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Toxicity Studies on Anti-fungal Essential Oils Extracted from Selected Aromatic Plants from Mabira and Kakamega Forests, East Africa

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Authors' contributions

This work was carried out in collaboration between all authors. Author JLN supervised the toxicity studies, was involved in aromatic plant collection; designed the study, managed the literature searches and prepared the manuscript drafts. Authors JCM and INW were involved in aromatic plant collection. Authors JK and LA were involved in toxicity studies. All the authors have read and approved the final manuscript.

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ABSTRACT

Aim: In a bid to explore for grain bio-preservatives, essential oils extracted from *Cymbopogon citratus*, *Rosmarinus officinalis*, *Monanthataxis littoralis* and *Aframomum angustifolium*, that were earlier established to have anti-mold activity, were evaluated for their biosafety by determining the oral LD_{50}.
Methods: The essential oils were extracted by hydro-distillation from aromatic plants collected from Kakamega and Mabira forests in Kenya and Uganda, respectively. Acute oral toxicity was established using mice by determining the LD_{50}; after which sub-acute toxicity studies were performed. The animals were observed for behavioural changes; and the gross and

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histopathological effects, if any, on the intestinal mucosa, spleen, lungs, liver, kidney and heart were noted.
Results: The oral LD_{50} for *Cymbopogon citratus*, *Rosmarinus officinalis*, *Monanthataxis littoralis* and *Aframomum angustifolium* essential oils were established as 7,046.90; 4,723.33; 13,335.82; and 17,539.82 (mg/kg body weight), respectively. In all cases, increased breathing rates were observed, however Rosemary also caused lethargy and convulsions. Grossly, no changes were seen in the liver, kidney, lungs, heart and spleen from both the control and the treated mice except for *Monanthataxis littoralis* where the lungs and liver seemed changed; and the urinary bladder distended. However, the latter effects were noted at higher doses than the established oral LD_{50}. Histopathologically, thickened intestinal mucosa lining; tubular degeneration and proteinuria in the kidneys; vascular congestion, focal necrosis and hydropic degeneration of hepatocytes in the liver, were encountered.
Conclusion: Basing on the oral LD_{50} in mice, all oils were safe and can be explored further as anti-mold grain preservatives. However, *Rosmarinus officinalis* was marginally safe as per the OECD guidelines. The histopathological effects of *Monanthataxis littoralis* essential oil need to be investigated further.

Keywords: LD_{50}; Cymbopogon citrates; Rosmarinus officinalis; Monanthataxis littoralis; Aframomum angustifolium.

1. INTRODUCTION

Since time immemorial, several plants and/or their products have been used in folk medicine to treat several medical complications [1-3]. The same plant products could be put to several new uses, including preservation and control of post-harvest losses [4,5]. Various researchers have ascertained the potential benefit of using essential oils in the control of microbial contamination of foods [6-19]. The essential oils could effectively replace synthetic chemicals, which are becoming unpopular due to global environmental and public health concern, in controlling pest and microbial spoilage of agricultural produce. Plant derived products as disease control agents at pre- and post- harvest stages exist; and tend to have low mammalian toxicity, less environmental effects and are widely publically accepted [20-22]. However, due to safety concerns arising from use of some plants and/or pure extracts of their components, there is need for comprehensive assessment of the toxic, environmental and economic consequences of these alternatives.

Cymbopogon citratus, commonly referred to as lemon grass, is a herbaceous plant widely used as a flavouring agent and possess various pharmacological activities [23]. Lemon grass extracts are generally considered safe since oral toxicity LD_{50} in rats of >1500 mg/g and dermal toxicity (LD_{50}) on rabbit skin of >5000 mg/kg has been reported [24]. Fandohan et al. reported functional damage to the stomach and liver of rats when essential oils were administered at doses >1500 mg/kg, however in general, a no adverse effect level (NOAEL) was established [25,26]. Chronic ingestion did not also have any adverse effect, and the treated rats gained more body weight in comparison to the untreated group [27]. In addition, low toxicity was reported in rats exposed to fresh leaf extracts of the lemon grass [28]. However, some of the hazards reported elsewhere include skin and eye irritation [24].

Rosmarinus officinalis, in addition to its utilization as a food spice, is widely used in folk medicine for treatment of liver, intestinal, renal and respiratory problems. Fahim et al. reported toxicity of rosemary essential oil as having a LD_{50} of 5.5 g/kg BW intragastrically in rats, and a lethal effect on all animals at an intragastric dose of 0.9 g/100 g BW [29]. Chronic exposure to rosemary oil in high concentration has been reported to cause contact dermatitis; and is contraindicated in pregnancy due to embryo toxic effects [9,30]. Different rosemary extracts exhibited NOAEL values in the range of 180 to 400 mg extract/kg BW/day equivalent, but this depended on the carnosol and carnosic acid content of the respective extracts. Ingestion of 20-60 mg/kg BW/day of carnosol plus carnosic acid is considered safe [31]. In this context, the chemical composition of rosemary plants from different ecological regions need to be established since this could influence their toxicity.

Aframomum angustifolium is a perennial herb indigenous in Uganda. The fruits and seeds are

gathered from the wild for medicinal and ethnodietary purposes [32]. However, no reports of toxicity has been claimed. In addition, no safety reference data exist for *Aframomum angustifolium* although the oil is documented to be in the category of flavor and fragrance agents with antioxidant and antimicrobial activity [24]. The seeds of a related species, *A. melegnata*, contain piperine alkaloid that resulted in blurred vision among humans and modified sexual behavior of male rats [33].

Previous studies demonstrated growth-inhibitory activity against grain fungi by the essential oils from *Cymbopogon citratus*, *Rosmarinus officinalis*, *Monanthataxis littoralis* and *Aframomum angustifolium* [14,18]. With the aim of sourcing for a safe and efficient product for use as a bio-preservative for grains destined for human food and animal feed processing, there was need to assess and evaluate the toxicity of these essential oils. Information on toxicity of the essential oils extracted from the plants of interest is scanty or non-existent; more especially so for *Monanthataxis littoralis*. In addition, variations in toxicity depending on plant species or variety and geographical region have been reported. This study was therefore set out to establish the toxicity of the essential oils of *Cymbopogon citratus*, *Rosmarinus officinalis*, *Monanthataxis littoralis* and *Aframomum angustifolium*, by establishing their acute oral (LD_{50}) and sub-acute oral toxicity effects, before recommendation for use in controlling the grain molds and associated mycotoxins.

2. MATERIALS AND METHODS

2.1 Source of Essential Oils

The essential oils that showed growth-inhibitory activity against molds in previous studies were selected for evaluation of their toxicity to the mammalian host. Fresh leaves of the selected aromatic plants were collected from Kakamega and Mabira forests during April-May (rainy season) and subjected to steam distillation for extraction of the essential oils. The plants included *Cymbopogon citratus* Stapf, *Rosmarinus officinalis* L., *Monanthotaxis littoralis (*Bagshawe & Bak. f.) Verdc. Annonaceae; and *Aframomum angustifolium* (Sonn.) K. Schum. For *Cymbopogon citratus* and *Rosmarinus officinalis*, voucher specimens were deposited for identification at the Department of Biological Sciences, Egerton University; while for *Monanthataxis littoralis* and *Aframomum angustifolium* this was done at the Department of Botany, Makerere University. The essential oil yields for *Cymbopogon citratus*, *Rosmarinus officinalis*, *Monanthotaxis littoralis* and *Aframomum angustifolium* were on average, 0.58%, 0.48%, 0.05% and 0.32% v/w, respectively.

2.2 Experimental Animals

Mice weighing 22-24 g were obtained from the animal house at the College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University; and were used to establish the acute and sub-acute oral toxicity effects of the oils. Instead of rats that are recommended by the OECD [34], mice were used due to the limitations in availability of the essential oils. The mice were housed in standard cages, under closely monitored conditions (humidity of 50-70%, temperature at 24±2℃, 12 hours day light and 12 hours darkness) and fed on a special enriched diet. Before the experiments, the animals were placed in particular cages and allowed to acclimatize for three days. The mice were fasted but allowed to have water during the 12 hours before the experiment. Oral administration of the oil in ½ mL doses at various concentrations was done by gavage; where the oil was directly introduced into the stomach through gastric tubing.

2.3 Acute Oral Toxicity Study

2.3.1 Preliminary estimation of the LD_{50}

Preliminary estimation of the LD_{50} was done as described by Akhila et al. [35]. The oils were administered orally to pairs of mice in ascending and widely spaced doses. The mice were observed for four hours, and finally overnight mortality was recorded. Doses killing one out of two mice in such experiments gave an approximate estimate of LD_{50}. The lowest dose which killed one animal and the highest dose which did not kill any animal were noted, and their geographical means were calculated to obtain the tentative LD_{50}.

2.3.2 Determination of the LD_{50} by the probit method

The proper LD_{50} and the standard error were established by the graphical method of Miller and Tainter (Probit) [36]. Seven dose levels chosen basing on the tentative LD_{50}, that is; three dilutions above and four below it were used. Groups of six mice each, constituted by equal

number of males and females were orally administered with the different doses of the oil diluted with 2% Tween 80®, but a control group was administered with the diluent. The animals were observed for behavioral changes; any other sign of toxicity and for mortality; and the number of the dead mice per group within 24 hours were noted. The dead mice had their vital organs subjected to gross and histopathological examinations.

2.4 Sub-acute Toxicity

Sub-acute toxicity studies were performed as recommended by OECD testing guidelines [34]. Six animals were used for each test group. Four separate double dilutions below the actual LD_{50} value were prepared. Test mice were divided into five groups comprising six randomly selected animals each, with equal numbers of males and females. The females were all non-pregnant and nulliparous. Each group was orally administered with a particular test dilution, daily for 28 days; as described above. The fifth group received 2% Tween 80® (negative control). Moribund animals and those that survived to the 28th day were sacrificed and the vital organs, that is, liver, kidneys, lungs, heart, intestines, stomach and brain; were removed, examined macroscopically for any lesions and then fixed in 10% formalin for histopathological examination.

3. RESULTS

3.1 The LD_{50} and the General Appearance and Behavioural Observations

The tentative and Probit LD_{50} doses; and the behavioural changes associated with essential oils from *Cymbopogon citratus*, *Rosmarinus officinalis*, *Monanthataxis littoralis* and *Aframomum angustifolium*; as established by oral administration to mice are presented in Table 1. *Rosmarinus officinalis* oil seemed to be marginally safe as compared to the others tested.

3.2 Toxicity of *Cymbopogon citratus* Essential Oil and the Associated Behavioral and Pathological Changes

Mice exposed to the EO presented dose-dependent alterations in general behavior. Those exposed to 32,000 mg/kg and 16,000 mg/kg showed increased breathing rates within the first hour of administration; were lethargic while the lower doses and the control did not exhibit any of such changes.

For the 32,000 mg/kg group, all mice died; five died for the 16,000 mg/kg group; while one died for the 8,000 mg/kg group; within the 24-h period. There were no deaths at lower concentrations. The tentative LD_{50} for EO was estimated as 8,105 mg/kg BW while the calculated LD_{50} by the Probit test was 7,046.9±3,798.6 mg/kg BW.

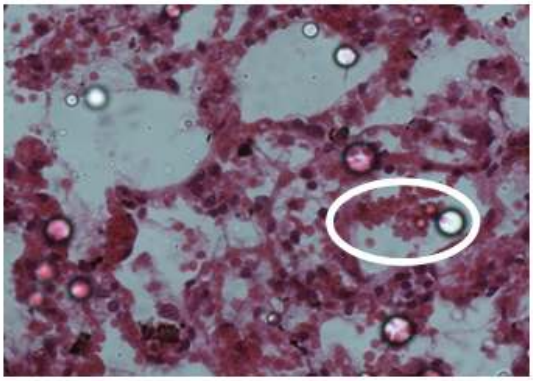
There were no substantial gross lesions in the selected tissues of mice that were exposed to the EO. In addition, there were no major histological changes in the brain, heart and spleen except at the highest dose (32,000 mg/kg BW); where degeneration of the neurons in the white matter; vascular constriction; and increased number of macrophages and foreign body giant cells occurred in the different organs.

From the acute-toxicity studies, dead or euthanized mice had multi-focal heamorrhages and peri-bronchial mono-nuclear infiltration in the lungs. In addition, multi-focal and diffuse heamorrhages occurred in the kidneys, whereas squamous metaplasia and diffuse mucosal infiltration were seen in the intestines. Following the 28-day exposure, no effects were seen in the mice exposed at the test doses, that is, < 8,000 mg/kg BW. However, as presented in Fig. 1, histological changes were seen in the lungs, liver, kidney and intestines. Emphysema, vascular congestion, lymphatic infiltration and haemorrhages were encountered in the lungs in mice administered with 8,000 mg/kg BW and above. In the liver, diffuse hydropic degeneration, vascular congestion and focal necrosis was seen in mice administered with doses of 8,000 mg/kg BW and above, but severity was dose-dependent. In the kidney, vessel constriction, tubular degeneration and proteinuria, focal lymphatic infiltration were observed, but no significant changes were seen at doses less than 8,000 mg/kg BW.

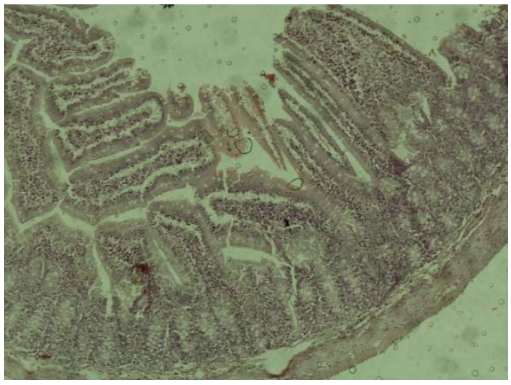
3.3 Toxicity of *Rosmarinus officinalis* Essential Oil and the Associated Behavioral and Pathological Changes

During acute toxicity studies, the dose-dependent behavioural changes that were observed for *Rosmarinus officinalis* essential oil included lethargy, convulsions and spasms, pilo-erection, ataxia, vertigo, increased heart rate, loss of appetite and respiratory failure. After 24 h, death was observed in all mice at 8,500 mg/kg;

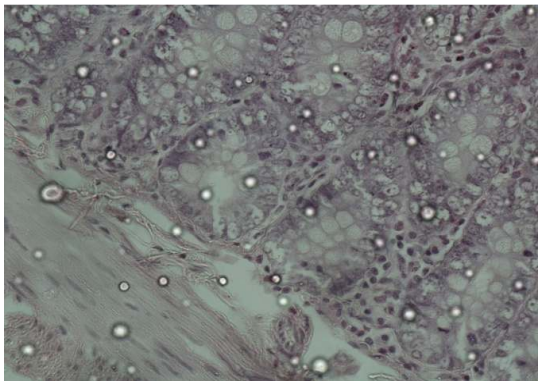
five at both the 7,500 and 6,500 mg/kg groups; four at 5,500 mg/kg; three at 4,500 mg/kg, one at 3,500 mg/kg while none died at 2,500 mg/kg. The tentative LD_{50} was estimated as 5,943.92 mg/kg BW while the calculated LD_{50} by the Probit test was 4,723.33±1027.2 mg/kg.



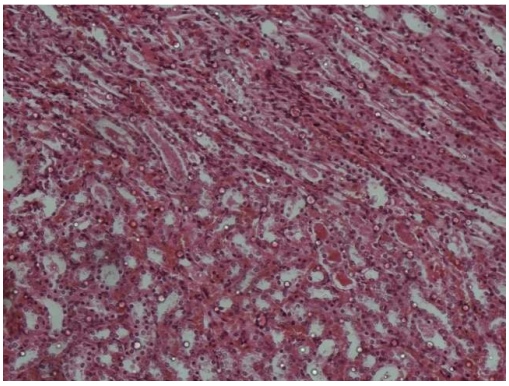
Lung ×3,300: Note RBCs in air sac (white circle)



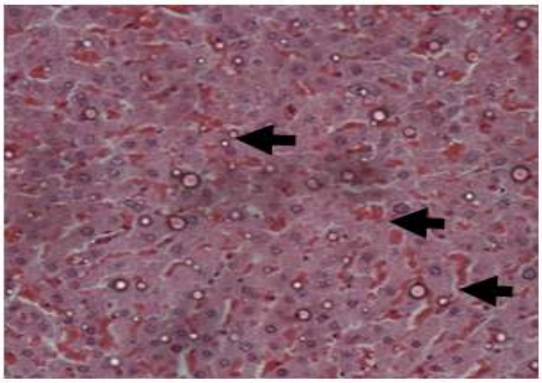
Intestine × 550: Note the thickened mucosal lining



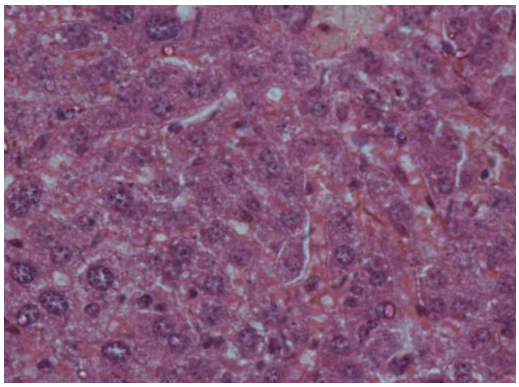
Intestine × 2,200: Sub-chronic exposure resulted in bloated cells



Kidney × 1,100:Tubular degeneration and proteinuria, and vessel constriction



Liver × 1,100: Vascular congestion (arrows), focal necrosis, diffuse hydropic degeneration



Normal liver × 3,300

Fig. 1. Histopathological changes in the lungs, intestines, kidneys and liver tissues following oral exposure of *Cymbopogon citratus* EO to mice (at doses >8,000 mg/kg BW)

Table 1. The tentative and probit LD_{50} doses and the behavioural changes associated with *Cymbopogon citratus*, *Rosmarinus officinalis*, *Monanthataxis littoralis* and *Aframomum angustifolium* essential oils

Essential oil	Tentative oral LD_{50} mg/kg BW	Probit oral LD_{50} mg/kg BW (SE)	Dose-dependent behavioural changes
Cymbopogon citratus	8,105.00	7,046.90 (3,798.64)	Increased breathing rate and lethargy
Rosmarinus officinalis	5,943.92	4,723.33 (1,027.18)	Lethargy, convulsions, pilo-erection, ataxia, vertigo, increased heart rate, respiratory failure
Monanthataxis littoralis	13,080.00	13,335.82 (8,089.79)	Difficulty breathing and sluggish movements
Aframomum angustifolium	23,000.00	17,539.82 (7,519.65)	Difficult breathing

At post mortem, gross examination revealed no apparent changes in the liver, kidney, lungs, heart and spleen organs from both the control and the treated mice. Microscopically, no significant changes were noted in the stomach, spleen, brain and heart tissues of exposed mice. However, changes were noted in the liver, lungs and kidneys. In the kidneys, multifocal hemorrhages were observed in both the dead and surviving subjects at the treatment dose of 8,500 mg/kg (data not shown). As presented in Fig. 2, mild to severe focal or diffuse hemorrhage and collapsed lungs/alveoli were observed in dead subjects at doses of 5,500 mg/kg and above, but this was also encountered in sections of lungs of control group. Significant histological damage to the liver was noted where enlarged hepatocytes; congestion, diffuse hydropic hepatocellular damage or vacuolation and necrosis were encountered (see Fig. 2). For mice administered with 3,500 and 2,500 mg/kg of body weight, the survivors of the 24 hour acute toxicity study fully recovered.

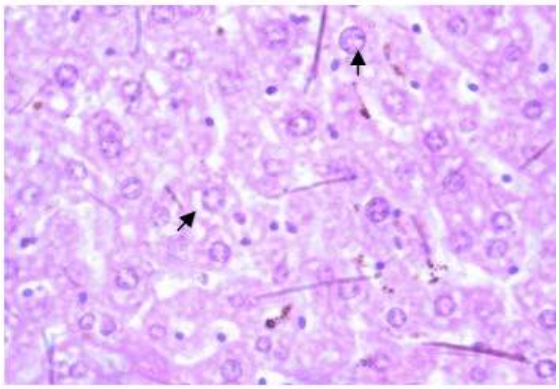
3.4 Behavioral Alterations and Pathological Changes Associated with *Monanthataxis littoralis*

The only behavioural changes that were observed included difficult breathing and sluggish movement, which were dose-dependent. After 24 h of oral exposure, death of the mice was seen in all the six; five; four; one; and none; at concentrations of 60,000 mg/kg; 30,000 mg/kg; 15,000 mg/kg, 7,000 mg/kg and 4,000 mg/kg, respectively. The tentative LD_{50} was estimated as 13,080 mg/kg BW while the calculated LD_{50} by the Probit test was 13,335.82±8,089.8 mg/kg.

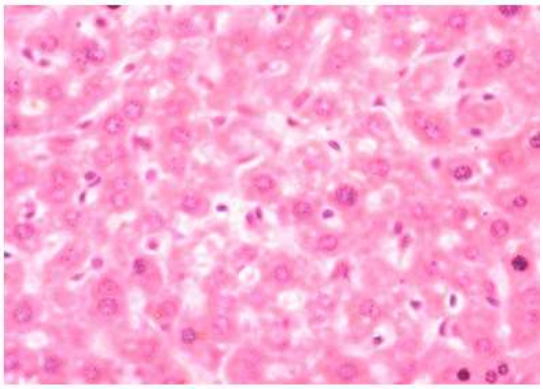
Gross examination of the internal organs of mice exposed to the EO revealed emphysematous lungs, distended urinary bladder, pin-point necrotic foci on the liver; and heamorrhages in the lungs, the spleen and the kidneys at a dose of 30,000 mg/kg BW and above. Microscopically, no significant changes were seen in the liver, lungs, heart, brain, kidneys and intestines at doses less than 30,000 mg/kg BW doses. However, at higher doses, congested vessels were seen in the heart, the brain, the lungs and the spleen. The histological changes that were associated with the oral administration of the oil in mice are presented in Fig. 3. Lung oedema and neutrophilia; and stratified squamous metaplasia of the intestinal epithelia lining were also seen. In addition, the kidneys had dense lymphatic infiltration around the blood vessel, proteinuria and tubular hydropic change.

Fig. 4 presents the histopathologic changes that occurred in the liver of mice administered with doses of *Monanthataxis littoralis* EO higher than 30,000 mg/kg. Vascular congestion, perivascular necrosis of the liver cells around central vein, diffuse necrosis and hydropic degeneration of the hepatocytes were seen.

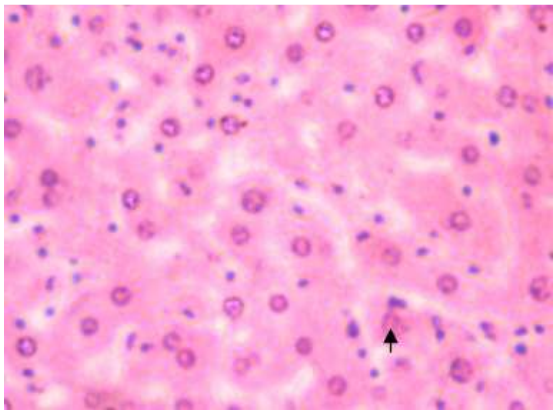
On sub-chronic exposure, there were haemorrhages in the kidneys, congestion of the heart blood vessels, diffuse hemorrhage and vascular congestion in the lungs; and stratified squamous metaplasia of the villus epithelium with increased number of goblet cells.



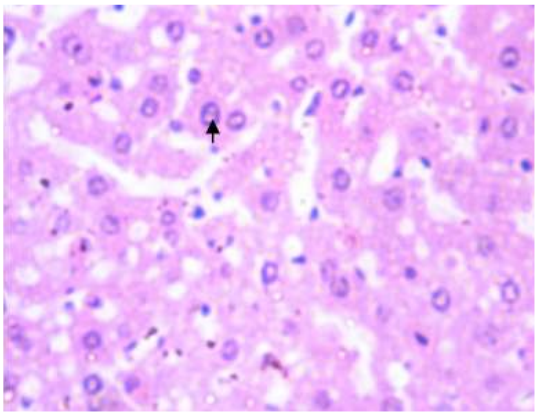
Liver × 40: Clear cytoplasm and enlarged hepatocytes



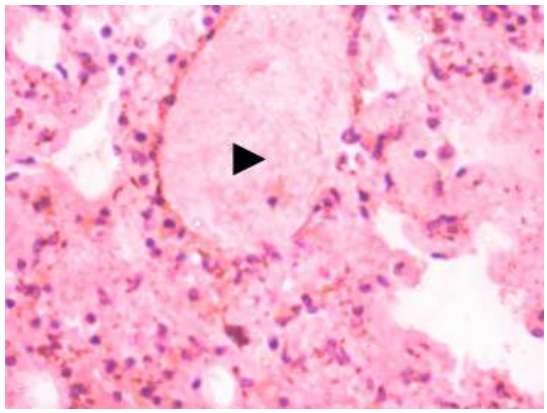
Liver × 40: Normal hepatocytes. Note the even-sized cells and filled extracellular spaces



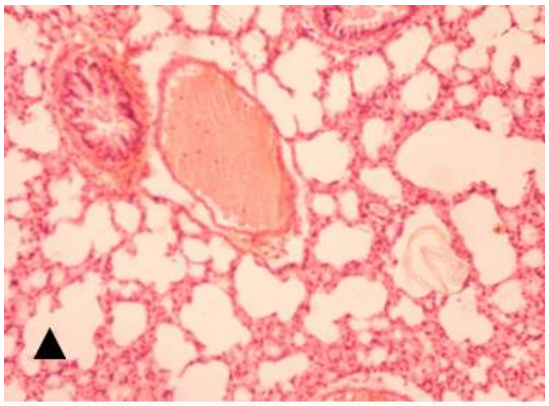
Liver × 40: Degenerated nuclear material (necrotic foci)



Liver × 40: Degenerated nuclear material (necrotic foci)

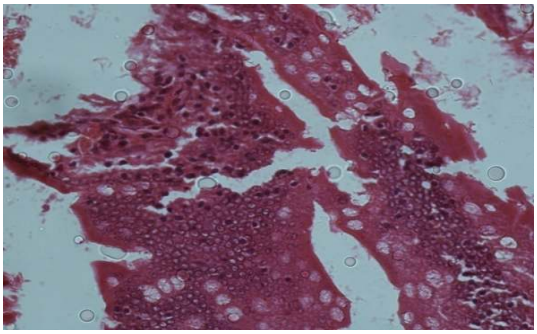


Lung × 40: Collapsed alveoli and hemorrhage- (control group)

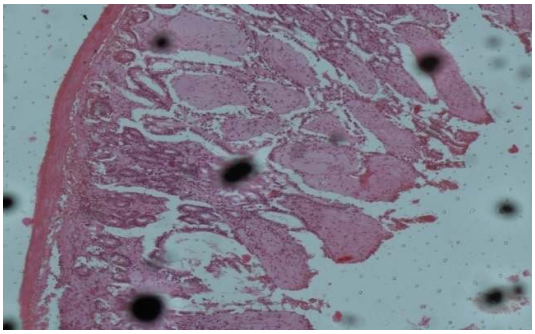


Lung × 10: Collapsed alveoli and hemorrhage

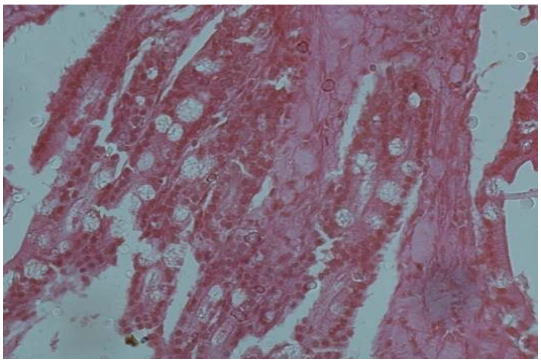
Fig. 2. Histopathological changes in the liver and lung tissues associated with oral exposure to *Rosmarinus officinalis* to mice (at doses above 5,500 mg/kg bwt for the liver tissue)



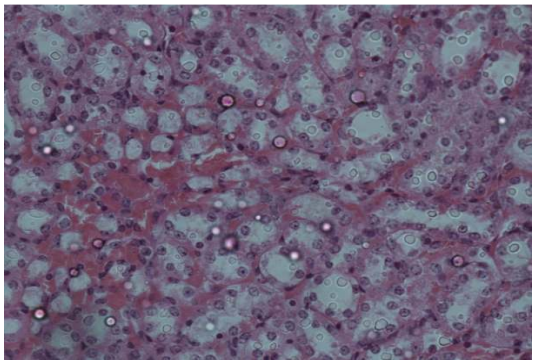
Intestine ×2,200: Metaplasia (on chronic exposure)



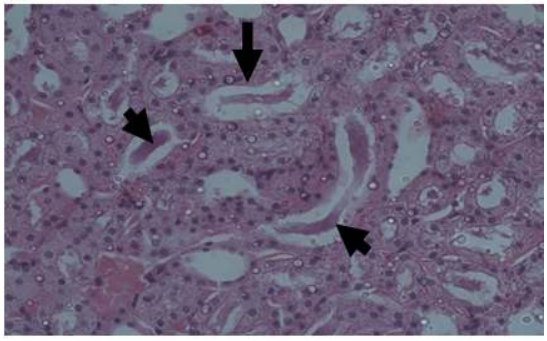
Intestine ×550: Necrotising enteritis



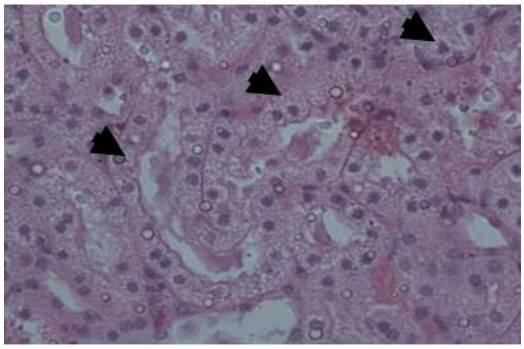
Intestine ×2,200: Intestinal mucosa with necrosis and many goblet cells



Kidney ×3,300: Control



Kidney ×2,200: Kidney proteinuria (arrows)

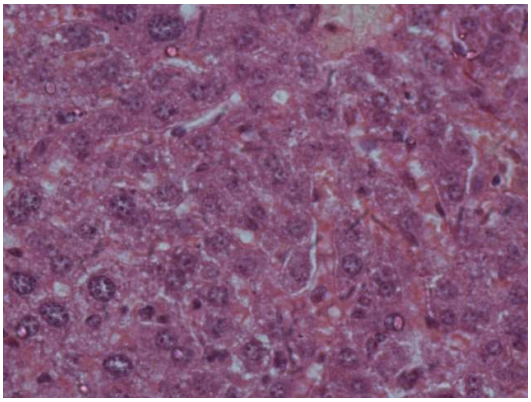


Kidney ×3,300: Kidney tubular hydropic change (arrows)

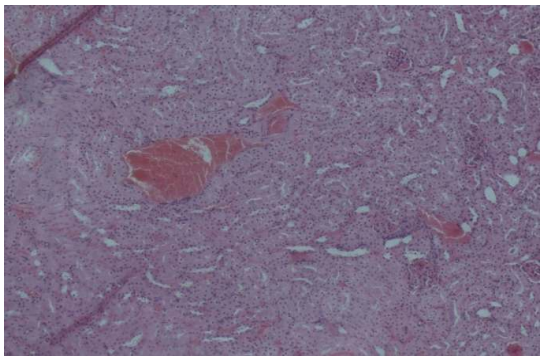
Fig. 3. The intestinal and kidney histological changes that were associated with *Monanthataxis littoralis* oral administration in mice

3.5 Acute Toxicity of *Aframomum angustifolium* Essential Oil and Associated Behavioral Alterations

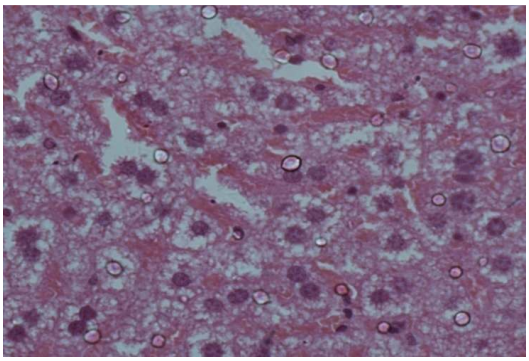
The essential oil induced difficult breathing as the behavioural change, which was dose-dependent. After 24 h of oral exposure, death of the mice was seen in all the six; five; two; one; and none; at concentrations of 37,782.61 mg/kg; 29,391.30 mg/kg; 21,000 mg/kg, 12,608.70 mg/kg and 4,217.39 mg/kg, respectively. The tentative LD_{50} for the EO was estimated as 23,000 mg/kg BW while the calculated LD_{50} by the Probit test was 17,539.82±7,519.7 mg/kg.



Liver ×3,300: Control



Liver × 550: Vascular congestion



Liver ×3,300: Hydropic change

Fig. 4. The histological changes in the liver that were associated with *Monanthataxis littoralis* oral administration in mice

4. DISCUSSION

This study established that *Cymbopogon citratus* EO is a safe product, since its LD_{50} was higher than the 5,000 mg/kg BW recommended as a cut-off by the OECD guidelines. These findings are in agreement with what was reported by Adeneye and Agbaje; although the latter tested fresh leaf aqueous extracts [28]. However, Costa, *et al*., reported a single-LD_{50} oral toxicity dose of 3,500 mg/kg BW in mice [26]. The increased breathing rates after administration of *Cymbopogon citratus* EO, just as observed in case of the other EOs that were tested could have been due to the stress of receiving the oral administration. There were no substantial gross lesions seen in tissues of mice that were exposed to the EO, which is in agreement with findings by Costa et al. where mice were exposed to a repeated dose in a 21-day oral toxicity study at up to 100 mg/kg BW [26]. The histopathological effects seen in the intestines could be associated with probably the irritation by the oil since functional damage to the stomach and liver was reported in rats by Fandohan et al. [25].

The LD_{50} for *Rosmarinus officinalis* essential oil was established as 4,723.33±1027 mg/kg, which is comparable to the 5.5g/kg BW as reported by Fahim et al. [29]. The LD_{50} of the essential oil indicate that it is marginally safe as per the OECD guidelines. Previous studies by Anadón et al. [37] reported toxicity doses as more than 2,000 mg/kg BW, but leaf extracts were tested. However, Alnamer et al. [38] reported an oral toxicity in mice of LD_{50} of 897.85 mg/kg; which is much lower than that established during this study. Our findings indicate that the essential oil from *Rosmarinus officinalis* plants collected from the fringes of Kakamega forests had low toxicity and fall in category 5 of toxic hazards, which are said to be of “low acute toxicity” and expected to have an oral or dermal LD_{50} in the range of 2000-5000 mg/kg [39].

Some of the behavioural changes observed during this study could be due to the various components found in the oil. The major constituents of *Rosmarianus officinalis* oil used in this study were α - Pinene (26.46%); 1, 8 - cineole (24.20%), Verbenone (9.41%), geraniol (3.38%), linalool (3.12%), and limonene (3.02%) (Un-published data). In contrast, the EO used by Alnamer et al. [38] had its composition as α - pinene (15.82%), camphene (6.80%), ß-pinene (4.75%), myrcene (1.70%), p-cymene (2.16%), 1, 8 - cineole (50.49%), camphor (11.61%), broneol (2.58%), and broneol acetate (2.08%). *In vitro* studies found strong evidence that 1,8-cineole controls inflammatory processes and mediator production of infection- or inflammation-induced mucus hypersecretion by its action as anti-inflammatory modifier rather than a simple mucolytic agent. Probably, 1, 8 - cineole played a role in the recovery of mice administered with 3,500 and 2,500 mg/kg of body weight after the acute toxicity studies. Camphor is one of the components of Rosemary oil, which has been associated with epileptiform convulsions if taken orally in sufficient quantity [40]. According to EFSA, the intoxication of camphor in humans, includes central nervous stimulation, oral and gastric irritation, nausea and vomiting, excitement, hallucinations, delirium, muscular excitability, tremors, convulsions and urinary retention [31]. This could be the same reason as to why such behavioral changes were experienced in mice. However, the concentration of camphor in the essential oil used in this study was below detectable levels; and may not be associated with the nervous symptoms observed.

Lack of gross and microscopic changes in the mice tissues and organs reveal that the *Rosmarinus officinalis* EO is probably not toxic at the doses that were used. However, the multifocal hemorrhages that were observed in kidneys of both the dead and surviving subjects at the dose of 8,500 mg/kg could be due to individual animal differences in tolerating the toxic substances. Ingestion of large amounts of the essential oil leading to a danger of gastroenteritis and nephritis has been reported; and is probably the reason for the effects seen in the kidneys. Centrilobular hypertrophy and reversibility of hepatic changes have been reported, which is more of an adaptive response and therefore not of toxicological concern [30]. From the sub-acute studies, no substantial tissue changes were noted. However, previous researchers reported effects on the reproductive system although Alnamer et al. reported none for the male system [38,41]. Hence, more research on the effects on the various body systems is recommended.

Traditionally, the *Monanthataxis littoralis* plant is used by some Ugandan communities by chewing it for good luck; and to the best of our knowledge, there are no previous studies on toxicity of *Monanthataxis littoralis*. Nonetheless, given the oral LD_{50} dose of 13,335.82 mg/kg as established by the current study, the oil is safe by the OECD guidelines. The necrotizing enteritis and thickening of the intestinal mucosa due to oral administration of *Monanthataxis littoralis* EO could have been due to irritation by the oil and the sub-chronic exposure. The microscopic changes in the kidneys are probably the explanations to the distended bladder that was seen macroscopically. The toxic effects of a substance depend on the chemical components; however the major constituents of *Monanthataxis littoralis* essential oil, as reported by Chepkirui et al. [18], have not been associated with undesirable properties. Hence, the toxic effects that were observed cannot be explained; and thus the causes of the microscopic changes need to be investigated further.

Comparisons for the established LD_{50} of 17,539.82 mg/kg for *Aframomum angustifolium* EO cannot be done. This is because no safety reference data exist; although the oil is documented to be in the category of flavor and fragrance agents with antioxidant and antimicrobial activity [24]). The oral LD_{50} dose of 17,539.82 mg/kg, indicate that the oil is significantly safe by the OECD guidelines. Indeed the fruits of the plant are edible and no ill-effects have been reported. However, the seeds have been reported to contain 1,8-cineole, which was described as lethal to humans in doses as low as 0.05 mL [33]. A related species, *A. melegnata*, has seed oils reported to be mildly toxic and contains piperine alkaloid that resulted in blurred vision among humans and modified sexual behavior of male rats [33,42]. However, Juergens reported that the anti-inflammatory and anti-oxidative properties of 1,8-cineole are of beneficial use in prevention of asthma and chronic obstructive pulmonary disease [43]. The histopathological changes in the vital organs, which may be associated with oral administration of the essential oil, were not investigated. Hence, more comprehensive studies need to be carried out to establish the safety of the oil extracted from these plants.

Drawing from the traditional post-harvest practices, a lot of research has been carried out to establish the pesticidal and anti-microbial activity of various essential oils; where immediate effects basing on acute toxicity or repellency on different insect pests are reported. Hence, there is limited information on the amount of oils used in controlling pest and microbial spoilage of agricultural produce. This calls for more research into this area if these products are to be registered and used widely.

5. CONCLUSIONS AND RECOMMENDATIONS

All the essential oils investigated during this study were safe according to the OECD guidelines; however, Rosemary essential oil should be used with caution since borderline toxicity levels were obtained. The oils should be tested in rats and by other routes, to establish the effects in the mammalian hosts and the inhalation toxicity and skin sensitivity, which are likely to be the portals of entry in personnel handling the treated grain produce. The effects on the reproductive system should be explored as lack of such information can negatively affect adoption of the technology by the end-users. Since the previous studies on these oils revealed their anti-mold activity and the current one has established their safety; more research is recommended to avail the innovation to the relevant stakeholders.

STATEMENT OF ANIMAL RIGHTS

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

ETHICAL APPROVAL

The research was recommended by the College of Veterinary Medicine, Animal Resources and Biosecurity Institutional Review Board; and approved by the Uganda National Council of Science and Technology (A459 dated 8/4/2011).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Takaki I, Bersani-Amado LE, Vendruscolo A, Sartoretto SM, Diniz SP, Bersani-Amado CA, Cuman RK. Anti-inflammatory and antinociceptive effects of *Rosmarinus officinalis* L. essential oil in experimental animal models. J Med Food. 2008;11(4): 741-6.
   DOI: 10.1089/jmf.2007.0524
2. Teimouri M. Antimicrobial activity and essential oil composition of *Thymus daenensis* Celak from Iran. Journal of Medicinal Plants Research. 2012;6(4): 631-635.
   DOI: 10.5897/JMPR11.1347
3. Minaiyan M, Ghannadi AR, Afsharipour M, Mahzouni P. Effects of extract and essential oil of *Rosmarinus officinalis* L. on TNBS-induced colitis in rats. Research in Pharmaceutical Sciences. 2011;6(1): 13-21.
   PMCID: PMC3203268
4. Isman MB, Miresmailli S, Machial C. Commercial opportunities for pesticides based on plant essential oils in agriculture, industry and consumer products. Phytochemistry Reviews. 2001; 10(2):197-204.
   DOI: 10.1007/s11101-010-9170-4
5. Aguilar F, Autrup H, Barlow S, Castle L, Crebelli R, Dekrant W, Toldrá F. Use of rosemary extracts as a food additive-Scientific opinion of the panel on food additives, flavourings, processing aids and materials in contact with food. EFSA J. 2008;721:1-29.
6. Elgayyar M, Draughon FA, Golden DA, Mount JR. Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. Journal of Food Protection. 2001;64:1019-24.
   PMID: 11456186
7. Ponce AG, Del Valle C, Roura SI, Shelf life of leafy vegetables treated with natural essential oils. Journal of Food Science 2004;69(2):50-56.
   DOI: 10.1111/j.1365-2621.2004.tb15515.x
8. Angioni A, Barra A, Cereti E, Barile D, Coisson JD, Arlorio M. Chemical composition, plant genetic differences, antimicrobial and antifungal activity

investigation of the essential oil of *Rosmarinus officinalis*. L. J Agric Food Chem. 2004;52(11):3530-3535.

DOI: 10.1021/jf049913t

9. Miresmailli S, Bradbury R, Isman MB. Comparative toxicity of *Rosmarinus officinalis* L. essential oil and blends of its major constituents against *Tetranychus urticae* Koch (Acari: Tetranychidae) on two different host plants. Pest Management Sci. 2006;62:366–371.

PMID: 16470541

10. Fu Y, Zu Y, Chen L, Shi X, Wang Z, Sun S, Efferth T. Antimicrobial activity of clove and rosemary essential oils alone and in combination. Phytother Res. 2007;21(10): 989-94.

PMID: 17562569

11. Lee SO, Choi GJ, Jang KS, Lim HK, Cho KY, Kim JC. Antifungal activity of five plant essential oils as fumigant against postharvest and soil-borne plant pathogenic fungi. Plant Pathol. J. 2007; 23(2):97-102.

DOI: 10.5423/PPJ.2007.23.2.097

12. Faixová Z, Faix Ś. Biological effects of Rosemary (*Rosmarinus officinalis* L.) essential oil. A Review. Folia Veterinaria. 2008;52:135-139.

13. Koul O, Walia S, Dhaliwal GS. Essential oils as green pesticides: Potential and Constraints. Biopestic. Int. 2008;4:63-84.

14. Matasyoh JC, Wagara IN, Nakavuma JL, Kiburai AM. Chemical composition of *Cymbopogon citratus* essential oil and its effect on mycotoxigenic *Aspergillus* species. African Journal of Food Science 2011;5:138–142.

Available:http://www.academicjournals.org/ajfs;

ISSN 1996-0794 ©2011 Academic Journals

15. Silva NC, Barbosa L, Seito LN, Fernandes Junior A. Antimicrobial activity and phytochemical analysis of crude extracts and essential oils from medicinal plants. Natural Product Research. 2012;26(16): 1510-4.

DOI: 10.1080/14786419.2011.564582

16. De Azerêdo GA, De Figueiredo RCBQ, De Souza EL, Stamford TLM. Changes in *Listeria monocytogenes* induced by *Origanum vulgare* L. and *Rosmarinus officinalis* L. essential oils alone and combined at sub-inhibitory amounts. Journal of Food Safety. 2012;32:226–235. DOI: 10.1111/j.1745-4565.2012.00372.x

17. Negi PS. Plant extracts for the control of bacterial growth: Efficacy, stability and safety issues for food application. International Journal of Food Microbiology. 2012;156(1):7-17.

DOI: 10.1016/j.ijfoodmicro.2012.03.006

18. Chepkirui C, Matasyoh JC, Wagara IN, Nakavuma J. Antifungal activity of *Monathotaxis littoralis* essential oil against mycotoxigenic fungi isolated from maize. Int. J. Microbiol. Res. Rev. 2013;2(6):103–109.

ISSN: 2329-9800

Available:www.internationalscholarsjournals.org © International Scholars Journals

19. Prakash B, Singh P, Kedia A, Dubey NK. Assessment of some essential oils as food preservatives based on antifungal, antiaflatoxin, antioxidant activities and *in vivo* efficacy in food system. Food Research International. 2012;49(1):201-8. DOI: 10.1016/j.foodres.2012.08.020

20. Soliman KM, Badeaa RI. Effect of oil extract from some medicinal plants on different mycotoxigenic fungi. Food Chem. Toxicol. 2002;40:1669-1675.

21. Abdolahi A, Hassan A, Ghosta Y, Javadi T, Meshkatalsadat MH. Essential oils as control agents of postharvest *Alternaria* and *Penicillium* rots on tomato fruits. Journal of Food Safety. 2010;30:341–352. DOI: 10.1111/j.1745-4565.2009.00211.x

22. Torlak E, Nizamlıoğlu M. Antimicrobial effectiveness of chitosan-essential oil coated plastic films against foodborne pathogens. Journal of Plastic Film & Sheeting 2011;27(3):235–248.

DOI: 10.1177/8756087911407391

23. Shah G, Shri R, Panchal V, Sharma N, Singh B, Mann AS. Scientific basis for the therapeutic use of *Cymbopogon citratus*, stapf Lemon grass. J Adv Pharm Technol Res. 2011;2(1):3-8.

DOI: 10.4103/2231-4040.79796

PMCID: PMC3217679

24. Available:http://www.thegoodscentscompany.com/data/es/1025531.html

[Accessed 29 January 2016]

25. Fandohana P, Gnonlonfina B, Laleyec A, Gbenoub JD, Darbouxc R, Moudachiroub M. Toxicity and gastric tolerance of

essential oils from *Cymbopogon citratus*, *Ocimum gratissimum* and *Ocimum basilicum* in Wistar rats. Food and Chemical Toxicology. 2008;46:2493-2497. DOI: 10.1016/j.fct.2008.04.006

26. Costa CA, Bidinotto LT, Takahira RK, Salvadori DM, Barbisan LF, Costa M. Cholesterol reduction and lack of genotoxic or toxic effects in mice after repeated 21-day oral intake of lemongrass (*Cymbopogon citratus*) essential oil. Food and Chemical Toxicology. 2011;49(9): 2268-72.
DOI: 10.1016/j.fct.2011.06.025
27. Mishra AK, Kishore N, Dubey NK, Chansouria JPN. An evaluation of the toxicity of the oils of *Cymbopogon citratus* and *Citrus medica* in rats. Phytotherapy Research. 1992;6:279-281.
DOI: 10.1002/ptr.2650060512
28. Adeneye AA, Agbaje EO. Hypoglycemic and hypolipidemic effects of fresh leaf aqueous extract of *Cymbopogon citratus* Stapf. in rats. J Ethnopharmacol. 2007;25: 440-4.
29. Fahim FA, Esmat AY, Fadel HM, Hassan KF. Allied studies on the effect of *Rosmarinus officinalis* L. on experimental hepatotoxicity and mutagenesis. Int J Food Sci Nutr. 1999;50(6):413-27.
DOI: 10.1080/09634899100987;
PMID: 10719582
30. Lemonica IP, Damasceno DC, di-Stasi LC. Study of the embryotoxic effects of an extracts of rosemary (*Rosmarinus officinalis* L.). Brazilian Journal of Medical and Biological Research. 1996;29(2): 223-227.
31. Use of rosemary extracts as a food additive: Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food, (Question No EFSA-Q-2003-140). The EFSA Journal. 2008;721:22-29.
32. Anywar GU, Kirimuhuzya C. Phtochemical profile and antibacterial activity of crude extracts of the pod o *Aframomum angustifolium* (Sonn) K. Schum. European Journal of Biological Research. 2015;5(2): 36–41.
ISSM 2449 -8955
33. European Food Safety Authority (EFSA). Compendium of botanicals that have been reported to contain toxic, addictive, psychotropic or other substances of concern. The EFSA Journal. 2009;7(9): 281-379.
DOI: 10.2903/j.efsa.2009.281
Available: www.efsa.europa.eu
[Accessed 15 January 2016]
34. OECD. Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris; 2008. DOI:http://dx.doi.org/10.1787/9789264070684-en
35. Akhila JS, Shyamjith D, Alwar MC. Acute toxicity studies and determination of median lethal dose. Current Science. 2007;97:917–920.
36. Randhawa MA. Calculation of LD_{50} values from the method of Miller and Tainter, 1944. Journal of Ayubeda Medical College, Abbottabad. 2009;21(3):184-185.
Available:http://www.ayubmed.edu.pk/JAMC/PAST/184
37. Anadon A, Martinez-Larranaga MR, Martinez MA, Ares I, Garcia-Risco MR, Senorans FJ, Reglero G. Acute oral safety of rosemary extracts in rats. J Food Prot. 2008;71(4):790-5.
PMID:18468034 iafp/jfp/2008/00000071/00000004/art00016
38. Alnamer R, Alaoui K, Bouidida E, Benjouad A, Cherrah Y. Psychostimulant activity of *Rosmarinus officinalis* essential oils. J Nat Prod. 2012;5:83-92.
39. Jamshidi R, Afzali Z, Afzali D. Chemical composition of hydro-distillation essential oil of Rosemary in different origins in Iran and comparison with other countries. American-Eurasian J. Agric. & Environ. Sci. 2009;5(1):78-81.
ISSN 1818-6769; © IDOSI Publications, 2009
40. Narayan S, Singh N. Camphor poisoning – An unusual cause of seizure. Case Report. Medical Journal of Armed Forces India, 2012;68:252-3.
DOI: 10.1016/j.mjafi.2011.11.008
41. Sá RCS, Leite MN, Oliveira LEG, Toledo MM, Greggio TC, Guerra MO. Preliminary assessment of *Rosmarinus officinalis* toxicity on male Wistar rats' organs and reproductive system. Brazilian Journal of Pharmacognosy. 2006;16(3):324-332.
Jul./Set. ISSN 0102-695X

42. Akpanabiatu MI, Ekpo ND, Ufot UF, Udoh NM, Akpan EJ, Etuk EU. Acute toxicity, biochemical and haematological study of *Aframomum melegueta* seed oil in male Wistar albino rats. Journal of ethnopharmacology. 2013;150(2):590-4.

43. Juergens UR, Anti-inflammatory Properties of the Monoterpene 1.8-cineole: Current evidence for co-medication in Inflammatory Airway Diseases Drug Res (Stuttg). 2014; 64(12):638-646.
DOI: 10.1055/s-0034-1372609

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