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Privet Leafhopper, *Fieberiella florii* Aqueous Extract Demonstrates *In Vitro* and *In Vivo* Neuromuscular Potentials in Mice and Chickens

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Abstract

Introduction: The ability of naturally occurring carotenoid pigments of *Fieberiella florii* (Cicadellidae, Hemiptera), a privet leafhopper, and its phytoconstituents were assessed.

Methods: Thus, the neuromuscular potentials of *F. florii* Aqueous Extract (FFAE) were investigated in mice and Chickens.

Results: FFAE contains higher alkaloids and tannin than phenols, flavonoids, terpenoids, phlebotanins and saponin. Also *in vitro* 2,2-diphenyl-1-picryl-hydrazyl, nitric oxide, lipid peroxidation scavenging activities, and ferric reducing antioxidant power demonstrated efficacies between 25 µg to 100 µg respectively. Vitamin A and carotenoid levels were 61.21 ± 1.83 and 146.11 ± 2.05 mg/100 g respectively. Validated pharmacologic models including traction, climbing and inclined plane tests were used to assess neuromuscular effects. FFAE oral gavage in mice up to 2 g/kg was non-lethal while median lethal dose in mice was 446.5 mg/kg intraperitoneally. FFAE increased ($P<0.05$) gamma aminobutyric acid actions and showed competitive depolarizing actions at 10 mg/kg in mice. In contrast to suxamethonium, FFAE-induced flaccid paralysis was qualitatively similar to that obtained after pancuronium administration. A co-administration of FFAE potentiated the actions of neuromuscular drugs. Bioactive compounds present in FFAE included linoleoyl chloride, n-hexadecanoic acid, palmitin, 1,2-di-,2-aminoethyl hydrogen phosphate and methyl esters of lauric acid, tridecanoic acid, tetradecanoic acid.

Conclusion: Overall, these present novel experimental findings suggest FFAE as a potential neuromuscular agent.

Keywords: Bioactive compound; *Fieberiella florii*; Neuromuscular action; Privet leafhopper; Pharmacology

Introduction

The puzzle that carotenoid biosynthesis genes from a fungus to some protist parasites and aphids are not the remnants of past endosymbiotic gene transfer but are instead the product of horizontal gene transfer has been resolved [1-6]. Also, studies have suggested this probable in situ carotene synthesis to account for the uniqueness of aphids in the insect class [1]. *Acyrthosiphon pisum* is the first known animals to have acquired the carotenoid biosynthetic machinery to produce carotenoid [2]. This corroborates with the hypotheses driven light-dependent production of adenosine triphosphate [7] in aphids. Although, a gene may be selfish in a limited metaphorical sense, the catalogue of justification on nature traverses a range of possibility. This species of insect has been known to take advantage of coevolution and exploited the dual ability of light absorption and reflection inherent in retinoids and carotenoid to accomplish their diversify actions. Toomey et al. [8] have linked these fitness-enhancing activities and carotenoid allocation to play crucial roles in sexual behavior, reproduction and avoiding predation while enhancing parasitism in their domain. The relationship between entomology and drug discovery remains under-utilize. Insects can be classified based on plants they visit [9]. Since, an order such as Hemiptera has been found to colonize and feed on different species of plant including green pea, soybean, melon, rose, giant bark, tulip tree, rice and white pine plants. The *Fieberiella florii*, a privet leafhopper, visits and feeds on a bitter tree (*Vernonia amygdalina*) in Southwestern Nigeria. The privet leafhopper, *F. florii*, is a Hemiptera that belongs to suborder Auchenorrhyncha and the family of Cicadellidae [10]. This present study was carried out for two major reasons. Firstly, there is a quest for new drugs and or toxins but not too many attentions have been given to insects. Secondly, mass rearing of *F. florii* has been carried out using nymphs and adults [10]. Currently, there is no study to show the medical importance and or modulatory potential of *F. florii* in an experimental animal model. In this preliminary study, therefore, we assessed the phytochemicals present in (FFAE), its possible neuromuscular effects in rodents as well as the bioactive components with a view to ascertaining its involvement in these processes.

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Materials and Methods

Drugs and chemicals

Diazepam sodium benzoate was a product of F. Hoffmann Roche Ltd Basel Switzerland by Cenexi Sas, Fontenay-sous-Bois, France. Pancuronium bromide was purchased from Healthcare Pvt. Ltd, India and suxamethonium chloride was obtained from Rotex Medica Trittau, Germany respectively. Ascorbic acid and gallic acid were purchased from Sigma Chemical Co. (USA). All other chemicals used were of analytical grades.

Collection of leafhopper

Using a forceps, morph leafhopper (average weight, 0.30 g and 2-4 weeks old) was collected at summer time around May 2015 (i.e., handpicked) from the bitter tree (*V. amygdalina*) stems around residential areas at Ilishan, Ogun State, Nigeria into modified aerated universal bottles containers and were immediately transported to the laboratory where they were washed with clean sterile water. Leafhopper identification was carried out by Dr. Anikwe JC, and Mr. Faton OM Seyon, both in the Department of Zoology, University of Lagos, Lagos State, Nigeria. After collection, the leafhoppers (adults and nymphs), were placed under controlled laboratory conditions (22°C ± 2°C, 12 h light/dark photoperiod) and fed with bitter leaf (*V. amygdalina*) stem stalks, until being processed for biological experiments.

Classification

Classification followed the description of Beirne [11].

Privet leafhopper: *Fieberiella florii*

Animals: Kingdom Animalia

Arthropods: Phylum Arthropoda

Hexapods: Subphylum Hexapoda

Insects: Class Insecta

Winged and Once-winged Insects: Subclass Pterygota

True Bugs, Cicadas, Hoppers, Aphids, and Allies: Order Hemiptera

Free-living Hemipterans Suborder Auchenorrhyncha

Infraorder: Cicadomorpha

Leafhoppers and Treehoppers: Superfamily Membracoidea

Leafhoppers: Family Cicadellidae

Subfamily: Deltocephalinae

Genus: Fieberiella

Extraction of pigments

F. florii (nymph leafhopper, green) (Plate 1) was extracted successively in Ringers's buffer (6.5 g, NaCl; 0.42 g, KCl; 0.25 g, $CaCl_2$; and 0.2 g of sodium carbonate) [12], and pH is 6.85 prior to phytochemical assessments. Twenty-two (22) nymphs green (2.5 mg/ml, variants weighing between 250 mg to 350 mg) of *F. florii* were centrifuged at 20 min at 3000 x g and the supernatant containing light green layer was collected in a glass potter. The supernatants were stored at -4°C normalized and used for phytochemical screening. The crude green pigments were facilitated by their known rapid crystallization and obtained spectral absorbance properties confirmed in accordance with carotenoid molecules as well as vitamin A (peak of absorbance was between 425 nm and 480 nm). Similarly, an aqueous extraction was prepared and preserved and stored for pharmacological experimental studies as well as bioactive characterization.

Plate 1: Privet leafhopper, *Fieberiella florii*.

Phytochemical assessments

The Ringer's portion was used for preliminary screenings to perform the standard phytochemical analysis following the protocol described by Harborne [13] and Sofowora [14] respectively. In addition to the phytoconstituents, Vitamin A and carotenoid levels were determined using the methods of Rosenheim and Webster [15] and Dugan et al. [16].

Assessments of antioxidant activity and free radical scavenging activities

The *in vitro* free radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was measured as described previously by Brand-Williams et al. [17]. The lipid peroxidation inhibition activity and nitric oxide scavenging activity were assayed by the methods described by Nuutila et al. [18] and Green et al. [19] respectively. Ferric reducing antioxidant power was determined according to the method of Ozgen et al. [20].

Pharmacological Experiments

Animals

Adult mice of both sexes (8-9 weeks old) weighing 24 g ±

3.0 g were obtained from inbreeds within the experimental animal handling facility of the Department of Pharmacology, Benjamin Carson (Snr.) School of Medicine, Babcock University, Ilisan-Remo, Ogun State, Nigeria at ambient temperature and humidity with a 12 h light/12 h dark schedule. One day old chicks were obtained from the Obasanjo farm, Challenge, Ibadan, Oyo state, Nigeria. They were fed with commercially available pelleted diets (Vita Feeds, Jos, Plateau State, Nigeria) and water *ad libitum* during the period of acclimatization and throughout the period of the experiment. Studies were carried out in strict compliance with established guidelines for care and use of laboratory animals in biomedical research and the procedures as documented by Kilkenny et al. [21] for reporting animal research.

Oral acute toxicity test

Thirty fasted albino mice were weighed (average weight 18 g, 8-9 weeks old), grouped into six of five animals/group. The toxicity study of FFAE was done according to the for Economic Co-operation and Development (**OECD**) guidelines [22]. Control animals received distilled water while others were orally administered 200, 400, 800 and 2000 mg/kg body weight of FFAE. The animals were observed at 2, 12, 24 and 48 h following administration of extract and were further observed for 14 days for reversibility of adverse effects.

Intraperitoneal acute toxicity test

Thirty fasted albino mice (average weight 18 g, 8-9 weeks old) were divided into six (6) groups (1-6) of five animals per group. The animals in group 1 served as control and received distilled water while groups 2-6 were administered intraperitoneal 100, 300, 500, 1000 and 2000 mg/kg body weight of FFAE respectively. The animals were observed similarly as mentioned for oral acute toxicity for reversibility of drug effects, however, data obtained at the end of 24 h were used to extrapolate median lethal dose [23].

Assessment of muscle relaxant effects of FFAE

Climbing test: Mice were trained to climb a chain 50 cm long, suspended from a clamp 90 cm high, by placing the fore paws of each animal on the free end of the chain [24]. A normal mouse would grasp the chain with the fore paws and when allowed to hang free, placed the two feet on the chain and climb until it attained a mark of 2 cm from the top of the chain. Mice which successfully reached the mark within 30 seconds were recruited and used for this experiment. Animals in different groups (n=6) received distilled water (10 ml/kg, p.o), FFAE (5 mg/kg and 10 mg/kg), diazepam (1 mg/kg, i.p.), pancuronium (1 mg/kg, i.p.), and suxamethonium (1 mg/kg, i.p.). Diazepam is a sedative-hypnotic drug. Pancuronium and suxamethonium are typical non-depolarizing and depolarizing mimetic muscle relaxant respectively. One hour post-oral or 30 min. post-intraperitoneal treatment, the mice were subjected to the climbing test and the time taken to climb to the designated mark were taken and recorded per animal.

Inclined screen test: The modified method of Randall et al. [25] was used in this experiment. Sixty (60) minutes after the administration of distilled water 10 ml/kg, p.o., FFAE (5 mg/kg and 10 mg/kg, p.o.), and 30 min after diazepam (1 mg/kg, i.p.) and pancuronium (1 mg/kg, i.p.), and suxamethonium (1 mg/kg, i.p.) treatments, each group (n=6) of mice was left for 30 min following oral gavage or 1 h post-intraperitoneal treatment on a flat, slippery rectangular glass plate (42 cm × 37 cm) inclined at 30° to the horizontal. Mice were observed on the time taken for the paralyzing effects capable of causing the mice to slide off the screen within the 1 h.

Traction test: The method of Rudzik et al. [26] was adopted in this study. The fore paws of a mouse were placed on a small twisted wire rigidly supported above a laboratory bench top. Normal mice grasped the wire with the fore paws and when allowed to hang free, placed at least one hind foot on the wire within 5 sec. Inability to fulfill the later disqualified the animal for the traction test. Initially screened mice were assessed after the administration similar to those of climbing and inclined tests. The response time for each mouse and percentage changes relative to the control were determined.

Assessment of neuromuscular effects of FFAE in one-day old chicks

The modified method of Barrett and Harvey [27] was used in this experiment. One day old chicks (Brawlers) (24 g to 35 g) were divided into different groups (n=6) and administered distilled water (10 ml/kg), pancuronium (1 mg/kg), suxamethonium (1 mg/kg), FFAE (5 mg/kg and 10 mg/kg), pancuronium (1 mg/kg) with FFAE (10 mg/kg) and suxamethonium (1 mg/kg) with FFAE (10 mg/kg) respectively. The chicks were observed for 5 min (digital camera, OLYMPUS, FE-280). Scoring was done by recording onset and duration of paralysis and the types of paralysis elicited by these agents.

Statistics

Results were expressed as mean ± SEM differences between groups were determined by T-test and one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS, version 20) software for windows. Post hoc testing was performed for inter-group comparisons using the least significant difference [28] and a P value <0.05 was considered significant. Data were converted to graph using Graph Pad Prism 6.

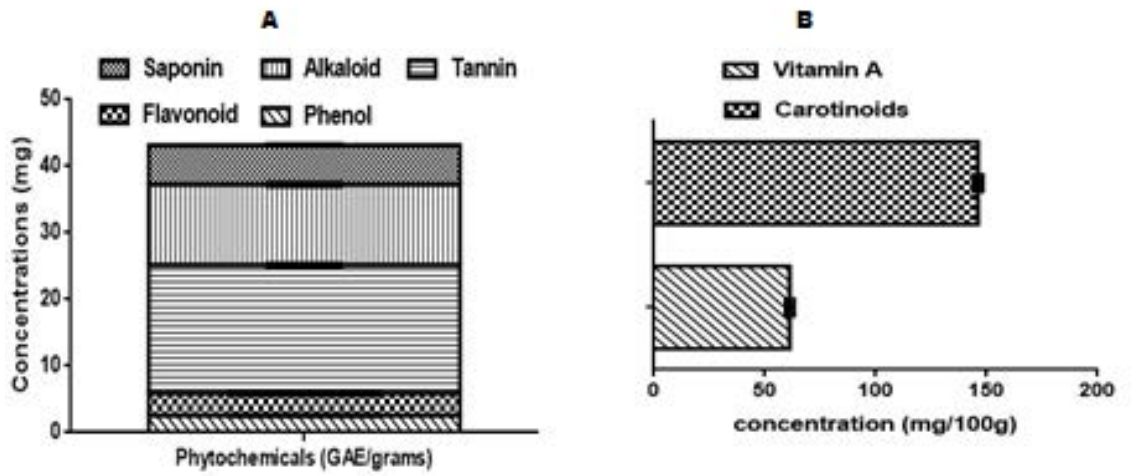
Results

From the results obtained in our experiments, Table 1 shows the presence of phenols, flavonoids, phlebotanin, alkaloids, terpenoids, steroid and saponin. Also, in Figure 1A, the amounts of tannin, alkaloid, saponin, flavonoid and phenol were in order of concentrations, although, tannin

Test	Phenol	Flavonoid	Phlobatanin	Tannin	Alkaloid	Terpenoid	Steroid	Saponin
Result	+	+	+	+	+	+	+	+

+: presence

Table 1: Qualitative phytochemical analysis of FFAE.



GAE: Gallic Acid Estimate; FFAE: *Fieberiella florii* Aqueous Extract.

Figure 1: Mean ± SEN, n=3. **A)** Quantitative Phytochemicals. **B)** Levels of vitamin A and carotenoid present in FFAE.

Treatments	Probit	Sign of toxicity
Control	0	No toxic changes observed
FFAE (2.0000)	0	Flaccid paralysis of lower limb observed in in first 2 h.
FFAE (2.4771)	5.2533	Spastic paralysis of lower limb observed in in first 2 h
FFAE (2.6990)	5.8416	Spastic paralysis of lower limbs observed in first 2 h
FFAE (3.000)	7.4949	Spastic paralysis of lower limbs observed in first 2 h
FFAE (3.3010)	7.7000	Spastic paralysis of lower limbs observed in first 2 h

Value in parenthesis represent log dose. Control (distilled water, 0.1 ml/kg); h: hour.

Table 2: Effects of acute intraperitoneal toxicity study after 24 h of administration of FFAE in mice.

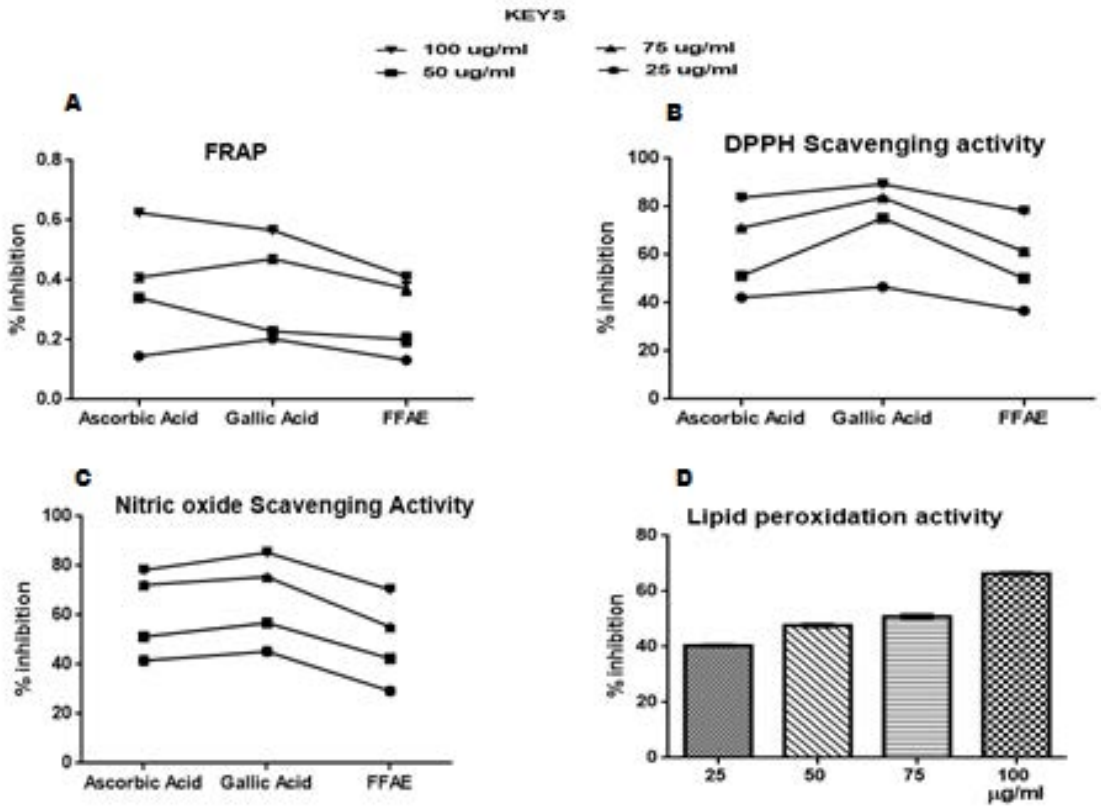
(44.7%) and alkaloid (28.0%) were most abundant. In Figure 1B, the levels of vitamin A and carotenoid in FFAE per mg 100 g were compared with standard. Here, FFAE contains double the levels of vitamin A (61.21 ± 1.83) for carotenoid (146.11 ± 2.05). Further, residual light-dependent production capacity (vitamin A – carotenoid – vitamin A) in mg per 100 g is 58.11% relative to carotenoid contents.

Acute toxicity testing

Table 2 results show the acute toxicity testing of FFAE. An oral gavage of FFAE in animals up to 4 g/kg was non-lethal while median lethal dose in mice was 446.5 mg/kg intraperitoneally. Mice administered 100 mg/kg FFAE intraperitoneally demonstrated stretching, flaccid paralysis, gripping, leaning on hind limbs, lying on abdomen and sedation. Animals that received doses of 300 mg/kg and above showed signs of restlessness, weakness, flattened abdomen, spastic paralysis, and death 2 h of administration. Mortality was observed and recorded for each group of animals following 24 h of administration. We obtained a median lethal dose (LD_{50}) of 446.5 mg/kg.

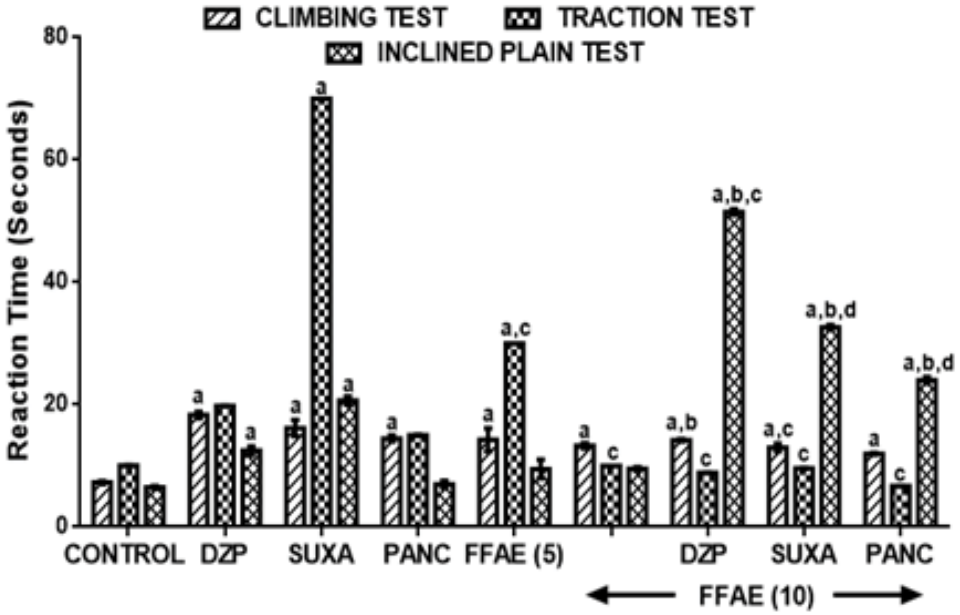
Results in Figure 2 effects of FFAE on *in vitro* antioxidants and free scavenging activity of percentage inhibition of DPPH, nitric oxide and lipid peroxidation at doses 25, 50, 75 and 100 µg/ml respectively. Ferric reducing antioxidant power (FRAP) also increased dose-dependently. An assessment of FRAP, NO and lipid peroxidation (LPO) LPO scavenging activities were compared with ascorbic and garlic acid standards.

Figure 3 shows the effects of FFAE on skeletal and smooth muscle relaxation in mice. In the traction test, diazepam, pancuronium, suxamethonium increased ($P<0.05$; $F=2.1$-24.5) reaction times by 98%, 50%, and 600% respectively when compared with control distilled water group. FFAE (5 mg/kg) also increased reaction time by 200% relative to control distilled water and suxamethonium (0.2 mg/kg) groups. In contrast, increased dosage of FFAE (10 mg/kg) produced no significant change ($P>0.05$) as observed in our results. In respect, a co-administration of FFAE at 10 mg/kg with either a centrally acting agent (diazepam, 0.2 mg/kg, i.p.), and peripheral acting neuromuscular drugs such as suxamethonium or pancuronium (0.2 mg/kg, i.p.) after 30 min interval did not produce any significant change when compared with control distilled water group. More so, FFAE at 10 mg/kg was able to reverse the action of suxamethonium



DPPH: 2,2-diphenyl-1-picryl-hydrazyl; FRAP: Ferric Reducing Antioxidant Power; FFAE: *Fieberiella florii* Aqueous Extract.

Figure 2: Assessments of antioxidants and free radical scavenging activities. Mean ± SEN, n=3.



DZP: Diazepam; SUXA: Suxamethonium; PAN: Pancuronium; FFAE: *Fieberiella florii* Aqueous Extract. [a]p<0.05 when compared with control (distilled water group). [b]p<0.05 when compared with diazepam group. [c]p<0.05 when compared with suxamethonium group. [d]p<0.05 when compared with pancuronium group. Climbing Test; Traction Test; Inclined Plane Test.

Figure 3: Effects of *Fieberiella florii* Aqueous Extract on neuromuscular activities in mice. Results expressed as Mean ± SEN, n=6.

significantly (P<0.05) when administered 30 min later. During the inclined plane test, diazepam and pancuronium increased reaction times by 92.31% (P<0.05), 7.69% (P> 0.05) while suxamethonium increased (P<0.05) the same by 218.46%.

Treatment	Onset of action (Sec)	Duration of action (Sec)	Papillary contraction (Sec)	Observed paralysis
Control	-	-	22.00 ± 0.89	-
PAN (1 mg/kg)	30.67 ± 2.54	60.00 ± 1.76	41.67 ± 1.42[a]	Flaccid
SUXA (1 mg/kg)	25.25 ± 1.90	157.50 ± 2.01	19.50 ± 1.00	Flaccid/spastic
FFAE (5 mg/kg)	45.44 ± 2.00[b,c]	18.12 ± 1.00[b,c]	23.00 ± 0.68[b,c]	Flaccid
FFAE (10 mg/kg)	33.88 ± 1.53[c]	6.30 ± 1.88[b,c]	44.50 ± 0.84[a,c]	Flaccid
PAN (1 mg/kg)+FFAE (10 mg/kg)	93.75 ± 2.70 (205.7)[b]	186.00 ± 2.13 (-210)[b,c]	5.50 ± 0.56[a,b,c]	Spastic
SUXA (1 mg/kg)+FFAE (10 mg/kg)	14.25 ± 3.20 (43.6)[c]	402.75 ± 1.67 (-156.1)[b,c]	10.25 ± 0.10[a,b,c]	Spastic

Results expressed as Mean ± SEN, n=6. Control (distilled water, 10 ml/kg). FFAE: *Fieberiella florii* Aqueous Extract; SUXA: Suxamethonium; PAN: Pancuronium. [a]p<0.05 when compared with control (distilled water group). [b]p<0.05 when compared with PAN group. [c]p<0.05 when compared with SUX group.

Table 3: Effects of FFAE on neuromuscular activity in a day old chicks.

The effects of FFAE in divided doses when administered alone increased but not significantly (P>0.05) different from distilled water control groups. In the climbing test, diazepam, pancuronium or (suxamethonium) (0.2 mg/kg) as well as FFAE (5 mg/kg or 10 mg/kg) when administered alone and separately increased (P<0.05) the climbing reaction times when compared with control distilled water group. All the treated animals however show significantly increased (p<0.05) in climbing reaction times by 94.5%, 71.8% and 64.4% (p<0.05; F=3.2-21.8) respectively when compared with control distilled water group.

Table 3 indicated the effects of FFAE on neuromuscular activity of one-day old chicks. Animals administered with distilled water did not show neuromuscular effect. However, pancuronium and suxamethonium showed the quick onset of actions which ranges from twitching (fasciculation) to paralysis (flaccid: staggered movement; spastic: twisted hind limbs) more than 5 mg/kg or 10 mg/kg FFAE administered, although, not insignificantly (P<0.05). Also, FFAE (10 mg/kg) alone demonstrated a highly significant (P<0.05) onset of drug action similar to standard control drugs. FFAE (10 mg/kg) when administered together with suxamethonium (1 mg/kg) and pancuronium (1 mg/kg) showed delayed onset of neuromuscular blockade. The duration of action was most prolonged in chicks that received a combination of FFAE (10 mg/kg) and pancuronium (0.2 mg/kg) by 210% when compared with pancuronium group, although, increased onset was also observed (205.7%). A similar result was obtained when FFAE was combined with suxamethonium in the duration (156.1%) but not the onset of action (43.6%). Suxamethonium but not pancuronium significantly (P<0.05; F=13.2) increased papillary contraction when compared with control distilled water group. Interestingly, FFAE (P<0.05) significantly increased papillary contraction in the treated animals when compared with control group. Either a 5 mg/kg or 10 mg/kg showed twitch-like static movements. However, pancuronium, when administered with a 10 mg/kg FFAE showed transient twitch-like effect unlike sustained fasciculation observed during suxamethonium (1 mg/kg) co-administration.

Discussions

A man continues to enjoy mother-nature by making good use of photosynthetic plants. Interestingly, animals such as the family of Hemiptera particularly the aphids have been demonstrated to also possess photosynthetic machinery. Historically, Homoptera is a suborder of order Hemiptera, which soon became obsolete [29] and split, in part, due to long branch attractive effects in phylogenetic analyses that involved some rapidly evolving DNA regions [30]. This species of insect has been known to take advantage of coevolution and exploited the dual ability of light absorption and reflection inherent in retinoids and carotenoid to accomplish their diversify actions. Thus, carotenoids are now available as food sources of tolerable intakes, stability and bioavailability [31,32]. The present study assessed the phytochemistry and neuromuscular potential of FFAE in rodents. From the results obtained following phytochemical analysis, *F. florii* contains flavonoids, phlobatanin, steroid, saponin, alkaloids, tannins, terpenoid and saponins (Table 1). Also, the amounts of tannin, alkaloid, saponin, flavonoid and phenol were quantified. However, tannin was present in highest amount followed by alkaloid (Table 2). The presence of these compounds in FFAE provides its suitability for a potential pharmacological candidate. In addition, the presence of vitamin A and carotenoids in FFAE provides such impetus for assessing its therapeutic yields (Table 3). The protective role of various compounds found in FFAE in human health has been elucidated [31-33]. However, these opportunities have long been neglected by scientists. Most importantly, these hallmark phytochemicals as found in FFAE lack *in vivo* study. Thus, this present study hypothesized that pharmacologic manipulation of these compounds could influence disease modulation and may open a new vista of research. For instance, the tannic acid which has been found to be relatively high in the aphids was also present in large amount in FFAE (Table 2). This may become favorable because tannin acts directly on inflammatory related diseases and has been used to stop bleeding [34,35]. Although, suggestions are that there are no true toxicological studies of tannin in insects, however, reports of beneficial, toxicity and dietary deficiency symptoms have been associated with tannin use. Some clinically embraced areas of interest are chronic diarrhea, dysentery, bloody urine, painful joints, persistent coughs and cancer [34]. In homes, tannic acid serves as a flavoring agent in tea,

astringent and coloring [35]. Further, it has been used in ointments and suppositories for the treatment of hemorrhoids [36] while non-clinically available for hides and ink and to kill dust mites [37]. Tannin gives taste to wine and beer and may help reduce the viscosity of drilling mud for oil wells [34]. It has been administered internally to check diarrhea and intestinal bleeding, as an antidote, alkaloidal and glycosidic poisons with which it forms insoluble precipitates [35,37]. In addition, FFAE presents an appreciable large amount of alkaloids (Table 2) which are naturally occurring chemical compounds containing basic nitrogen atoms [38]. They have physiological effects that make them useful as medicines. Most of these are the powerful muscle relaxant, cosmetics, analgesics, and local anesthetic [39]. In respect, drugs such as quinine, atropine, caffeine, serotonin, pilocarpine, barbiturates are alkaloids **[**40**].** Similarly, anticancer vinca alkaloids also inhibit cancer cell growth by stopping cell division [41]**.** Thus**,** an increasing understanding of FFAE involvement may offer a therapeutic opportunity in remediating the oxidative damage. Currently, there is no study in the literature that demonstrates the potential of FFAE when administered to experimental animals. However, there are studies to show that carotenoid accumulations in light-exposed tissues have gained popularity and uses in the cosmetic industries which currently serve as antioxidants and anti-inflammatory purposes for photoprotection amongst others [31,33]. The FFAE *in vitro* demonstrates antioxidants and free scavenging activity with some percentage inhibitions of DPPH, nitric oxide and lipid peroxidation in a dose-dependent manner. Similarly, its ferric reducing antioxidant power also increases when compared with ascorbic acid and gallic acid (Table 4). This implies that FFAE may offer an antioxidant defense to any substance whose presence, even at low concentrations may cause oxidation damage. More so, FFAE was also effective in skeletal and smooth muscle relaxation in mice (Table 5). The traction test, climbing as well as inclined plane show increase reaction times that were comparable to standard drugs used in this study at the doses administered. Although, these methods demonstrated muscle relaxant effects of FFAE, traction test and climbing further scored agility and exploratory movements [26] respectively. More so, an inclined plain assesses the total strength of the forelimbs and hind limbs in order to show energy balance [27]. In this study, a co-administration of FFAE with either a centrally or peripheral neuromuscular drugs did not produce any significant changes in muscle relaxation. Rather, FFAE shows reversibility to the action of pancuronium, suxamethonium and or diazepam. These may, in part, demonstrate possible potentials of the involvement of FFAE in neuromuscular activities with a tendency to interact with skeletal muscle and or GABAergic receptors in laboratory animals. The effect of FFAE was further assessed on neuromuscular activity of a day old chicks (Table 6). Moreover, 10 mg/kg FFAE used in this study demonstrates a potency similar to pancuronium and suxamethonium which showed the quick onset of actions that ranges from twitching (fasciculation) to paralysis. FFAE when combined with either suxamethonium or pancuronium shows delayed onset of neuromuscular blockade. Although, the duration of action was most prolonged with suxamethonium, but, a sustained increased onset and duration of actions were obtained in treated animals. Pupillary contraction in the treated animals was improved following FFAE administration. This ability could afford FFAE to help quench medically related problems associated with reactive oxygen species as well as muscular dysfunction which may damage membranes or proteins that are required for fueling biochemical processes in the body. We explored further the biological compounds in FFAE. Some of the bioactive compounds present in FFAE (Table 4) in large amount include linoleoyl chloride, n-hexadecanoic acid, palmitin, 1,2-di-,2-aminoethyl hydrogen phosphate and methyl esters of lauric acid, tridecanoic acid, tetradecanoic acid respectively. Lauric acid methyl ester, the most abundant compound in FFAE has been associated with synaptosomal-associated protein which mediates methionine-rich polypeptide in rapid axonal transport as well as palmitoylation [42]. Linoleoyl chloride is used as a reagent for the synthesis of fatty acyl glyceric acids such as dilinoleoyl-D-glyceric acid which has potent cytotoxic action [43]. Also, both linoleoyl chloride and methyl tridecanoate involvement in synaptic transmission at neuromuscular junctions have been reported [44]. Additionally, myristic acid, a common saturated fatty

RT	Compound Name	Chemical Formula	Peak Area %
10.473	Dodecanoic acid, methyl ester/Lauric acid, methyl ester	$C_{13}H_{26}O_2$	33.56
23.474	9,12-Octadecadienoyl chloride, (Z,Z)-/Linoleoyl chloride	$C_{18}H_{31}ClO$	14.92
8.443	Tridecanoic acid, methyl ester/Methyl tridecanoate	$C_{14}H_{28}O_2$	11.82
7.412	Tetradecanoic acid, methyl ester/ Myristic acid, methyl ester	$C_{15}H_{30}O_2$	8.93
11.518	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	7.46
21.592	Palmitin, 1,2-di-, 2-aminoethyl hydrogen phosphate	$C_{37}H_{74}NO_8P$	7.18
8.552	Hexadecane	$C_{16}H_{34}$	6.47
22.588	13-Docosenamide, (Z)-/Erucylamide	$C_{22}H_{43}NO$	4.34
12.892	Hexadecanoic acid	$C_{18}H_{36}O_2$	3.30
10.023	Tridecane	$C_{13}H_{28}$	2.02

RT: Reaction Time; GC-MS: Gas-Chromatography/Mass-Spectrometry analysis

Table 4: GC-MS analysis of bioactive compounds present in FFAE.

acid, functionally acts as a lipid anchor in biomembranes [45]. Palmitin enhances secreted internalization of stimulus into motoneuron-like cells [46]. Other compounds in FFAE have been documented for various uses. For instance, n-hexadecanoic acid, erucylamide, palmitin are good sources of antioxidants, anti-inflammatory, antimicrobial, immunosuppressants, antihyperglycemic, antispasmodic and anticancer effects. Comparable neuromuscular actions were observed in mice and chickens despite the different models. Although, at this point, it may be too early to translate FFAE actions into those of clinical drugs, however, further investigation to show the molecular basis of interaction particularly at the neuromuscular system is essential.

Conclusion

Overall, data from this study present novel experimental findings that suggest FFAE as a potential pharmacological agent particularly involved in neuromuscular interactions. Further studies to unravel these actions are therefore essential.

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