African Journal of
Biochemistry and Molecular Biology Research

ISSN : 1595-7926

Index: Harvard, Boston, Sydney University, Dimensions, Lens, ResearchGet Scilit, Semantic, Google, Base etc

https://doi.org/10.58578/AJBMBR.v3i3.12146

# Toxico-Phytochemical Profiling of Hydro-Alcohol *Mangifera indica* Leaf Extract in *Drosophila melanogaster* Harwich Strain

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| Submitted: | Revised: | Accepted: | Published: |
|---|---|---|---|
| Aug 5, 2026 | Sep 2, 2026 | Sep 14, 2026 | Sep 19, 2026 |



**Abstract**

Acute toxicity and median lethal dose ($LD_{50}$) data for *Mangifera indica* leaf extracts in *Drosophila melanogaster* remain limited, constraining evaluation of their biological safety. This study investigated the acute toxicity of crude hydro-alcoholic *Mangifera indica* leaf extract (CHAMILE) in the Harwich strain of *D. melanogaster*. Qualitative phytochemical screening was conducted using standard phytochemical analytical protocols. Acute toxicity was evaluated in 1–3-day-old flies of mixed sexes (n = 225), distributed across nine replicate treatment vials containing 25 flies each and orally exposed for four days to CHAMILE at 0.00, 0.16, 62.50, 125.00, 250.00, 500.00, or 1000.00 mg/10 g corn meal. Daily and cumulative mortality were recorded, while behavioral toxicity was assessed through negative geotaxis deficits and light–dark preference using the Rapid Iterative Negative Geotaxis (RING) and Two-Choice Tube Light–Dark (TTLD) assays. Lethality percentages and corresponding probit values were plotted against log-transformed extract doses using regression analysis based on Finner's table and method, while behavioral data were analyzed using one-way ANOVA at $P < 0.05$. Phytochemical screening detected 12 secondary metabolites, including alkaloids, flavonoids, saponins, and phenolic compounds. All flies exposed to the highest extract concentration died within 72 hours, and the estimated $LD_{50}$ was 524 mg/10 g corn meal. Significant wall-climbing deficits were also observed in the behavioral assessments. These findings indicate dose-related acute toxicity and behavioral impairment associated with high-dose



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CHAMILE exposure in *D. melanogaster*. The detected bioactive compounds may contribute to the observed toxic and physiological effects. This study may represent an initial $LD_{50}$ assessment of *M. indica* leaf extract in *D. melanogaster* and provides preliminary toxicological evidence supporting further safety profiling of different *M. indica* plant parts across *D. melanogaster* strains before broader toxicological implications are considered.

**Keywords**: Acute Toxicity; *Drosophila melanogaster*; $LD_{50}$; *Mangifera indica*; Phytochemical Screening

## INTRODUCTION

*Mangifera indica* (MI), commonly known as mango in English and mangoro by most Nigerian ethnicities, of the plant class Magnoliopsida and plant family Anacardiaceae, is the commonest of the genus Mangifera [1]. This evergreen-leaf and edible fruit-producing flowering Tropical plant, whose height increases with age from a few meters when young and tender to hundreds of meters as it advances in age [2], is widely distributed throughout the world where it often exists as mixed cultivated and wild, and its nutritional and medicinal uses recognized [3], [4], [5], [6].

Apart from the nutritional usefulness for which its fruit is well known [3], [4], [5], [7], the several disease spectrums for which the root, bark, fruit and especially leaf extracts are ethnomedicinally effective include abdominal colicky pain, depression, diabetes mellitus, diarrhea, dysentery, indigestion and dyspepsia, respiratory infections, soar throat, earache, sepsis and abscesses. Others are hepatitis, wounds and skin eczema, hypertension, cancers, malaria, sexual transmitted diseases, bone fractures, snake bites, insect and scorpion stings, menorrhagia, dog bite, irregular, heavy and painful menses, calonorrhagia etc.[8], [9], [10], [11]. Analgesic, antimicrobial, vasodilatory and anti-hypertensive, antineoplastic, antifungal and antiviral, wound-healing, ulcer-healing, antidiarrhea, anti-inflammatory, antidepressant, antihyperglycaemic activities are some of the pharmacological activities attributed to extracts/preparations from different parts of MI.

Most toxicological evaluations on MI extracts which have been largely carried out in rodents indicate the plant may be well tolerated. Previous acute toxicity assessments in rodents show mild or nil toxicity with the LD50s of *Mangifera indica* leaf, fruit, seed kernel,

bark, and root being > 2000 mg/kg body weight [12], [13], [14][15], [16], [17], and in most cases with no gross or behavioural toxic signs observed. Similarly, administrations of extracts from different parts of the plant to rodents at 100 - 1000 mg/kg body weight for durations ranging from 28-90 days did no show significant gross, behavioural or biochemical changes in the experimental animals [13], [14], [15], [16], [17], [18]. But a previous acute exposure safety evaluation of an MI seed extract in *Clarias gariepinus* fish juveniles indicate a 96-hour $LC_{50}$ of MI extract on the fish was 0.7079 g/L – with some deleterious histological lesions observed on fish - suggesting MI seed extract may not have been well tolerated orally by this fish specie.

These pharmacological and toxicological properties of MI extracts are believed be due to the rich presence of several vital bioactive compounds such as mangiferin, flavonoids, flavones, polyphenolic compounds, essential oils, terpenes, sterols, saponins, tannins, alkaloids, structural carbohydrates, key amino acids, organic acids, fatty acids, vitamins, minerals [8], [9], [10], [19], [20], [21], [22] in them.

However, evaluation of MI toxicity in DM is scanty. Our literature search showed only one study had so far been carried out on this subject, in which Pam et al. (2021) found the LC50 of crude MI stem bark extract to be 373 mg/10 g corn meal – with the extract exerting some reproductive, locomotory and oxidative toxic effects [23]

In view of the numerous ethnopharmacological activities of MI extracts – particularly leaf, and the advantages of Drosophila model for most human disorders - some of which were slated to be investigated in our lab, there was a need to determine MI acute toxicity and its LD50 for a benchmark and to aid our dose selection for subsequent efficacy and chronic toxicity studies. Thus, this study's aim was to determine acute toxicity, the LD50 and relevant locomotory toxicity in DM Harwich strain and the phytochemical composition of CHAMILE.

## MATERIALS & METHODS

### Materials

Materials used in this study are as follows:

i. *Drosophila melanogaster* Harwich strain

ii. *Mangifera indica* leaves

iii. Standard corn meal ingredients [a full load of which comprised dry maize flour 100 g, methyl paraben 4 g, bakers' yeast 20 g, and aga-agar 10 g].
iv. Distilled water, absolute ethanol, and routine lab hard wares

## Methodology

### Plant harvesting, preparation & extraction

Fresh MI (mango) leaves were harvested at about 1400 HRS from a MI tree within the University of Abuja Teaching Hospital, Gwagwalada Abuja, Federal Capital Territory, Nigeria in the month of January 2026. Immediately thereafter, the leaves were briskly washed with clean water and subsequently spread for air-drying on well cross-ventilated platforms within the Department of Pharmacology & Therapeutics laboratory within the same teaching hospital. The leaves were allowed to dry completely before it was pulverised into fine green powder. About 350 g of this powder was later macerated into a 4-L mixture of 70% distilled water-absolute ethanol for 72 hours with intermittent shaking of the mixture. At 72 hours the mixture was filtered with Whatman's filter paper and the filtrate air-dried with assisted mechanical fanning to a dry chocolatey brown CHAMILE paste weighing 45.8 g (13.1%). This was kept in a brown plastic container till use.

### Preliminary Phytochemical Screening

Qualitative phytochemical screening of CHAMILE was conducted using the underlisted standard procedures as outlined by the World Health Organization (WHO, 2011) and Trease & Evans' *Pharmacognosy* (16th ed.) employing established pharmacognostic methods. Outcomes of all assays performed on CHAMILE were judged as negative or positive for the metabolite being tested based on the observation or lack of observation of characteristic indicators, which might be color changes, precipitate formation, frothing, ring development, and oily stains, etc.

Three pre-assay CHAMILE stock solutions were made – acidic, neutral and alcohol. Acidic CHAMILE preparation for the phytochemical screening was made by dissolving 1g portion of the crude extract in 6ml of diluted hydrochloric acid (HCL)and filtered. The filtrate was divided into portions for the screening of alkaloids, flavonoids and glycosides in the study.

Aqueous CHAMILE stock solution was made by dissolving 1g of the crude extract in 75ml of distilled water and filtered. The filtrate was used to conduct the screening of

tannins, proteins, carbohydrates, saponins, resin, phenolic compounds and cardiac glycosides. Phytochemical screened for in this study.

Alcohol CHAMILE stock solution preparation was constituted by dissolving 1 g of CHAMIE in 20ml of methanol and the mixture filtered. The filtrate was divided into portions to screen CHAMILE for the presence of terpenoids, steroids, anthraquinones, Phlabotamins, .

**Test for Alkaloids**

Mayer's test was done to determine presence of alkaloids in CHAMILE. To 1 - 2 ml of acidified CHAMILE in a test tube, 3 drops of Mayer's reagent were added along the inner walls of the test tube and gently shake the mixture to combine. The immediate development of a creamy-white or pale-yellow precipitate indicated the presence of alkaloids. Alkaloids were absent if the solution remained clear or only showed slight, transparent turbidity without a distinct precipitate

**Test for Flavonoids**

Alkaline reagent or Sodium hydroxide test: 2-3 drops of 10% NaOH solution were added to 2 mL of CHAMILE. The immediate formation of an intense yellow color indicated the presence of flavonoids.

**Test for Glycosides**

General glycoside test: 2ml of HAMI was neutralized with 2ml of dilute sodium hydroxide solution in a test tube. 2ml of Fehling's solution was then added and heated for 5 minutes. Formation of a rick red precipitate indicated the presence of glycosides.

Test for Tannins

Braeners test: To 2ml of crude CHAMIE extract, 2ml of 10% alcoholic ferric chloride solution was added. Formation of a blue black coloration indicated presence of tannins.

**Test for Carbohydrate**

Molisch test: To 2ml of CHAMILE extract 3 drops of Molisch reagent were added and shaken then 3 drops of concentrated H2SO4 were added to the test tube wall slowly. A purple or violet ring forming at the junction of the two layers confirmed the presence of carbohydrates in the extract.

**Test for Saponins**

Froth test: To 2ml of the crude CHAMILE extract in a test tube, 5ml of distilled water was added and was shaken vigorously for 5 mins, formation of persistent froth that lasted for 15 minutes clearly indicated the presence of saponins.

**Test for Proteins**

Biuret Test: To 2 ml of crude CHAMILE extract in a test tube, 2 ml of sodium hydroxide solution (dilute, about 10%) was added. Then 3 drops of copper (II) sulfate solution were added. The mixture was fairly shaken well. A change in color from blue to pink, purple, or violet indicated the presence of proteins.

**Cardiac glycoside**

Killiar kiliani: Evaporate 2 ml of CHAMILE to dryness in a test tube. Then the dried residue was dissolved in 2 mL of glacial acetic acid, then 1 drop of a 5% ferric chloride solution was added, and the mixture was gently under layered with 1 mL of concentrated sulfuric acid ($H_2SO_4$) along the side of the test tube. Formation of a distinct brown, blue or blue-green ring at the interface of the two layers indicated the presence of a cardic glycosides.

**Test for Phenolic Compounds**

Ferric Chloride Test: 2ml of CHAMILE neutral was treated with 3 drops of ferric chloride solution. The appearance of a deep blue coloration clearly indicated the presence of phenolic compounds.

**Test for Resin**

Copper Acetate Test: 3 drops of copper acetate solution were added to CHAMILE in a test tube and the mixture was shaken vigorously for 30 seconds and then allowed to settle or separate into phases. Observation of an emerald green or deep green colored solution indicated the presence of resins or diterpenes. Failure of the original extract-mixture colour to change or a green colour to form (i.e., a green colour did not form) in the process, indicated no resin in CHAMILE.

**Test for Terpenoids**

Salkowski test: 2ml of CHAMILE was mixed with 1ml chloroform, followed by careful addition of 2ml concentrated sulfuric acid along the side of the test tube. Observation of a reddish brown interface indicated the presence of terpenoids.

**Test for Steroid**

Salkowski test: 2 ml of CHAMILE was mixed with 2 ml of chloroform. 2 ml of concentrated sulfuric acid ($H_2SO_4$) was then gently added to the mixture along the side of the test tube. The was then shaken well and finally allowed to stand, Formation of a red/brown chloroform layer and greenish-yellow fluorescence acid layer indicated presence of steroids.

**Test for Anthraquinones**

Borntrager's test: To 2 ml of CHAMILE, 2 ml of dilute hydrochloric acid. The mixture was boiled on a water bath for about 10 minutes and then filtered while hot. The filtrate was transferred to a separatory funnel and partitioned with 2 ml of chloroform (carbon tetrachloride or benzene). The resulting mixture was shaken well for 1 minute and thereafter allowed to settle. Finally, the upper organic (chloroform) layer was collected and an equal volume to the collected mixture of ammonia (10%) or NaOH was added and the mixture gently shaken and then finally rested. The formation of a pink, red, or violet color in the lower aqueous/ammoniacal layer indicated the presence of anthraquinones. Failure of this mixture to develop this colouration indicated absence of anthraquinones.

**Test for Volatile oil**

NaOH & HCl test: To 2ml of CHAMILE 0.1 ml of diluted NaOH and 0.1 ml of diluted HCL were serially added in that order in a test tube which was then shaken together. Formation of a white precipitate indicated presence of volatile oils.

**Test for Phlobatannins**

To 2ml of CHAMILE 2ml diluted hydrochloric acid was added and boiled. A few drops of the CHAMILE from the stock solution to 2 mL of 1-2% aqueous hydrochloric acid (HCl). The mixture in the test tube was boiled in bath water for 5-15 minutes. The test tube was then carefully removed and observed. Formation of a red-colored precipitate or sediment at the bottom indicated presence of phlobatannins while maintenance of the

original mixture colour or failure of the mixture to form a clear reddish colour indicated absence of phlobatannins.

**Drosophila strain culture & study conditions**

*Drosophila melanogaster* Harwich strain wild-type (1 to 3-day olds; both sexes raised on standard corn meal and cultured in standard bottle vials were used for this study. They were kept at a $26\pm1^{0C}$ room temperature, about 60% humidity and under 12h/12hr light/dark cycles in the Fly lab extension of the Department of Pharmacology & Therapeutics, Faculty of Basic Clinical Sciences of the College of Health Sciences, Yakubu Gowon University (formerly University of Abuja) Abuja, Nigeria. The corn meal consisted of appropriate composition of yellow corn starch, bakers' yeast, agar-agar and methyl paraben sourced from local reputable suppliers.

**Extract acute toxicity testing**

This was carried out between 800-1800 HRS in the first week of February 2026 in the Drosophila Fly lab extension of the Department of Pharmacology & Therapeutics of the University of Abuja (aka Yakubu Gowon University) Abuja. Briefly, groups (n = 25; N = 100) of *Drosophila* flies were exposed to a standard treatment vial containing 0.00, 0.01, 62.50, 125.00, 250.00, 500.00 or 1000 mg extract per 10.0 g corn meal for four days. Flies in vials were acutely observed for the first 24 hours for any gross behavioural defects (i.e., immobility, lying on the back, reduced flying, aggression) and mortalities in the treatment vials compared to control groups. For the rest of the test lethality and behaviours were observed and recorded accordingly in all vials.

**Behavioural assessment of extract's potential toxic on fly locomotion**

Behavioural effects of the extract on the experimental flies were carried out at the end of four (4) completed days. Extract's potential toxicity on the neuromuscular system of the experimental flies was determined at the end of the 4-day acute toxicity testing using two different iterative Drosophila melanogaster behavioural assays - the rapid iterative negative geotaxis (RING) and the Two-choice tube light-dark (TTLD) assays. Both have been shown to be effective in investigating Drosophila melanogaster behaviors, especially its locomotor activity [24], [25] – and were adopted in this study with minor modifications. In brief, for the RING assay 60 flies for each acute toxicity treatment group were sorted under anaesthesia from the larger pool into 6 vials of 10 containing equal number of sexes. In this way, each assay batch contained six 100-ml test tubes – one for each treatment dose

group except for the 1000 mg/10 g fly corn meal group in which flies had all died 72 hours into the study. These flies-containing test tubes were mounted firmly into the holes of a plastic rack which was subsequently raised and its flat base uniformly hit on a flat surface so that all the flies in the tubes would fall to their bottoms. Fractions of the treated and untreated fly groups which crossed a 9-cm high landmark on the test tubes within a specified time, 8 seconds, was recorded and compared. For the TTLD assay, two types of behavioural data – locomotor and % light-dark distribution parameters can be generated from the same fly samples in the apparatus. The latter parameter is generated by simply making the device to lie horizontal to make the light-dark portion to be level with each other. The distribution/preference of the flies in a sample is simply captured at intervals. In brief, 40 flies (equal sexes) in 4 batches of 10 were manoeuvred into a locally fabricated 20-cm long 250-ml Drosophila melanogaster TTLD apparatus partitioned into two equal light-dark parts. Each batch of 10 flies uploaded into the tube apparatus was allowed to acclimatize to the new environment for 5 minutes before percent light-dark fly distributions were video-taped every 5 minutes for 3 readings. 5-10 minutes following completion of fly light-dark distribution aspect of this assay, locomotor activity of the same samples was assessed by standing the behavioural tool vertically upright with its dark portion uppermost. This was done until all flies in a sample moved by negative geotaxis into the dark part of the tube. Following this, the vertical positions of the parts were quickly flipped iteratively. Fractions of flies in treated and untreated groups that attained a landmark 2 cm to top of the light part of tube within 5 seconds were video-recorded and compared. Each procedure was repeated 3 times and the mean percent light-dark distributions in the test apparatus of treated and untreated fly groups were thus recorded and compared.

**Acute toxicity, LD50 and other behavioural data analysis**

Regression analysis of CHAMILE treatment group probit mortalities versus log doses per 10 g of corn meal (Table 2) was made sing Finney's table, method and formula of probabilities [26] where Y = ax + b, where Y = probit mortality, x = log dose, a = log dose variable (gradient) and b = Y intercept. Data from RING and TTLD assays were expressed as percentages (%) and means±S.E.M. Comparisons of data were made using one-way ANOVA of IBM SPSS statistics 2.0 software with *P* value was set at 0.05.

## RESULTS

### Phytochemical analysis results

Out of the 16 secondary metabolites screened for, presence of alkaloids, flavonoids, tannins, saponins, glycosides, cardiac glycosides, terpenoids, phenolic compounds, anthraquinone, carbohydrates, proteins and steroids were detected while resin, phlobatannins, anthraquinone and volatile oils were not detected in CHAMIE (Table 1).

**Table 1: Secondary metabolites in CHAMIE**

| S/n | Secondary metabolites | Result |
|---|---|---|
| 1 | Alkaloids | + |
| 2 | Flavonoids | + |
| 3 | Tannins | + |
| 4 | Saponin | + |
| 5 | Glycosides | + |
| 6 | Cardiac glycoside | + |
| 7 | Terpenoid | + |
| 8 | Phenolic compounds | + |
| 9 | Anthraquinone | - |
| 10 | Volatile oil | - |
| 11 | Carbohydrate | + |
| 12 | Protein | + |
| 13 | Resin | - |
| 14 | Steroid | + |
| 15 | Phlobatannins | - |

### Acute toxicity testing results

### Lethality results

All flies exposed to 1000 mg CHAMIE/10 g of corn diet died within 72 hours of the acute toxicity study (Table 2). CHAMIE Dose-response regression analysis yielded a Y-intercept, b, of 2.83, and dose variable, a, of 0.80, and an LD50 of 524.00 mg/10 g of corn diet.

### Locomotor and other behavioural toxicity result

Significant wall climbing deficits were observed in the 500 mg/10 g treatment flies compared to the normal controls and other treatment groups (Table 3).

There were no significant differences in the light preference behaviours in the treatment compared to the control groups (Table 3).

**Table 2: CHAMIE acute exposure doses and mortalities**

| S/n | Dose (mg/ 10g diet) | Log Dose /10 g | Total Rx group observations | Rx group Mort Probit values |
|---|---|---|---|---|
| 1 | 1000.00 | 3.00 | 225.00 | 8.72 |
| 2 | 500.00 | 2.70 | 225.00 | 4.06 |
| 3 | 250.00 | 2.40 | 225.00 | 3.56 |
| 4 | 125.00 | 2.10 | 225.00 | 3.35 |
| 5 | 62.50 | 1.80 | 225.00 | 3.19 |
| 6 | 0.16 | -0.80 | 225.00 | 3.08 |
| 7 | 0.00 | - | 225.00 | 3.08 |

(Rx, treatment; Mort, Mortality)

**Table 3: Effect of acute doses of CHAMIE on flies' wall climbing & light preference.**

| S/n | Dose (mg/ 10g diet) | RING assay % wall climbing | Two-choice tube light-dark (TTLD) assay | |
|---|---|---|---|---|
| | | | % light preference | % wall climbing |
| 1 | 0.00 | 95.83±2.28 | 75.00±4.69 | 83.33±4.32 |
| 2 | 0.16 | 99.17±0.83 | 76.67±6.07 | 90.83±3.98 |
| 3 | 62.50 | 97.50±1.79 | 68.33±5.05 | 89.17±3.98 |
| 4 | 125.00 | 96.67±2.25 | 77.50±3.29 | 88.33±3.44 |
| 5 | 250.00 | 94.17±3.36 | 64.17±5.43 | 85.83±4.34 |
| 6 | 500.00 | 65.83±5.14* | 77.50±5.66 | 60.83±5.14* |
| 7 | 1000.00 | - | - | - |

(*= significant, level of significance $P \leq 0.05$; RING, Rapid Negative Iterative Geotaxis; -, all group samples died within 72 of study]

## DISCUSSION

Our study's first significant finding appears to be the high (total) lethality at 1000 mg/10 g corn meal of the extract. This was unexpected judging from the general presumption of safety of MI (Mango) along with its edible and inedible products. This finding suggests our deliberate choice of wide graded acute toxicity dose ranges from zero to 1000 mg extract/10 g clearly paid off. The 4-day LD50 of 524 mg/10 g corn meal for CHAMILE may be the first or one of the few reports on the toxicity of MI leaf extracts in DM particularly Harwich strains as far as our literature search revealed. CHAMILE LD50

of 540 mg/10 g corn meal is comparable to a 7-day 373 mg/10 g corn meal LD50 found for crude MI stem extract in one of the only two previous MI toxicity studies found for DM [23]. It is reasonable to expect CHAMILE acute toxicity (and LD50) may approach, equal or even surpass crude stem extract toxicity (or LD50) were our study extended to 7-day used for toxicity testing of the latter extract. Apart from potential toxicity indicated by their limited LD50 values in both our and previous studies in DM, there are other findings supporting the fact that high or even moderate extract doses MI extracts may not be very safe in Drosophila flies and potentially in humans. Our study found significant wall climbing deficits at 500 mg/10 g corn meal level as evaluated by RING and TTLD assays, suggesting CHAMILE exposure may be associated with neurobehavioural toxicities in the experimental DM. Likewise, Pam et al. (2021) observed a dose-dependent negative impact of MI crude stem extract on the experimental fly in-vivo antioxidant profile, longevity, reproduction and wall climbing [23]. This latter finding points to a more systemic toxicity potential of high doses of MI extracts.

However, above-stated toxicity profiling of our crude leaf and previous stem MI extracts may be different from one or two available studies in DM and Brine shrimp, as well as several available toxicity studies in rodents. A previous in-*vivo* Somatic Mutation and Recombination Test (SMART) study of aqueous MI leaf extract showed no significant genotoxicity/cytotoxicity risks but rather cytoprotective activity in experimental DM flies and rats [27]. Similarly, aqueous and ethanol MI leaf extract has been previously shown to be non-toxic to Brine shrimp larvae and exhibiting an LD50 equal or greater than 2000 - 5000 mg/1 kg body weight in rats [28], [29], [30], [31]. The lesson from all this is that more toxicity profiling of different medicinal parts of MI is indicated in this Harwich and other strains of the fly to form a broad information pool for future research/academic benchmarking.

Nevertheless, a key goal of this study was achieved in the determination of the CHAMILE LD50 for the first time in Drosophila melanogaster. This value would be a critical guide in dose selection in subsequent efficacy and other studies.

Another key study goal was the qualitative profiling of CHAMILE to give an idea of the bioactivity and biodiversity present in the crude extract. Qualitative screening of CHAMILE indicates the presence of 12 important classes of secondary metabolites including alkaloids, flavonoids, saponins, cardiac glycosides, terpenoids and phenolic compounds. The rich qualitative phytochemical profile obtained from our study is similar

to findings from previous studies indicating MI leaf extracts possession of potent bioactive compounds such as phenolics (e.g., mangiferin) and flavonoids that may be account for most of the ethnomedicinal and ethnopharmacological activities of MI leaf extracts [5], [32], [33], [34], [35]

## CONCLUSION

This study found CHAMILE LD50 was estimated to be 524 mg/10 g corn meal in DM Harwich strains. High doses may be potentially lethal and toxic to experimental flies. MI ethnomedicinal, pharmacological, and toxic activities may be due to several secondary metabolites detected in the crude extract. There is the need to intensify/broaden toxicity profiling of MI and its products in several DM strains.

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