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Phytochemical, Acute Toxicity, Analgesic, *in vitro* Antioxidant Studies and GC-MS Investigation of Essential Oil of the Methanol Leaf Extract of *Momordica charantia*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SOO and ASA carried out the study, authors AAO and TOO performed the statistical analysis and managed the analysis of the study, authors MAY and AAA designed the study and wrote the protocol, while author AAA managed the literature searches and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Medicinal plants have bioactive compounds which play an important role in the healing of various diseases. They are the best sources for chemical ingredients, antimicrobial and antioxidant agents for cure of different diseases. Most medicinal plants possess pharmacological activities (anti-

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inflammatory, antidiabetic, antioxidant, antibacterial, antifungal etc.) due to the presence of these phytochemicals in them. In this study, we intend to conduct qualitative phytochemical screening and to quantitatively evaluate the total phenol, flavonols, tannins, proanthocyanidins and flavonoids contents of the plant, *Mormodica charantia*. The acute toxicity studies of the methanol leaf extract of *M. charantia* on mice was conducted to evaluate how safe the plant is in relation to dosage, and this is intended to establish the safety of the plant in mice with reference to OECD guidelines. Furthermore, the analgesic activity of the methanol leaf extract of *M. charantia* and the free radical scavenging activities of the plant *in vitro* were demonstrated using various standard procedures. We also intend to profile the major compounds present in the essential oil of the plant understudied using Gas Chromatography-Mass Spectrometry Analysis. Summarily, this study was aimed to investigate the phytochemical screening, acute toxicity, analgesic, *in vitro* antioxidant, as well as the chemical constituents in the essential oil of the methanol leaf extract of *M. charantia.*

Keywords: Medicinal plants; Phytochemicals; Mormodica charantia; anti-oxidant; anti-inflammation activities; acute toxicity; GC-MS.

1. INTRODUCTION

Medicinal plants are the greatest asset to human health and represent a viable treasure for discovering new potential compounds with various therapeutic effects [1], factors such as the availability, affordability and accessibility of medicinal plants have led to their high demand and usage [2]. Plant-derived medicines are relatively cheaper than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment [3]. Their medicinal properties are largely due to the phytochemicals that are present in these plants which produce a definite physiological action on human body [4,5]. These plants constitute the main source of new pharmaceuticals and healthcare products [6]. Extraction and characterization of several active phyto-compounds from these plants have given birth to some high activity profile drugs [7]. It is believed that crude extract from medicinal plants are more biologically active than isolated compounds due to their synergistic effects [8]. Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances [9,10].

Moreover, botanicals are enjoying widespread use of plants for treatment of several ailments, but little is known about their toxicity and safety issue which are always a concern. Investigations on functional plants provide evidence for the presence of substances that offer potential human health benefits. However, it should be a vital requirement to determine the toxic effects of some of the substances contained in the plants [11].

Toxicity is defined as the expression of being poisonous, indicating the state of adverse effects caused by the interaction between toxicants and cells. This interaction may vary depending on the chemical properties of the toxicants and the cell membrane, as it may occur on the cell surface, within the cell body, or in the tissues beneath as well as at the extracellular matrix. The toxic effects may take place prior to the binding of the toxicants to the vital organs such as liver and kidneys. Hence, evaluation of toxic properties of a substance is crucial when considering public health protection because exposure to chemicals can be hazardous and results to adverse effects on human being. In practice, the evaluation typically includes acute, sub-chronic, chronic, carcinogenic and reproductive effects [12]. The present study aims to determine the toxicity of methanol leaf extract of *M. charantia* using an acute oral toxicity test in animal models [13]. Toxicological studies help to make decision whether a new drug should be adopted for clinical use or not. Therefore, evaluating the toxicological effects of any medicinal plant extract intended to be used in animals or humans is a crucial part of its assessment for potential toxic effects.

Nociception, initiated by pain receptors, is known as the neural process of encoding and processing noxious stimuli. It is the afferent activity produced in the peripheral and central nervous system by stimuli that have the potential to damage tissue. Plants reportedly used to relief pain are confirmed to be rich in active principles such as alkaloids, flavonoids, tannins, terpenoids and steroids [14]. The plant, *Momordica charantia* contains these secondary metabolites which may possess analgesic properties.

Free radicals of different forms are generated at a low level in cells to help in the modulation of several physiological functions and are quenched by an integrated antioxidant system in the body [15]. However, if free radicals are produced in excess amount they can be destructive leading to inflammation, ischemia, lung damage and other degenerative diseases [16,17]. Antioxidant activity is the ability and potential of a plant to reduce oxidative reactions generated by the activities of the free radicals [18].

Essential oils are used in traditional therapies, in medicine, the food industry as flavouring additives and in cosmetics as fragrance [19,20,21]. The main constituents of essential oils are mono and sesquiterpenes, carbohydrates, phenols, alcohols, ethers, aldehydes and ketones which are responsible for biological activities.

Momordica charantia is a tropical and subtropical vine of the family Cucurbitaceae, widely grown in Asia, Africa and the Caribbean for its edible fruit. The plant grows to about six to eight feet in height and each node on the vine has a lobed leaf and a tendril. The male and female flowers that this plant bears are yellow in color and grow at the axils of the leaves. The fruit of the vine is oblong and with a rather rough exterior [22]. The plant is cultivated throughout the tropics, particularly in India, China, East and West Africa and Central and South America [23,24]. The use of the essential oil of this plant in the preparation of various commercial products mainly as antimicrobial and antioxidant agents have been reported [25,26,27]. Extensive clinical studies have been done and reported by several scientists on this plant species and showed that they have very strong antibacterial [28], antiulcer [29], antiviral [30], anthelmintics [31], antimalarial [32], immunomodulatory [33], antitumour [34], antiprotozoal [35] antioxidant [36], antifungal [37], insecticidal [38] and anti-platelet activities [39]. *M. charantia* has been proven to be beneficial in various pathological conditions of animal models through its antioxidative and analgesic properties [36] hence the need to explore its pharmacological potentials.

Therefore, the present study was aimed at determining the quantitative and qualitative phytochemical constituents, acute toxicity, analgesic, free radical scavenging activities and characterization of the components of the essential oil of the methanol leaf extract of *M. charantia* using GC-MS technique as this will help to determine the mechanism of actions of this plant results in its biological or pharmacological activities.

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant Materials

Fresh whole plant of *Momordica charantia* was collected from the botanical garden, University of Ibadan. The plant materials were properly identified and authenticated at the Department of Botany, University of Ibadan and the Voucher Specimen Number was (VSN: UIH-22563). The voucher specimen was maintained at the Herbarium of the Department of Botany, University of Ibadan.

2.2 Preparation of Plant Extract

The leaves were dried at room temperature (27 ± 2℃) and pulverised to fine powder using an electric blender. The fine powder (400 g) was soaked and extracted in 90% methanol (1L) using Soxhlet extractor for 3days until complete extraction. The extracts were filtered through Whatman no 1 filter paper and the filtrate was evaporated to dryness by rotary evaporator (Yamato, Rotary Evaporator, model-RE 801, Japan) at 190-220 rpm and 40 - 50^0c for 24 h under reduced pressure to give amorphous solid mass. The extract yield was 12%.

2.3 Experimental Animals

Male adult albino Wistar rats (150 – 200g) and Swiss albino mice (15 – 30g) bred in the experimental animal house of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria were used for the study. The animals were kept in cages within the animal house and allowed free access to water and standard livestock pellets. All experimental protocols were conducted in compliance with the University of Ibadan Ethics Committee on Research in Animals as well as internationally accepted principles for laboratory animal use and care. Treatments were performed at the experimental animal facility of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria.

The animals were housed and maintained under standard laboratory conditions at 22 ± 2℃ with 12 hr light/dark cycles and humidity at 55 ± 5%. Standard laboratory animal feed and water were

provided *ad libitum* and animals were acclimatized to the experimental conditions for a period of one week before the commencement of the experiment.

2.4 Ethical Approval

The study protocol was approved by the Animal Care and Use Research Ethics Committee, University of Ibadan (UI-ACUREC/App/2015/044). All the animals received humane care in accordance to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science (NAS) and published by the National Institute of Health, NIH [40]. All procedures in this study were performed according to the Animal Ethics Committee, University of Ibadan.

2.5 Phytochemical Analysis

The standard methods of Wall et al [41,42]; Trease and Evans [43]; Kokate et al. [44] and Wadood et al. [45] were used in the analysis of the phytochemical components aerial parts of *M. charantia*. The constituents analysed for were alkaloids, flavonoids, tannins, reducing sugars, anthraquiniones, terpenes and cardiac glycosides. Quantitatively, total phenol was determined by the Folin-Ciocalteu method [46,47]; the total flavonoids content was estimated by aluminium chloride colorimetric method as described by Chang et al. [48] and Hossain et al. [49]. Total flavonol content was determined by adopting the procedure described by Kumaran and Karunakaran [50]; total proanthocyanidin was determined based on the procedure of Sun et al. [51] and Oyedemi et al. [52]. Tannin determination was done according to the method of AOAC [53] with some modifications.

2.6 Acute Toxicity Studies

The acute toxicity study of *Momordica charantia* was determined according to the method of Sawadogo et al. [54]. 30 mice fasted for 16 hours were randomly divided into 6 groups of 5 animals each. The crude extract was suspended in a vehicle (2.5% Tween 80 in normal saline) and graded doses of the extract (200, 400, 800, 1600, 3200 mg/kg) corresponding to groups B, C, D, E and F respectively were separately administered to the mice in each group. The control group representing group A was administered with the vehicle (2 ml/kg) only. The animals were observed keenly for about 30 minutes for any signs of toxicity or mortality and further observations were made every 8 hours for a period of 48 hours after administration of the extract for general behavioural changes, morbidity and mortality.

2.7 Analgesic Studies

Acetic acid-induced writhing response in mice was performed using the method described by Sawadogo et al. [54] and Collier et al. [55]. Four groups (A, B, C and D) of mice (n=5) each received orally administered vehicle control (Tween 80, 2 ml/kg) (i.e. control), ibuprofen (10 mg/kg), or plant extracts (100 mg/kg and 200 mg/kg) respectively. Sixty minutes later, 0.6% acetic acid (10 ml/kg) solution was injected intraperitoneally to all animals in the different groups. The number of writhes occurring between 5 and 20 minutes after acetic acid injection was counted. A reduction in the number of writhings compared with the control group was considered as evidence of analgesia. The percentage inhibition of the writhing response was calculated from the formula:

$$\% \text{ inhibition} = (Do - Dt)/Do \times 100$$

Where Do was the average writhing response of the control group, while Dt was the average writhing response of the treated group. A significant reduction of the writhes in the tested animals compared to those in the control group was considered as an antinociceptic response.

Furthermore, formalin induced paw lick test was done according to the modified method of Dubuisson and Dennis [56]. Four groups (A, B, C and D) of mice (n=5) each received orally administered vehicle control (Tween 80, 2 ml/kg) (i.e. control), ibuprofen (10 mg/kg), or plant extracts (100 mg/kg and 200 mg/kg) respectively. Thirty minutes after treatment, 0.05ml of 2.5% formalin was injected subcutaneously into the sub – planter surface of the mice left hind paw, then the number of paw licks by the mice were recorded both at the early (0 – 5mins) and late phases (15 – 30 mins), the time interval between the paw licks was also noticed [57]. The time that the animals spent licking the injected paws [58] and the number of times the rats licked the injected paws [59] were observed and measured as an index of pain.

The percentage inhibition of the paw licks for both phases was calculated from the formula:

% inhibition = (Do – Dt)/Do x 100

Where Do was the average number of paw licks of the control group, while Dt was the number of paw licks of the treated group.

2.8 *In vitro* Antioxidant Activity

The antioxidant activities of the of *M. charantia* were determined using ferric reducing antioxidant power (FRAP) [60], ABTS (2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt radical scavenging assay [61,62], DPPH (1,1-diphenyl-2-picrylhydrazyl) test [63], Nitric oxide (NO) radical scavenging activity [52].

2.9 Gas Chromatography and Mass Spectrometry (GC-MS) Analysis

The plant powder was subjected to hydrodistillation in order to extract the essential oils (Massada 1976). The essential oil was analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) (NRC-GC/El-MS Lab), according to method of Van den dool and Kratz [64] and Syarul et al [65].

2.10 Statistical Analysis

The results were expressed as Mean ± SD. The results were treated to a one- way analysis of variance (ANOVA) and subsequently to the Turkey multi comparison post-test using the statistical package Graph Pad prism version 5 (Graph Pad software, San Diego CA, USA). Differences between means were considered significant at $\alpha_{0.05}$.

3. RESULTS

3.1 Phytochemical Analysis

Phytochemical screening of methanol extract of *Momordica charantia* revealed the presence of alkaloids, flavonoids, saponins, tannins, anthraquinones, terpenes and cardiac glycosides. However, reducing sugars were absent. In terms of quantitative analysis, the total proanthocyanidins was the highest.

3.2 Acute Toxicity Studies

During the course of the acute toxicity study, no dose (treatment) related effect was observed on the general condition or behaviour of the experimental animals (at the doses 200mg/kg, 400mg/kg, 800mg/kg and 1,600mg/kg, 3200mg/kg). No mortality or obvious clinical signs were observed in all the groups throughout the experimental period. The results showed that methanol leaf extracts of *M. charantia* demonstrated high safety margin since the animals tolerated up to 3,200 mg/kg body weight of the extracts orally; thus oral administration of *M. charantia* extract did not produce any significant toxic effect in mice. Hence, the extract can be utilized safely for therapeutic use in pharmaceutical formulations.

3.3 Analgesic Studies

The peripherally mediated analgesic activities of *M. charantia* by acetic acid-induced writhing response in mice are shown in Table 2. The control mice were found to produce 55.4 writhes after the injection of acetic acid. For the doses studied, the methanol leaf extract showed better analgesic activity where the writhes were reduced to 21.4 and 11.2 at doses of 200 and 400 mg/kg, respectively. The methanol extract of *M. charantia* (at doses of 100, 200 and 400mg/kg) and ibuprofen caused a significant decrease in the number of writhes when compared to the control. The extract (200 and 400mg/kg) and ibuprofen (10mg/kg) exhibited a high antinociceptive power of 61.4%, 79.8% and 81.6% respectively. The percentage of inhibition at 400mg/kg was almost the same as that of the standard drug ibuprofen.

Table 1. Phytochemical analysis of *Mormodica charantia* (Quantitative)

Phytochemical constituents	Quantity
Total Phenols (mg Gallic acid eq./g extract)	12.07 ± 1.33
Total flavonoids (mg quercetin eq./g extract)	12.81 ± 5.04
Total flavonols (mg quercetin eq./g extract)	47.66 ± 1.11^{ab}
Total proanthocyanidins (mg catechin eq./g extract)	60.66 ± 3.58^{abc}
Total Tannins (mg tannic acid eq./g extract)	1.06 ± 0.04^{abcd}

(^{abcd}) significant difference at $\alpha_{0.05}$ relative to total phenols, total flavonoids, total flavonols and total proanthocyanidins respectively.

The result of formalin paw lick test showed that the methanol leaf extract of *M. charantia* and ibuprofen caused a significant decrease in the number of paw licks when compared to the control at both phases of the test. The antinociceptive effect of the extract (100, 200 and 400mg/kg) and ibuprofen was more pronounced at the late phase (second phase). The antinociceptive power of 400mg/kg dose of the aqueous extract was almost the same to the standard drug (ibuprofen) at the late phase of the test (Table 3).

3.4 *In vitro* Antioxidant Studies

The plant showed high percentage inhibition activity in all the free radical scavenging models evaluated in the present study.

The ferric reducing activities of *M. charantia* were significantly lower than the standard drugs used in this order: vitamin E > rutin > *M. charantia* at concentrations of 0.05 mg/ml and 0.1 mg/ml. At concentration of 0.2 mg/ml the absorbance value of *M. charantia* was comparable with rutin, while at concentrations 0.025 mg/ml and 0.5 mg/ml, the absorbance value of *M. charantia* were higher than that of rutin as depicted in Fig. 1, thereby establishing its antioxidant ability.

The percentage inhibition of 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS) radical by *M. charantia* and the standards (Vitamin E and rutin) is shown in Fig. 2. The % inhibition of ABTS by the plant was concentration dependent and compared favourably well with the vitamin E and rutin. The scavenging activity was in the order: rutin > Vitamin E > *M. charantia* but the difference was not significant. As the concentration increases, the % of ABTS radical scavenging activity of the plant also increases.

The DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging potential of *M. charantia* was concentration dependent as shown in Fig. 3. At concentrations of 0.025 mg/ml and 0.05 mg/ml, the percentage inhibition of DPPH by the plant and the standard was recorded in decreasing order: rutin > *M. charantia* > Vitamin E, while at concentrations of 0.1 mg/ml, 0.2 mg/ml and 0.5 mg/ml, the percentage inhibition was in the order rutin > Vitamin E > *M. charantia*. From the figure, as the concentration increases, the % inhibition of DPPH radical decreases.

The Nitric oxide (NO) radical scavenging activity of *M. charantia* was depicted as the % inhibition of nitric oxide in Fig. 4. At concentrations of 0.025 mg/ml, 0.05 mg/ml and 0.1 mg/ml, the activities of the extract and the standards were in the order: rutin > Vitamin E > *M. charantia*. At concentrations of 0.2 mg/ml and 0.5 mg/ml, the NO radical scavenging activity was in the order rutin > Vitamin E > *M. charantia*.

Table 2. Analgesic effect of methanol extract of *M. charantia* and ibuprofen on mouse writhing reflex induced by acetic acid (n = 5), mean ± SD

Parameters	MC				
	A (control)	B (Ibuprofen 10 mg/kg)	C (100 mg/kg)	D (200 mg/kg)	E (400 mg/kg)
No of writhes/20mins	55.4 ± 6.66	10.2 ± 4.76[a]	30.0 ± 6.89[ab]	21.4 ± 6.23[a]	11.2 ± 6.85[ac]
Inhibition of writhes (%)	0	81.6	45.8	61.4	79.8

([abc]) significant difference at $\alpha_{0.05}$ relative to control, standard drug and 100mg/kg MC extract respectively.

Table 3. Analgesic effect of methanol extract of *M. charantia* and ibuprofen on paw lick test on mouse induced by formalin (n = 5), mean ± SD

Parameters (early phase)	MC				
	A (control)	B (Ibuprofen 10 mg/kg)	C (100 mg/kg)	D (200 mg/kg)	E (400 mg/kg)
No of paw licks	29.8 ± 4.95	21.6 ± 4.73[a]	15.8 ± 2.86[a]	13.0 ± 2.74[ab]	15.6 ± 2.30[a]
Inhibition (%)	0	27.5	47.0	56.4	47.7
Parameters (late phase)					
No of paw licks	27.2 ± 6.73	7.0 ± 2.92[a]	13.6 ± 4.45[a]	11.2 ± 6.09[a]	8.1 ± 1.92[a]
Inhibition (%)	0	74.0	50.0	58.8	70.2

([ab]) significant difference at ($\alpha_{0.05}$) relative to control and standard drug (ibuprofen) respectively.

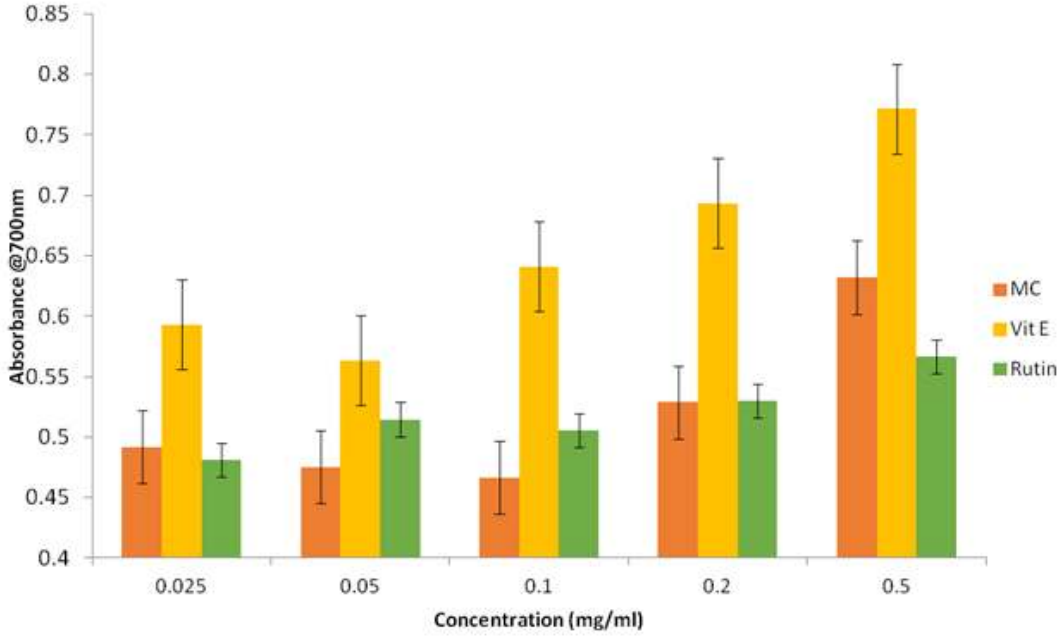


Fig. 1. Antioxidant status of *Mormodica charantia* using Ferric Reducing Antioxidant Power (FRAP). FRAP (*μ*mol AAE/L), AAE: ascorbic acid equivalent

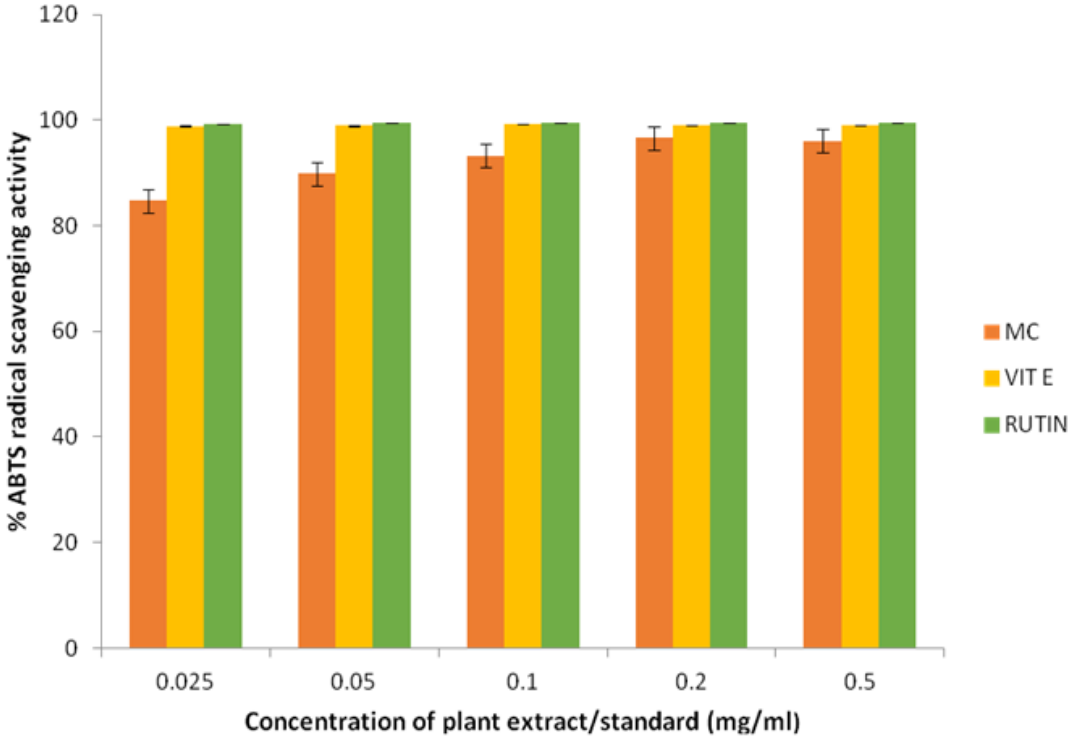


Fig. 2. ABTS radical scavenging activity of *M. charantia*

3.5 Gas Chromatography and Mass Spectrometry (GC-MS) Analysis

The result revealed various compounds (Fig. 5), identification of the compounds were carried out based on their retention times, matching with the library and their mass spectra with literature data. Of the compounds identified, those with higher percentage quality were trans-beta.-Ionone (91), trans 2-Hexenal (89), 1,6-Octadien-3-ol (62), 3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)- (91) (Fig. 6). In the present study, some compounds such as 3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)- (0.94), 1,4-Benzenedicarboxamide (0.79) and N, N'-bis (2-hydroxy-1-methyl-2-phenylethyl) Nitro-L-arginine (0.79) were detected for the first time in *M. charantia.*

4. DISCUSSION

The bioactive compounds isolated from herbal plants are harmless and do not cause any adverse effect on health, thus is widely used as OTC-medication [66]. Plant origin drugs are known to play vital roles in the management various chronic diseases and have received a great preference by researcher as alternatives to allopathic pharmaceutical drugs in recent times [67].

The phytochemicals investigated, as shown in this study, have been reported to possess strong antioxidant activities due to their ability to absorb, quench free radicals and decompose peroxides generated in the system [62]. Phenols exhibit antioxidant free radicals scavenging activities [52,68], flavonoids are potent water-soluble antioxidants which help in radical scavenging and prevention of oxidative cell damage [69], proanthocyanidins are known to improve cardiac recovery [70]. Tannins are useful for the prevention of cancer as well as treatment of inflamed or ulcerated tissues [71], alkaloids have analgesic, antimalarial, antiseptic and bactericidal activities but could be toxic to cells [72]. Saponins in medicinal plants are responsible for most biological effects related to cell growth and division in humans and have inhibitory effect on inflammation [73]. These phytochemicals are mostly responsible for the medicinal values of *M. charantia.*

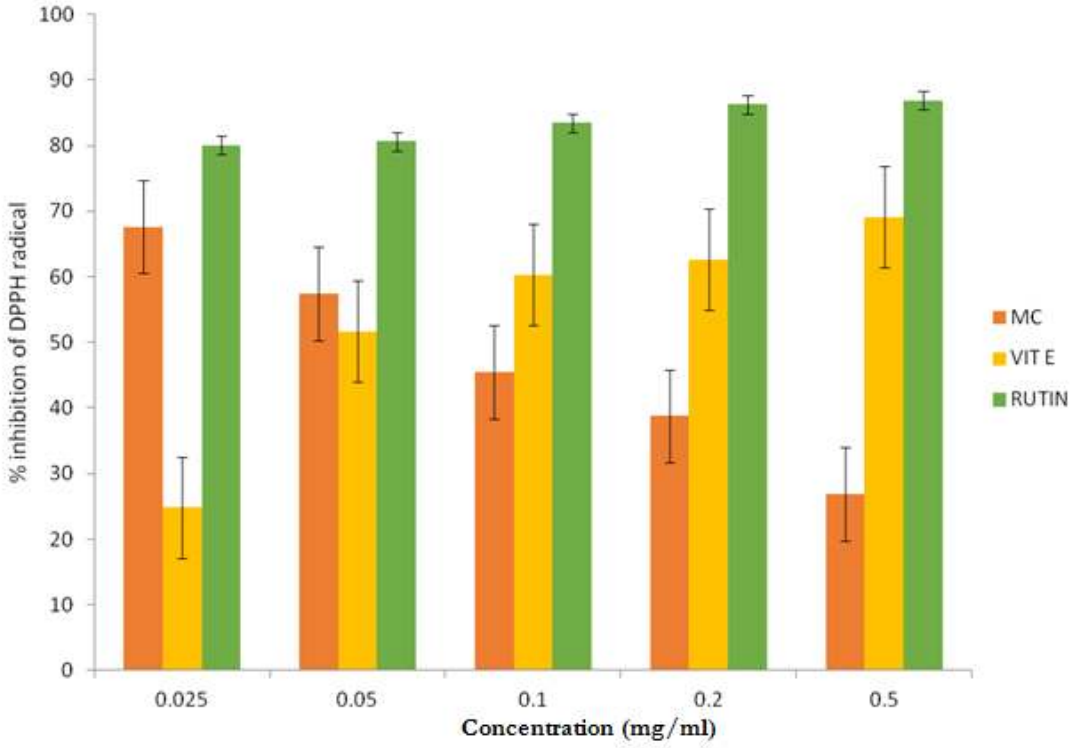


Fig. 3. DPPH radical scavenging activity of *M. charantia*

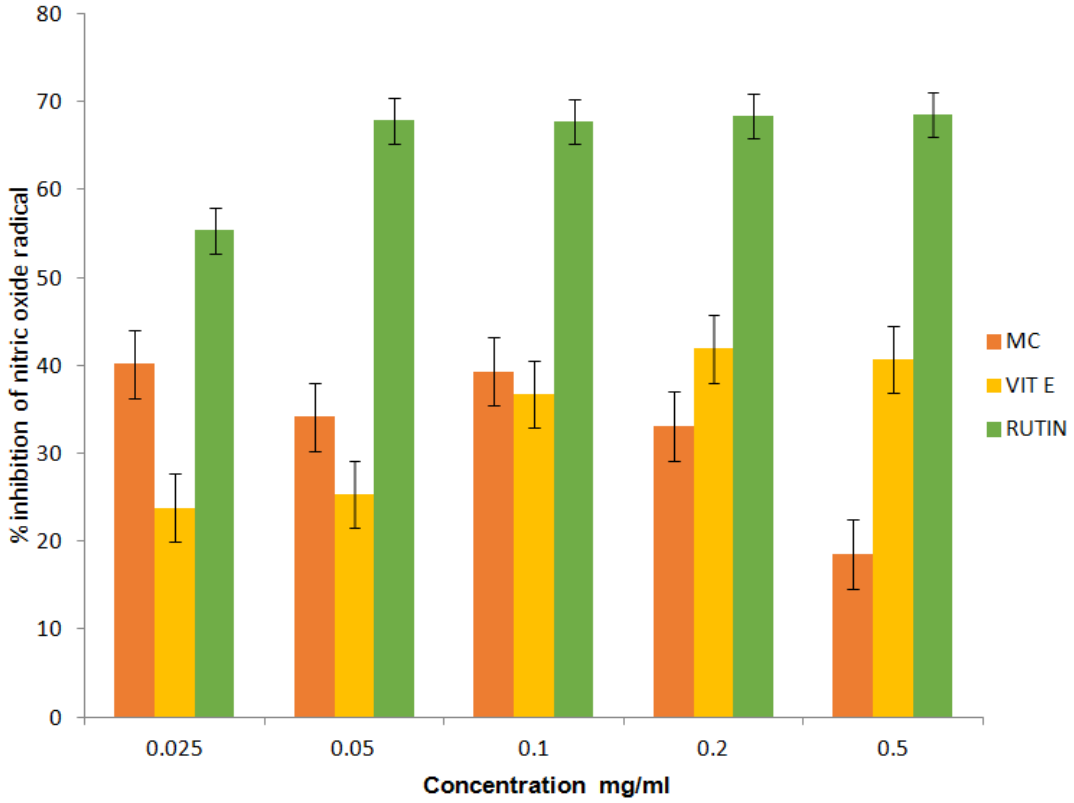


Fig. 4. Nitric oxide radical scavenging activity of *M. charantia*

Toxicity studies are crucial in judging the safety of medicinal plants to ascertain their potential for development into pharmacological products [74]. The acute toxicity study of *Momordica charantia* showed the absence of lethality or toxic side effect on oral administration at all the doses used in this study. This might be an indication of the non-toxic nature of the plant extract. Mbaka et al [75] reported that according to the FAO/WHO Expert Committee on food additives [76], if no death occurred at 2 g/kg of body weight, then it is assumed that the substance is non-toxic, thus in this context, the methanol leaf extract of *M. charantia* is considered a safe plant even at a dose of 3,200 mg/kg body weight and the lethal dose (LD_{50}) can be categorized under class 5 based on OECD guideline 423 [77]. According to Adeneye and Olagunju [78]; any pharmaceutical drug or compound with the oral LD_{50} higher than 1000 mg/kg could be considered safe and low toxic. This suggests that the methanol leaf extract of *M. charantia* is practically safe and non-toxic, attesting to the extensive use of the plant as a traditional medicine.

Acetic acid induced writhing test is a visceral pain model which is very sensitive for analgesic drugs, thus it is highly useful for analgesic drug development [79]. Intraperitoneal injection of the acid produced abdominal writhing by the activation chemosensitive nociceptors. With respect to the acetic acid-induced abdominal writhing, *M. charantia* produced a significant analgesic effect with dosage of 400 mg/kg being comparable to the standard drug ibuprofen. The ability of the extract to inhibit acetic acid-induced writhing in mice (a model of visceral pain) shows that it could be useful in the management of visceral pain. The dose dependent inhibition of acetic acid induced writhing by the extract indicated a peripheral effect and it's suggestive of the dose dependent manner of medicinal plants extracts in the treatment of pain and inflammation [80,81]. The inhibition of acetic acid writhing shows that the extract may have depressant effect on the nervous system since central nervous system depressants have been known to inhibit or reduce the number of writhing in acetic acid pain models [82,83].

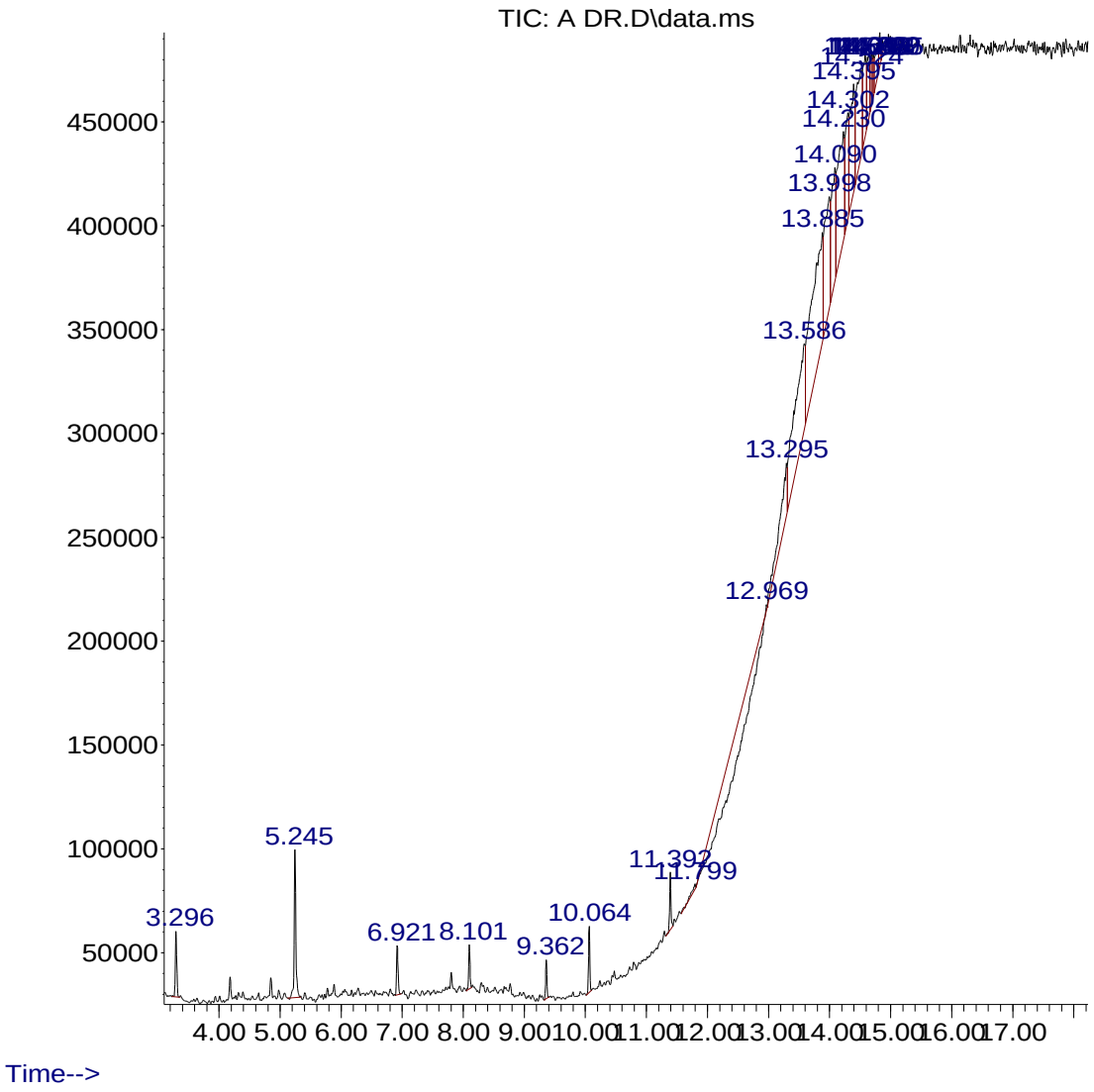
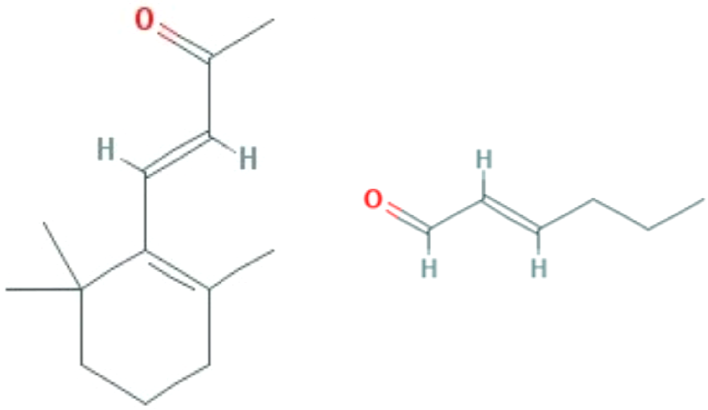


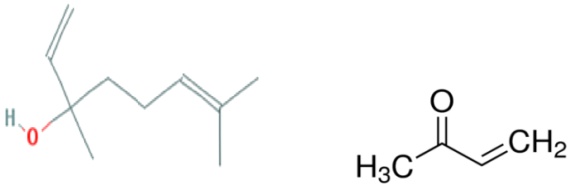
Fig. 5. GC-MS chromatogram of essential oils of methanol extract of *M. charantia*)

The formalin induced paw lick test is a model devised to evaluate analgesic effect of new drugs [84]. The test possesses two distinct phases possibly reflecting different types of pain. The early phase reflects a direct effect of formalin on nociceptors (neuropathic or non-inflammatory pain), whereas the late phase reflects the inflammatory pain mediated by the release of several inflammatory agents including prostaglandins [85,86]. The pain stimulus is continuous rather than transient and may bear some resemblance to clinical pain.

The extract provided a significant inhibition in both phases, suggesting the involvement of both neurogenic and inflammatory mechanisms; it reduced both the duration and the number of paw lick in both phases. This implies that the extract offered protection against the activation of chemoreceptors and the activities of chemo-irritants and inflammatory agents. The anti-nociceptive effect of the extract was probably mediated via both neurogenic and inflammatory mechanisms.



a) Beta-ionone **b) Trans-2-Hexenal**



c) 1-6-octadien-3-ol **d) 3-Buten-2-one**

Fig. 6. Structures of a) Beta-ionone b) Trans-2-Hexenal c) 1-6-octadien-3-ol d) 3-Buten-2-one

The mechanism of analgesic activities may be due to the presence of flavonoids and other plant constituents acting synergistically in the plant. Non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen act by the reduction of sensitization of pain receptors caused by prostaglandins at the inflammation site [87]. Although the active doses of the plant extract were higher than that of the reference drug, it should be noted that the extracts have different compositions of several substances.

The antioxidant activity of the plant extracts could be explained on the basis of their phenolic and flavonoids content [88,89,90]. Furthermore, the methanol leaf extract of *M. charantia* has high phenolic content (12.07 ± 1.33 mg gallic acid equivalent/g extract) and flavonoid content (12.81 ± 5.04 mg quercetin equivalent/g extract), this study is in agreement with previous studies which reported that there is a high a correlation between total phenolic content and flavonoid contents and antioxidant capacities of medicinal plants [91,92,93].

The ability of a substance to act as an antioxidant depends on its strength to reduce ROS by donating hydrogen atom [94]. There was transformation of Fe^{3+} to Fe^{2+} in the presence of the *M. charantia* showing its reductive capability in comparison to vitamin E and rutin. Scavenging of DPPH radical in this study indicates the potency of the plant in donating hydrogen proton to the lone pair electron of the radicals; it could be suggested that *M. charantia* extract contain compounds capable of donating protons to the free radicals. The scavenging activity of ABTS+ by the *M. charantia* was found to be higher than that of DPPH radical. The scavenging activity of ABTS+ radical by the methanol extract of *M. charantia* could imply that the extract may be useful for treating radical related pathological damage, especially at higher concentrations [95].

Nitric oxide is an important chemical mediator generated by endothelial cells, macrophages and neurons and is involved in the regulation of various physiological processes. Excess concentration of nitric oxide is associated with several diseases [96,97]. Oxygen reacts with the excess nitric oxide to generate nitrite and peroxynitrite anions, which act as free radicals [98]. In the present study, the plant showed a NO radical scavenging activity by competing with oxygen to react with nitric oxide and thus inhibits the generation of the anions. Therefore, the plant

has potent antioxidant properties to curtail progression of radical related diseases and thereby give credence to the traditional usage of *M. charantia* extract.

The present study showed that the essential oil has wide varieties of volatile oil terpene hydrocarbons (aliphatic and cyclic) and their corresponding isoprenoid derivatives, alkaloids and aromatic compounds which are responsible for its pharmacological activities. GC-MS analysis revealed many pharmacologically important phytochemicals in the essential oil of *M. charantia* including alkanols, phenols, aldehyde, ketones, sesquiterpenes and other hydrocarbons in the essential oil of *M. charantia*. Trans-beta-Ionone (4-(2,6,6-Trimethylcyclohex-1-en-1-yl)but-3-en-2-one) with molecular formula of $C_{13}H_{20}O$ and molecular weight of 192.302 g/mol has been shown to exhibit anti-proliferative function [99]. Beta-Ionone demonstrates potent anticancer activity both *in vitro* and *in vivo*; it has been demonstrated to have the potent capacity to suppress DMBA-initiated mammary cancer in rats [100]. Beta-ionone is known to be useful model inducer of P450 enzyme(s) in studying toxic mechanism of certain chemicals which require metabolic activation by P450s in mice [101]. Trans-2-Hexenal is an aldehyde with molecular formula $C_6H_{10}O$ and molecular weight of 98.145 g/mol. It is known to have antimicrobial and antifungal properties [102], at non-toxic concentrations; it produces a significant inhibition of glutathione S-transferase activity in intact human melanoma cells. The role of cytosolic glutathione S-transferase in resistance to alkylating anticancer drug is well documented [103]. Consequently, the exploitation of this molecule in the treatment of tumours has been proposed [104]. Also, this compound has been regarded as activator of the antioxidant response elements [105]. 1-6-octadien-3-ol (Geraniol) is a monoterpenoid and an alcohol with molecular formula $C_{10}H_{18}O$ and molecular weight 154.25g/mol. It has anticonvulsant properties. 3-Buten-2-one on the other hand is a ketone with molecular formula CH_2=$CHCOCH_3$ and molecular weight of 70.09 g/mol. All these compounds identified amongst others are known to act synergistically to elicit the various pharmacological actions of *M. charantia.*

5. CONCLUSION

In conclusion, the result of this present study showed that *M. charantia* possesses biologically active phytoconstituents which act synergistically to elicit various pharmacological activities. Furthermore, *M. charantia* extract is not toxic in all the doses studied and did not produce any significant toxic effect in mice. Hence, the extract can be utilized safely for therapeutic use in pharmaceutical formulations. This study also demonstrated that methanol leaf extract of *Momordica charantia* exhibited highly potent and dose related analgesic activities. These findings justify the folkloric use of the plant to treat pain and inflammatory conditions. The main finding of our study was the inhibitory effect of the extract on formalin-induced oedematous in hind paw probably due to its dose dependent effect on COX activity. Therefore, it seems that one possible mechanism by which *M. charantia* extract leads to pain relief is COX system inhibition. The present findings justify that *Momordica charantia* can be used as a natural source for alternative or supplementary therapeutic drug for the treatment of analgesic and inflammatory diseases.

The present study showed the scavenging activity of the methanol leaf extract of *M. charantia.* Thus the plant can curtail the progression of radical related diseases and thereby give credence to the traditional usage of *M. charantia*. However, further experimental analyses will be needed to confirm its efficacy in the treatment of free radical-mediated diseases. The GC-MS analysis of bitter gourd (*M. charantia*) showed that it contains hydrocarbons, alkaloids, triterpenoids and phenolic compounds which are responsible for its pharmacological activities. The preliminary results suggest promising alternatives for exploring therapeutic and pharmaceutical interest in *M. charantia* extract with minimal or no possible adverse effects.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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