends to decrease below the rate before administration of anaesthetic. The result shows that the freeze-dried leaf extract of *T. vogelii* at the level of concentrations used did not impact negatively on experimental subjects.

In the present experiment with *H. longifilis* injected with the freeze-dried leaf extract significantly differences were observed among the mean values of percentage change in opercular beat rate indicating that the opercular rate was affected by concentration. Imanpoor *et al*,(2010). The mean values of percentage change in OBR showed that values obtained after the administration of the extracts did not show marked deviations from the values obtained before administration. It has been observed that marked deviations in values of OBR from reference

(control) suggests an adjustment in physical fitness as a result of stress condition. Edwards and Fushur (1991); Leight and Van Dolah,(1999). Since the values of percentage change in OBR after administration of the extracts in the present study did not show marked deviations from the values before sedation it appears reasonable to suggest that the experimental fish were not badly affected by stress to warrant an adjustment in the physical condition of experimental fish. The result of the present study suggest that the freeze-dried leaf extract of *T. vogelii* could be adopted for used as tranquilizer in the transportation of fish over long distances.

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