Turk J Pharm Sci 2024;21(2):104-112
DOI: 10.4274/tjps.galenos.2023.14704

ORIGINAL ARTICLE

Antiseizure Activity of *Mitragyna inermis* in the Pentylenetetrazol-Induced Seizure Model in Mice: Involvement of Flavonoids and Alkaloids

Relwendé Justin OUÉDRAOGO[1,2*], Muhammad JAMAL[3], Lassina OUATTARA[1,2], Muhammad NADEEM-UL-HAQUE[3], Faisal KHAN[3], Shabana Usman SIMJEE[3], Georges Anicet OUÉDRAOGO[1,2], Farzana SHAHEEN[3]

[1]Nazi Boni University, Department of Biological Sciences, Life and Earth Sciences Training and Research Unit, Bobo-Dioulasso, Burkina Faso
[2]Nazi Boni University, Animal Health and Biotechnology Research and Teaching Laboratory, Bobo-Dioulasso, Burkina Faso
[3]Karachi University, Third World Center for Science and Technology, Hussain Ebrahim Jamal Research Institute of Chemistry, Karachi, Pakistan

ABSTRACT

Objective: This study aimed to investigate whether *Mitragyna inermis (Willd.)* Otto Kuntze organic and aqueous extracts are able to control seizures induced by pentylenetetrazol (PTZ) in mice based on flavonoid fingerprints and alkaloidal contents.

Materials and Methods: Ethanolic extract and decoction-derived fractions from roots, leaves, and stems were subjected to chromatographic fingerprinting using $AlCl_3$ and screening for their antiseizure effects using PTZ-induced acute seizure model. From the fractions that showed potent bioactivities, plausible antiseizure alkaloids were isolated using thin layer chromatography, and their structures were elucidated using ^{1}H NMR, 2D NMR, ^{13}C NMR, and FAB-HR (*+ve* or *–ve*).

Results: All fractions, with the exception of the dichloromethane and hexane fractions, revealed remarkable flavonoid fingerprints. An acute PTZ-induced seizure test revealed that ethanolic extract of stem bark [500 mg/kg body weight (bw)], ethyl acetate extract of stem bark (500 mg/kg bw), and aqueous extract of leaves (300 mg/kg bw) significantly delayed the occurrence of hind limb tonic extension (HLTE); however, a non-significant delay was observed in the onset of first myoclonic jerk compared with control animals. Isolation yielded four main alkaloids: that are, pteropodine (1), isopteropodine (2), mitraphylline (3) and corynoxeine (4). Corynoxeine is a new compound derived from *M. inermis*.

Conclusion: This study suggests that flavonoid fingerprints are tracers of *M. inermis* anticonvulsant ingredients. The stem bark ethanolic and ethyl acetate extracts and leaf aqueous extracts contain anticonvulsant bioactive principles that delay notifying the HLTE occurring in male naval medical research institute mice. Furthermore, alkaloidal contents also remain plausible bioactive anticonvulsant principles. All observations support the traditional use of *M. inermis* to manage epilepsy. However, further studies are needed to understand the effects of alkaloid fractions, flavonoids, and the isolated compounds as promising antiseizure agents derived from *M. inermis* in experimental animals.

Keywords: *Mitragyna inermis*, antiseptics, flavonoids, alkaloids, corynoxeine

INTRODUCTION

Epilepsy is neurological and seems to be systemic disorder (Yuen et al.[1]). Epilepsy presents as partial or generalized seizures along with psychiatric comorbidities (Banerjee et al.[2]). Furthermore, people suffering epilepsy are more represented in the middle and low-income countries due to the enormous treatment gaps. Social stigma, discrimination and misunderstanding are some prejudices to live with epilepsy (Moshé et al.[3]). In the recent development of various antiseizure drugs (AEDs), some prevents seizures by acting through multiple ways such as acting on sodium channels (valproic acid, phenytoin, carbamazepine), calcium channels (ethosuximide), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (parampanel), γ-aminobutyric acid (GABA) receptors (diazepam), or modulate the release of GABA (gabapentin,

*Correspondence: rjustino14@yahoo.com, Phone: +226 75386260, ORCID-ID: orcid.org/0000-0002-5036-2144
Received: 10.03.2023, Accepted: 13.05.2023

pregabalin or valproic acid) (Macdonald[4], Kobayashi et al.[5]). Unfortunately, some of them still exhibit adverse effects like hypersensitivity reactions, mood changes, hepatotoxicity and are also ineffective against drug-resistant seizures (Copmans et al.[6], Devinsky et al.[7]). Whereas, the primary goal of AED therapy is complete freedom from seizures without adverse side effects (Shorvon et al.[8]). So, the needs to develop new AEDs become imperative (Moshé et al.[3]). Pentylenetetrazol (PTZ) causes seizures on its administration by preventing inhibitory effects of GABA type A ($GABA_A$) receptor (Huang et al.[9]). Thus, this GABA receptor antagonist is widely used as a model for AED research. Natural sources like plants are suitable for development of various new drug candidates (Cragg et al.[10]).

Mitragyna inermis (Willd) Otto Kuntze (Rubiaceae) is a bushy tree or shrub (Arbonnier[11]). In Burkina Faso, its leaves, roots and stem barks are used in mental illness and epilepsy treatment (Arbonnier[11], Nacoulma[12]). Different modes of preparation such as maceration, infusion, decoction and leaching with various accessible solvents such as water, hydroalcoholic and acetonic solutions are used to obtain traditional medicines (Ouédraogo et al.[13]).

Moreover, previous studies were demonstrated anticonvulsant properties of leaf methanolic extract (300-1200 mg/kg) and stem bark ethanolic and aqueous extracts (62.5-500 mg/kg) (Timothy et al.[14], Atinga[15]). Those studies were reported on a moderate dose of convulsant agent (PTZ 60 mg/kg) and the anticonvulsant extract constituents were not specifically reported from this plant (Atinga et al.[15]). Therefore, these authors reported flavonoids, tannins, alkaloids, anthraquinone, glycosides, and terpenoids in these extracts. However, tube tests reported on these potent extracts are preliminary phytochemical screenings and can be confirmed or refuted by thin layer chromatography screenings (Ouédraogo et al.[13]). Also, extensive phytochemical report revealed that *M. inermis* contains polyphenols, triterpenoids, indole and oxindole alkaloids (Shellard and Sarpong[16,17], Toklo et al.[18], Ouédraogo et al.[19]). It has been reported that flavonoids and alkaloids or medicinal plant containing flavonoids and alkaloids interact with $GABA_A$ receptors leading to anticonvulsant activities (Copmans et al.[6], Jäger and Saaby[20], Zhu et al.[21]). Besides, several AEDs like diazepam, carbamazepine, lorazepam, midazolam, brivaracetam, piracetam, aniracetam, oxiracetam, pramiracetam, nefiracetam, nebracetam, fasoracetam and levetiracetam licensed or in clinical development have the common function like alkaloids (Shorvon et al.[8]). Nevertheless, the knowledge of the potential contribution of flavonoids and alkaloids patterns of *M. inermis* different part extracts on its therapeutic properties is unknown. In drug research based on traditional recipes, it is important to assay or highlight ubiquitous tracers of biological activity due to synergistic effects (Yuan et al.[22]). Indeed, flavonoids are generally followed in the standardization of raw materials from traditional medicine and chromatographic fingerprinting can be used in the quality control of medicinal plant materials (Ouédraogo et al.[13]).

This study aimed to investigate whether *M. inermis* organic and aqueous extracts are able to control seizures induced by PTZ in mice based on flavonoid fingerprints and alkaloidal contents.

MATERIALS AND METHODS

Phytochemical study

Plant materials and extract preparation

Based on a previous study, *M. inermis* parts (leaf, stem bark and root) samples were collected from Banfora, Dindérésso, and Boromo during September and November (Ouédraogo et al.[23]). A voucher specimen (UNB 939) is deposited in the Herbarium of University Nazi Boni. The collected samples were subjected to total phenolic compounds assays and antioxidant properties evaluation, which are sensitive to ecological factors (Ouédraogo et al.[23]). At the end of these analyses, three samples were categorized by plant part. Based on their phenolic contents and remarkable antioxidant potential, three samples by plant part were used to form composite samples *per* part, *i.e.* 40 + 40 + 40 = 120 g of stem bark, 40 + 40 + 40 = 120 g of root bark, and 30 + 30 + 30 = 90 g of leaves. The composite samples were used for different extractions, maintaining the ratio of plant matter. Different preparations have been made in relation to the traditional way of preparation and those reported from previous studies (Timothy et al.[14]; Ouédraogo et al.[19]).

Acetonic extraction

Ninety g of leaves, 120 g of root bark, and 120 g of stem bark were subjected to 10 fold 70% acetone (*v/w*) for 1 hour 30 min at 1500 rpm at 37 °C. Then, filtration and centrifugation at 3800 rpm/35 min/4 °C were carried out. The supernatant solvent were evaporated at 45 °C with rotary evaporator and stored at 4 °C until their use (Checkouri et al.[24]). This extraction yielded 32.74%, 13.76%, 22.87% from leaf, stem and root, respectively.

Aqueous decoction and fractionation

Five hundred g of each part of *M. inermis* was used for extraction. Thus, the extraction was performed with 10 fold distilled water (*v/w*) 100 °C/30 min (Ranilla et al.[25]). Filtrate pH was reduced to 3-4 and then subjected to fractionation successively with increasing polarity [hexane, dichloromethane (DCM), ethyl acetate to butanol]. The residual water and butanol were evaporated by freeze-drying and the other solvents at 45 °C using a rotary evaporator. This fractionation yielded 13.6%, 5.97%, and 4% for the leaf decoction, ethyl acetate, and butanol fractions, respectively. Also, 2% for the ethyl acetate fraction of stem bark and 3.10% for the ethyl acetate fraction of roots. Extracts and fractions were stored at 4 °C until use (Silva et al.[26]).

Ethanol extraction

Stem bark powder (120 g) was macerated for 24 hours with 1200 mL of ethanol (99%). Therefore, the raw material was macerated twice (the second with the recovered solvent). The solvent was then evaporated at 45 °C with a rotary evaporator (2% yield) and stored at 4 °C (Timothy et al.[14]).

Flavonoid fingerprinting, bioassay-guided fractionation, and alkaloids isolation

Extracts and fractions of each plant part were dissolved in methanol and applied on F254 silica plate. Then, elution was carried out over 8 cm with ethyl acetate: acetic acid: methanol: water (10:1.6:0.6:1.5) and the plates were viewed at ultraviolet (UV) 365 nm and after spraying with aluminum chloride (Ouédraogo et al.[19]). This first phytochemical screening is part of the confirmation of the presence of different subgroups of flavonoids reported as potential bioactive of traditional drugs. In addition, this screening allows the grouping of fractions according to the tracers that are flavonoids in a preliminary standardization guide. All extracts were subjected to anti-PTZ-induced seizures assays.

Furthermore, 20 g of the aqueous decoction of the leaves and 10 g of the ethanolic extract of the stem barks that exhibited potent antiseizure effects in PTZ-induced seizures were used for alkaloid treatments. Therefore, the extracts were treated with 10% acetic acid for 12 hours. At the end of the time, filtration was performed and the pH of the filtrate was adjusted to 10, followed by extraction with DCM three times. The DCM fraction was then concentrated in rotavapor and dissolved in 5% sulfuric acid, followed by extraction with hexane to remove non-alkaloids. The residual aqueous phase pH was increased to 10 using ammonia, followed by a new extraction with DCM. The last DCM fraction was concentrated in rotavapor, dissolved in methanol, and dried under the hood. Finally, DCM was used to recover the yellow-colored-soluble compounds. This process yielded 2.4% alkaloid fraction from the leaves and 7.8% alkaloid fraction from the stem bark. Similar spots were observed on both fractions after application on a precoated F254 silica plate and elution with DCM: acetone (9:1). Then, preparative thin layer chromatography (TLC) with the same system yielded four compounds: 1 (10 mg) and 2 (10 mg) from the leaves and 3 (6 mg) and 4 (5 mg) from the stem bark.

General methods

TLCs were performed on preheated Kieselgel 60 F254 (Merck) plates. Plates were developed using DCM-acetone and then UV 254 and 365 nm. The isolated compounds were subjected to ^{1}H NMR, 2D NMR, HMBC, HSQC, COSY and NOESY, ^{13}C NMR, and FAB-MS (*+ve* or *–ve*) analyses. Bruker Avance Neo NMR spectrometers operating at 400 and 600 MHz were used to run 1D and 2D NMR spectra, giving coupling constants (*J*) in Hz and chemical shifts (δ) in ppm relative to the residual CD_3OD signal with TopSpin.

Structure elucidation of pteropodine (1), isopteropodine (2), mitraphylline (3), and corynoxeine (4)

Elucidation was carried out by comparing ^{1}H NMR, 2D NMR, ^{13}C NMR, and FAB-HR (*+ve* or *–ve*) data with the reported data.

Pteropodine (1)

Amorphous white powder: FAB-HR (*+ve*): *m/z*= 369.1826 [M + H]$^{+}$; [M ($C_{21}H_{24}O_4N_2$) + H]= 369.1826. ^{1}H NMR (400 MHz, CD_3OD): δ = 8.53 (brs, NH), 2.34 (dd, *J*= 6.0, 2.4 Hz, H-3), 3.21 (dd, *J*= 8.2, 7.0 Hz, H-5a) 2.40 (t, *J*= 4.3 Hz, H-5b), 2.29 (dd, *J*= 3.2, 8.2 Hz, H-6a) 2.00 (ddd, *J*= 12.9, 7.9, 1.5 Hz, H-6b), 7.05 (td, *J*= 7.6, 1.1 Hz, H-9), 6.86 (d, *J*= 7.9 Hz, H-10), 7.19 (td, *J*= 7.7, 1.3 Hz, H-11), 7.27 (dd, *J*= 7.5, 1.2 Hz, H-12), 1.55 (d, *J*= 7.6 Hz, H-14a) 1.33 (dd, *J*= 3.7, 7.2 Hz, H-14b), 2.37 (dd, *J*= 3.3, 2.5 Hz, H-15), 7.50 (s, H-17); 4.47 (dq, *J*=12.4, 6.1 Hz, H-19), 1.59 (d, *J*= 4.6 Hz , H-20), 2.32 (d, *J*= 2.3 Hz, H-21a), 3.31 (H-21b), 3.57 (s, 3H, OCH_3), 1.39 (d, *J*= 6.1 Hz, CH_3). ^{13}C NMR (600 MHz, CD_3OD): δ = 183.36 (C-2), 75.31 (C-3), 55.91 (C-5), 34.98 (C-6), 57.76 (C-7), 134.60 (C-8), 123.79 (C-9), 110.57 (C-10), 129.19 (C-11), 124.18 (C-12), 142.78 (C-13), 30.84 (C-14), 32.31 (C-15), 110.57 (C-16), 156.83 (C-17), 73.72 (C-19), 39.29 (C-20), 54.27 (C-21), 51.67 (OCH_3), 169.66 (C=O), 18.95 (CH_3).

Infrared spectroscopy (IR) [potassium bromide (KBr)]: V_{max}= 3320.57, 2943.98, 2832.23, 668.42 cm^{-1}. UV/UV-visible (MeOH) A= 1.347 (218 nm) and 1.652 (247 nm).

Isopteropodine (2)

Amorphous white powder: FAB-HR (*+ve*): *m/z*= 369.1821 [M + H]$^{+}$; [M ($C_{21}H_{24}O_4N_2$) + H]= 369.1821. ^{1}H NMR (400 MHz, CD_3OD): δ = 8.54 (brs, NH), 2.42 (dd, *J*= 4.2, 2.8 Hz, H-3), 3.25 (td, *J*= 8.7, 2.7 Hz, H-5), 2.29 (ddd, *J*= 12.4, 9.6, 2.5 Hz, H-6b) 1.96 (dt, *J*= 13.0, 8.5 Hz, H-6b), 7.01 (td, *J*= 7.6, 1.1 Hz, H-9), 6.88 (d, *J*= 7.7 Hz, H-10), 7.18 (td, *J*= 7.7, 1.3 Hz, H-11), 7.27 (d, *J*= 6.7 Hz, H-12), 1.47 (dt, *J*= 13.1, 3.8 Hz, H-14a) 0.79 (q, *J*= 12.2 Hz, H-14b), 2.44 (dd, *J*= 8.2, 3.7 Hz, H-15), 7.42 (s, H-17), 4.32 (dq, *J*= 10.4, 6.2 Hz, H-19), 1.59 (ddd, *J*= 8.8, 7.7, 4.4, 3.6 Hz, H-20), 2.39 (dd, 3.6, 8.5 Hz, H-21), 3.36 (dd, *J*= 12.1, 2.1 Hz, H-21b), 3.58 (s, 3H, OCH_3), 1.40 (d, *J*= 6.24 Hz, CH_3). ^{13}C NMR (600 MHz, CD_3OD): δ = 183.17 (C-2), 72.77 (C-3), 55.10 (C-5), 35.36 (C-6), 58.51 (C-7), 135.09 (C-8), 123.43 (C-9), 110.83 (C-10), 128.98 (C-11), 125.47 (C-12), 142.35 (C-13), 31.41 (C-14), 31.92 (C-15), 110.97 (C-16), 156.50 (C-17), 73.64 (C-19), 39.36 (C-20), 54.50 (C-21), 51.56 (OCH_3), 169.40 (C=O), 18.78 (CH_3).

IR (KBr): V_{max}= 3320.43, 2944.11, 2832.23 cm^{-1}. UV/UV-Visible (MeOH) A= 0.790 (214 nm), 0.815 (244 nm).

Mitraphylline (3)

Amorphous white powder: FAB-HR (*+ve*): *m/z*= 369.1809 [M + H]$^{+}$; [M ($C_{21}H_{24}O_4N_2$) + H]= 369.1809. ^{1}H NMR (400 MHz, CD_3OD): δ = 8.50 (brs, NH), 2.45 (d, *J*= 2.5 Hz, H-3), 3.25 (d, *J*= 2.5 Hz, H-5a), 2.55 (d, *J*= 1.6 Hz, H-5b), 2.06 (ddd, *J*= 13.1, 8.1, 1.4 Hz, H-6a), 2.34 (dd, *J* = 4.6, 1.7 Hz, H-6b), 7.06 (td, *J*= 7.6, 1.0 Hz, H-9), 6.88 (d, *J*= 7.7 Hz, H-10), 7.21 (td, *J*= 7.7, 1.2 Hz, H-11), 7.29 (d, *J*= 7.6 Hz, H-12), 2.25 (dt, *J*= 3.1, 3.0 Hz, H-14a), 0.99 (q, *J*= 11.6 Hz, H-14b), 2.09 (d, *J*= 1.4 Hz, H-15), 7.44 (s, H-17), 4.42 (dd, *J*= 6.5, 3.5 Hz, H-19), 1.92 (dd, *J*= 4.6, 2.3 Hz, H-20), 3.11 (d, *J*= 7.3 Hz, H-21), 1.90 (s; H-21), 3.55 (s, 3H, OCH_3), 1.11 (d, *J*= 6.6 Hz, CH_3). ^{13}C NMR (600 MHz, CD_3OD): δ = 180.29 (C-2) , 75.56 (C-3), 54.97 (C-5), 35.48 (C-6), 57.07 (C-7), 134.71 (C-8), 123.73 (C-9), 110.56 (C-10), 129.23 (C-11), 124.14 (C-12), 143.17 (C-13), 30.72 (C-14), 31.56 (C-15), 108.17 (C-16), 155.63 (C-17), 75.43 (C-19), 42.91 (C-20), 54.92 (C-21), 51.39 (OCH_3), 168.91 (C=O), 15.08 (CH_3).

IR (KBr): V_{max}= 3323.82, 2943. 70, 2832.08, 1448.24, 1113.69 cm^{-1}. UV/UV-Visible (MeOH) A= 0.199 (230 nm), 0.303 (216 nm), 0.160 (243 nm), 0.104 (260 nm), 0.097 (267 nm).

Corynoxeine (4)

Amorphous white powder: FAB-HR (*+ve*): *m/z*= 383.1955 [M + H]$^+$; [M ($C_{22}H_{26}O_4N_2$) + H]= 383.1955. ^{1}H NMR (400 MHz, CD_3OD): δ = 8.55 (NH), 2.39 (d, *J*= 2.0 Hz, H-3), 2.45 (d, *J*= 9.1 Hz, H-5a), 3.28 (H-5b), 2.30 (m, H-6a), 2.00 (d, *J*= 11.9 Hz, H-6b), 7.02 (td, *J*= 7.6, 1.1 Hz, H-9), 6.87 (d, *J*= 7.7 Hz, H-10), 7.18 (td, *J*= 7.7, 1.3 Hz, H-11), 7.43 (d, *J*= 8.8 Hz, H-12), 1.88 (H-14), 2.50 (d, *J*= 3.3 Hz, H-15), 7.25 (s, H-17), 5.49 (m, H-18), 4.90 (d, *J*= 2.1 Hz, H-19), 2.84 (d, *J*= 11.0 Hz, H-20), 3.17 (dt, *J*= 11.0, 3.6 Hz, H-21a) 1.95 (m, H-21b), 3.55 (s, OCH_3), 3.72 (s, OCH_3). ^{13}C NMR (600 MHz, CD_3OD): δ = 180.26 (C-2), 73.88 (C-3), 55.02 (C-5), 36.06 (C-6), 58.12 -C-7), 135.0 (C-8), 123.45 (C-9), 110.72 (C-10), 128.86 (C-11), 125.94 (C-12), 142.3 (C-13), 24.17 (C-14), 73.37 (C-15), 112.56 (C-16), 161.48 (C-17), 140.87 (C-18), 115.97 (C-19), 43.6 (C-20), 60.05 (C-21), 51.29 (OCH_3), 61.85 (OCH_3), 170.31 (C=O).

IR (KBr): V_{max}= 3320.28, 2943.33, 2832.29, 1448.15, 1114.24 cm^{-1}. UV/UV-visible (MeOH) A= 0.987 (220 nm) and 1.058 (230 nm).

Compounds 1, 2, and 3 appeared as white amorphous powder with the respective molecular weights 368.1826, 368.1821 and 368.1809. All matched to the same calculated formula $C_{21}H_{24}O_4N_2$. In addition, the common fragment ions appeared at *m/z*= 339.3 [M-30]$^+$, 325.2 [M-44]$^+$, 291.2, 227.3, 164.1 [M-205]$^+$. Herein, they reveal similarity to pentacyclic oxindole alkaloids. On TLC with solvent system DCM: Acetone (9:1), these compounds showed different frontal reference high (1 > 2 > 3). All compounds (1, 2, 3) in ^{1}H NMR revealed presence of one methoxy group. That means, these compounds are the stereoisomers of pentacyclic oxindole alkaloids. Therefore, configuration assignment based on the chemical shift described by Beckett et al.[27] showed that compound 1 and compound 2 are on β configuration with C19, C20 but α configuration with C15. Also, compound 1 (δ*d*-CH_3= 1.39) and compound 2 (δ*d*-CH_3= 1.40) revealed *cis* D/E ring junction with three-proton doublet for the methyl (Beckett et al.[27]). Compound 3 three-proton doublet appeared at 1.11 ppm as D/E ring *trans* junction (Beckett et al.[27]). Correlation was observed between H-17 doublet and the proton H-15 *via* a long range in compounds 1, 2 and 3. However, according to asymmetric centers and their NMR spectra assignment by theoretical calculations of shielding constants of Paradowska et al.,[28] compound 1 (δC3 = 75.31) and compound 3 (δC3 = 75.56) appeared as *7R* alkaloids, but compound 2 (δC3 = 72.77) seemed like *7S* alkaloid. Also, compound 1 (δC20 = 39.29) and compound 2 (δC3 = 39.36) revealed to be *20S* alkaloid but *20R* alkaloid for compound 3 (δC3 = 42.91) (Paradowska et al.[28]). For that, compound 1 (*7R*, *20R*) *allo*-type isomer is identified to pteropodine (uncarine C), compound 2 (*7S*, *20R*) also *allo*-type isomer is identified to isopteropodine (uncarine E) and compound 3 (*7R*, *20R*) normal type isomer matched to mitraphylline (Paradowska et al.[28], Toure et al.[29], García Giménez[30], Salim and Ahmad[31]). Full ^{1}H and ^{13}C NMR signals assignment was made using HMBC, HSQC, COSY and NOESY correlation data. These compounds were previously reported from *M. inermis*.

Compound 4 appeared in molecular weight and formula 382.1955 and $C_{22}H_{26}O_4N_2$ respectively. Peaks at *m/z*= 339.2, 325.2 and 164.0 revealed the presence of olifinic and oxindole fragments. ^{1}H NMR revealed the presence of two methoxy groups at δH= 3.72, δC= 61.85 and δH= 3.55, δC= 51.29. Paradowska et al.[28] theoretical calculations of shielding constants were used for oxindole alkaloids asymmetric centers and NMR spectrum assignment. So, compound 4 (δC3 = 73.88; δC20 = 43.6) is (*7R, 20R*) alkaloid. The chemical shifts δC18 = 140.87, δH18 = 5.49 and δC19 = 115.97, δH19 = 4.90 are characteristic of the methylene group. It appeared to be a tetracyclic oxindol alkaloid. However, ^{1}H NMR, 2D NMR, ^{13}C NMR and FAB-HR compound **4** matched to those of rhyncophylline 18, 19 didehydro like corynoxeine reported from *M. speciosa* (Paradowska et al.[28], Flores-Bocanegra et al.[32]). Thus, in this study, we isolated new specific tetracyclic oxindole named corynoxeine from *M. inermis*.

Acute PTZ-induced seizure model in mice

All the procedures were performed according to the institutional animal care committee of the International Center for Chemical and Biological Sciences (ICCBS). The animal study protocol was submitted to the ICCBS/Karachi University Ethical Committee (date: 20.02.2019, approval number: 2019-006). Seventy-six male naval medical research institute (NMRI) mice with weights between 20 and 24 g were chosen for the experiment. Mice were sourced from ICCBS animal research facility. An experiment was conducted in the light of the daylight/darkness cycle at 25 ± 2 ˚C with appropriate moisture. Animals were brought into the experimental room 1 hour before the start of the experiment for the acclimatization and separated into control and test groups. 110 mg/kg body weight (bw) PTZ as convulsive dose was selected based on an acute crisis model. Mice were grouped together in batches of 7 mice for each dose and control. After acclimatization, the controls were treated with distilled water 10 mL/kg bw by intraperitoneal (*i.p.*). After 30 min, PTZ was injected into the animals, and the main stage of seizure development were observed. The extract ability to attenuate seizure threshold is indicated by absence of seizures at least 5 seconds (Atinga[15]). For the tests with the extracts and fractions of *M. inermis*, the 7 mice for each dose were subdivided into two sub-groups of 3 mice for the first test and 4 mice for the additional test. During the first phase, if no major capacity was observed to mitigate the effects of PTZ at a given dose, the additional test was not carried out. The following doses prepared in distilled water were considered based on data in the literature (Timothy et al.[14], Atinga[15]):

- Aqueous decoctate of leaves: 500, 300, and 150 mg/kg bw;
- Additional fractions acetone + ethyl acetate + butanol of leaves: 500 and 250 mg/kg;
- Stem bark ethanolic extract: 500 and 250 mg/kg bw;
- Additional stem bark ethyl acetate + acetone fractions: 500 and 250 mg/kg bw;
- Stem bark ethyl acetate fraction: 500 and 250 mg/kg bw;
- Stem bark acetone fraction: 500 and 250 mg/kg bw;
- Additional root bark ethyl acetate + acetone fractions: 500 and 300 mg/kg bw
- For the test groups, a suitable dose of the extract was injected intraperitoneally into the animals. After 30 min, a convulsive

dose of PTZ was administered, and the delay in the onset of first myoclonic jerk (FMJ) and hind limb tonic extension (HLTE) was observed and recorded for each animal. The flowchart of the entire study is presented in Figure 1.

Statistical analysis

Statistical analysis were performed by One-Way ANOVA followed by Dunnett's multiple comparison using Graph Pad Prism-8 Statistical Software Package at significance level set as $p < 0.05$.

RESULTS AND DISCUSSION

AED are more or less effective against generalized, myoclonic and absent tonic-colonial seizures (Macdonald[4]). Indeed, around 30% of patients do not respond to the currently available AEDs (Moshé et al.[3]). In traditional medicine, *M. inermis* is used to manage epilepsy. Unfortunately, there was no data on the required levels of herbal materials for therapeutic value. Chromatographic fingerprinting is used in the quality control of medicinal plant materials (Ouédraogo et al.[13]). This report focused first on flavonoid fingerprints as tracers of bioactive contain in various fractions of *M. inermis*. As a result, DCM and hexane fraction didn't show the remarkable flavonoid fingerprints (Figures 2-4). In the different parts, decoction, acetone, ethyl acetate and butanol fractions revealed various flavonoid fingerprints. Using flavonoid fingerprints, as fraction standardization, aqueous decoctate of leaves appeared atypical however, leaves ethyl acetate, butanol and acetone fractions appeared typical. Also, stem bark ethyl acetate and acetone fractions appeared typical, as well as root barks ethyl acetate and acetone fractions. Thus, for the same part, fractions may be combined for their similar flavonoid fingerprints. We noticed variability in flavonoids groups contain (Figures 2-4). Other studies reported the presence of flavonoids in *M. inermis* parts but still on content analyses (Nacoulma[12], Timothy et al.[14], Atinga[15], Ouédraogo et al.[33]).

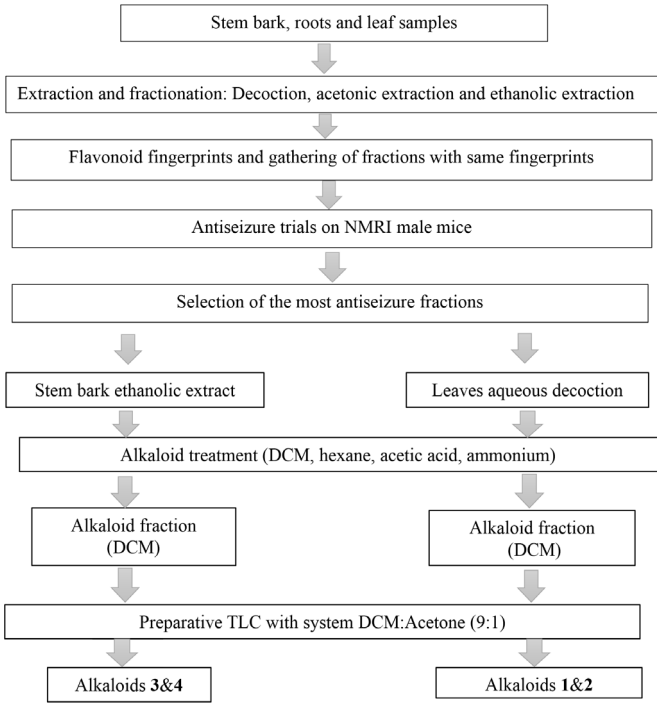

Figure 1. Flowchart of the entire study

NMRI: Naval medical research institute, DCM: Dichloromethane, TLC: Thin layer chromatography

Secondly, we conducted an investigation of *M. inermis* roots, leaves, and stem bark standardized fraction effects on acute PTZ-induced seizures in NMRI mice. All findings were presented in Table 1 and Figure 5. The preliminary study revealed that leaf decoction, stem bark ethyl acetate extract and ethanolic extract delayed the onset of PTZ-induced convulsions. In contrast, root fractions did not show antiseizure effects in the PTZ-induced seizure test in mice (Table 1). The using of

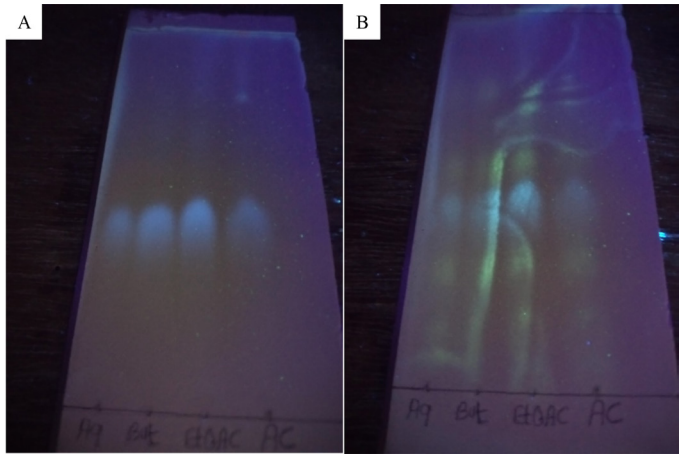

Figure 2. TLC of only leaves extract and fractions that contain flavonoids, A) UV light, B) AlCl3 spraying + UV light. Four additional yellow spots after spraying revealing flavonoids contain. But, EtOAc and Ac appeared as the same compound group contains

TLC: Thin layer chromatography, UV: Ultraviolet, Aq: Aqueous, But: Butanol, EtOAc: Ethyl acetate, Ac: Acetone fractions

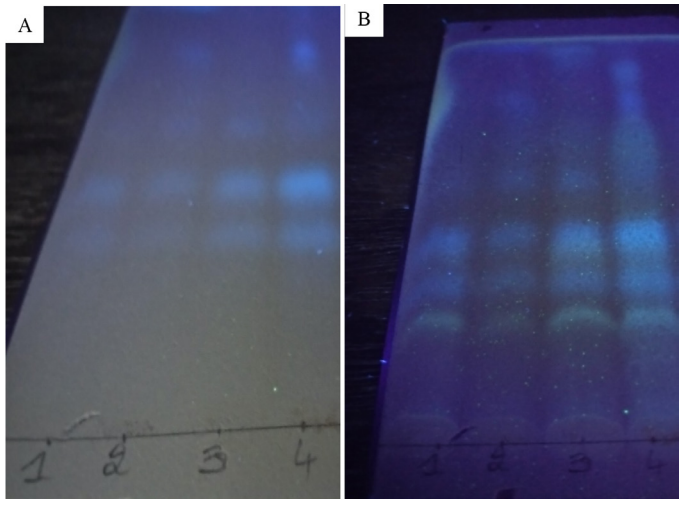

Figure 3. TLC of stem bark, A) UV light, B) AlCl3 spraying + UV light. 1) Hexane fraction, 2) DCM fraction, 3) EtOAc fraction, 4) AC. Three additional yellow spots after spraying revealing flavonoid contains. Most present in 3 and 4 that looked the same

TLC: Thin layer chromatography, UV: Ultraviolet, DCM: Dichloromethane, EtOAc: Ethyl acetate, Ac: Acetone fractions

M. inermis root in the management of epilepsy has not been reported in any ethnobotanical survey. This lack of antiseizure properties might justify its non-utility in traditional medicine. Herbal medicinal product must be manufactured from the indicated part of the plant and must be free of other parts of the same or other plants (Ouédraogo et al.[13]). The acetone stem bark fraction failed to demonstrated any effect, but the ethyl acetate stem bark fraction was found to be responsible of the combined antiseizure effects (Table 1). With the aim to confirm the abilities of *M. inermis*-derived fractions to reduce the seizures, more assays were carried out (Figure 5). PTZ 110 mg/kg bw showed FMJ at 49 ± 0.67 sec and HLT at 65.33 ± 4.22 sec in control group. So, ethyl acetate extract at 500 mg/kg bw, decoction at 300 mg/kg bw and ethanol extract at 500 mg/kg bw revealed the noticeable delay of HLTE occurs (more than 10 min). Moreover, no significant antiseizure effect was observed between stem bark ethanolic extract (500 mg/kg bw) and leaf decoction (300 mg/kg bw) ($p > 0.05$). In contrast, all the fractions failed to significantly delay the onset of first myoclonic seizures. These results suggested that *M. inermis* delay the seizures, specifically delayed HLTE occurred, but failed to show the abilities to delay first myoclonic seizures. The lack of abilities to delay the onset of FMJs suggests that *M. inermis* might require more time to exhibit antiseizures effects after the administration of the extract. However, the results observed in this study with the stem bark ethanolic extract are in agreement with the reported on temporary absence of seizures at 500 and 250 mg/kg bw (Timothy et al.[14]). Also, we reported that *M. inermis* leaves methanolic extract prevented seizures at the doses more than 600 mg/kg (Atinga[15]). But this latter study employed high doses of extracts. In traditional drug preparation methanol is subjected to usage limits (Ouédraogo et al.[13]). The high dose of PTZ used in this study to induced seizure may justify variability of anticonvulsant properties and lack of remarkable latency to FMJ occurred compared to the reported study (Timothy et al.[14], Atinga[15]). It has been reported that flavonoids or medicinal plant extracts containing flavonoids interact with $GABA_A$ receptors leading to anticonvulsant

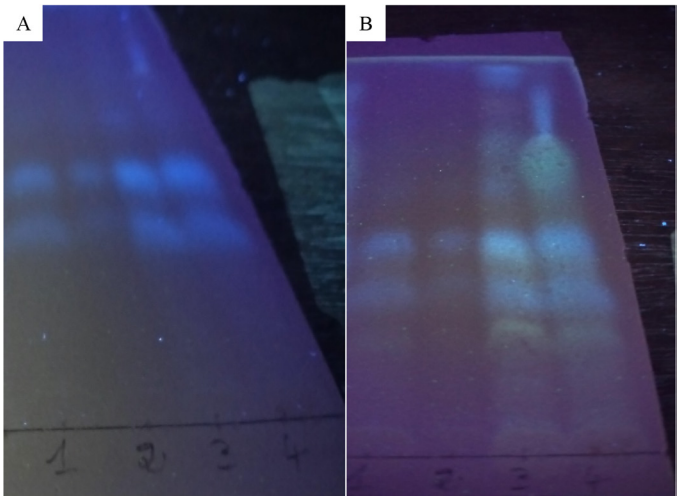

Figure 4. TLC of roots, A) UV light, B) $AlCl_3$ spraying + UV light. 1) Hexane fraction, 2) DCM fraction, 3) EtOAc fraction, 4) Ac. Three additional yellow spots after spraying revealing flavonoid content. Most present in 3 and 4 that appeared the same

TLC: Thin layer chromatography, UV: Ultraviolet, DCM: Dichloromethane, EtOAc: Ethyl acetate, Ac: Acetone fractions

Table 1. Screening of different parts extract and fraction of *M. inermis* for PTZ-induced seizures in mice

Parts	Extracts/fractions	Doses (mg/kg)	Delay in HLTE
Leaves	Decoction	500	++
		300	+++
		150	+
	Ethyl acetate + Butanol + Acetone	500	++
		250	+
Stem bark	Ethanol	500	++++
		250	+++
	Ethyl acetate + Acetone	500	+++
		250	++
	Ethyl acetate	500	+++
		250	+
	Acetone	500	-
		250	-
Roots	Ethyl acetate + Acetone	500	-
		300	-

Noticeable delay of HLTE was recorded by comparison with the group treated with distilled water as a control, n= 3. ++++: More than 10 min, +++: 5 to 10 min, ++: 2 to 5 min, +: less than 2 min, -: No activity, PTZ: Pentylenetetrazol, HLTE: Hind limb tonic extension

activities (Copmans et al.[6], Jäger and Saaby[20], Zhu et al.[21]). Indeed, the variability could arise from the difference of flavonoid fingerprints observed with leaves and stem bark leading to the variation of the HLTE delay. Moreover, it may be suggested that these active extracts might act as agonist of $GABA_A$ receptor, similar to the effects other AEDs that prevent seizures in PTZ-induced seizure test (Kobayashi et al.[5], Shorvon et al.[8],). So those derived fractions could be powerful sources of anticonvulsants. In addition, multiple studies reported lower toxicity of *M. inermis* different part-derived extracts in mice and rates (Timothy et al.[14], Monjanel-Mouterde et al.[34], Ouedraogo et al.[35]). Our latest research on these mixed fractions revealed that they are less hazardous to mouse fibroblast and human hepatocyte cell lines (Ouédraogo et al.[19]). Flavonoids could therefore be tracers to guarantee the quality and therapeutic value of *M. inermis* parts preparation in traditional use and the research of anticonvulsant phytomedicines.

A thorough phytochemical analysis was carried out on the most anticonvulsant extracts and stem bark ethanolic extract and leaf decostate. The fractionation revealed that the alkaloid fraction represented 2.4% of leaf decoction and 7.8% of stem bark ethanolic extract. Traore et al.[36] reported that alkaloid-rich fraction showed low toxicity on monocyte proliferation through inhibition of mammary cell protein production but no mutagenic or genotoxic activities. Subsequently, in order to know these fraction alkaloid ingredients, TLC on the derived alkaloid fractions offered the main oxindole alkaloids *viz* pteropodine, isopteropodine and mitraphylline. In addition, we isolated the new tetracyclic oxindol alkaloid, corynoxeine (Figure 6). Moreover, pteropodine and isopteropodine were reported for their lack of adverse effects on cell culture and DNA (Lee et al.[37], Aponte et al.[38], Ahmad and Salim[39]). Also, isopteropodine showed a neuroprotective effect without toxicity until 10^3 µM

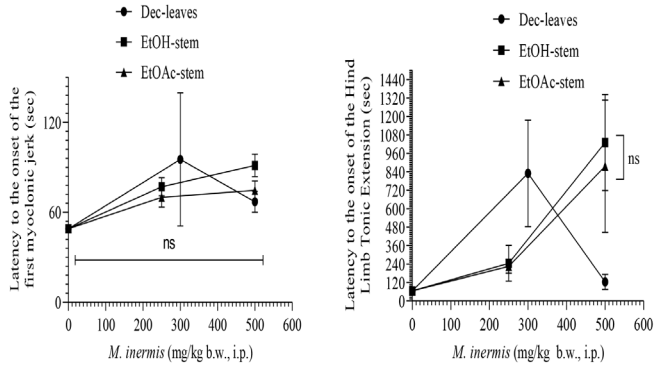

Figure 5. Anti-PTZ-induced seizures of some of the most active fractions. Onsets of FMJ and HLTE are expressed as mean ± SD, n= 7. The dose 0 mg/kg bw is the control (group treated with distilled water). No significant difference was observed between the FMJ onset of the control and treated groups. Compared with the control group, stem bark ethyl acetate and ethanol extract and leave decoction delayed noticeably HLTE ($p \leq 0.05$). Statistical analyses were performed by One-Way ANOVA with Dunnett's multiple comparison tests

Statistical significance levels: *$p \leq 0.05$, ns: Non-significant, Dec: Decoction, EtOH: Ethanol, EtOAc: Ethyl acetate, PTZ: Pentylenetetrazol, FMJ: First myoclonic jerk, HLTE: Hind limb tonic extension, SD: Standard deviation

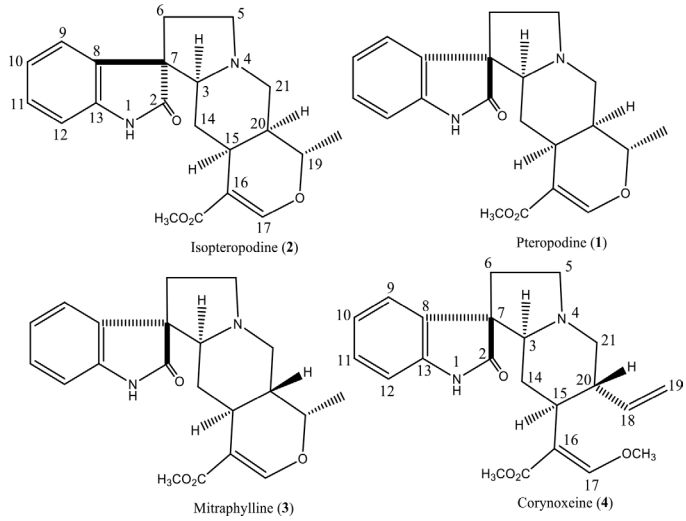

Figure 6. Structures of pteropodine (1), isopteropodine (2), mitraphylline (3), and corynoxeine

(Ahmad and Salim[39]). However, *M. inermis* alkaloid content was already reported (Shellard and Sarpong[16,17], Toure et al.[40]). Alkaloid extract and compounds of other *Mitragyna species* as well as *M. speciosa* are reported to exhibit benefit pharmacological effects on the brain, but require moderate doses (Nelsen et al.[41]). Indeed, alkaloid such as mitragynine interact with neurons by blocking calcium channel (Suhaimi et al.[42]). Therefore, neuronal Ca^{2+} channel-blocking effect abolish seizures (Kobayashi et al.[5]). Mitragynine is also reported in *M. inermis* leaf and stem bark (Shellard and Sarpong[16], Toure et al.[40]). However, indole alkaloids such as ibogaine similarly to pteropodine, isopteropodine and mitraphylline isolated in this study is reported to exhibit anticonvulsant activity by blocking *n*-methyl-D-aspartate (NMDA) receptors. Also, tetracyclic oxindole alkaloids such as rhynchophylline and isorhynchophylline present in *M. inermis* leave and stem bark have been reported with anticonvulsant activities by inhibiting NMDA (Zhu et al.[21]). Thus, the abilities of decoction and ethanolic extracts to exhibit acute antiseizure effects in PTZ-induced seizure test might be attributed to the alkaloid fractions with the plausible synergistic action of pteropodine, isopteropodine, corynoxeine and mitraphylline or due to flavonoid contains. Some AEDs such as phenytoin, carbamazepine and diazepam exhibit alkaloid functions (Kobayashi et al.[5]). However, the pro-seizure effect reported with the ingestion of *M. speciosa* alkaloids calls for having moderate look and use of *M. inermis* alkaloids for treating seizures.

CONCLUSION

This study suggests that flavonoid fingerprints are tracers of *M. inermis* anticonvulsant ingredients. Stem bark ethanolic and ethyl acetate extracts and leaf aqueous extracts contain anticonvulsant bioactive principles that delay HLTE in male NMRI mice. Furthermore, alkaloid contents also remain plausible bioactive anticonvulsant principles. All observations support the traditional use of *M. inermis* to manage epilepsy.

However, further studies might help understand the effects of alkaloid fractions, flavonoids, and the isolated compounds as promising antiseizure agents derived from *M. inermis* in experimental animals.

Acknowledgments

One of the authors would like to sincerely acknowledge The World Academy of Sciences-ICCBS/Karachi/Pakistan for providing research funding and analysis facilities through Sandwich Postgraduate Fellowship FR number 3240316605 to Relwendé Justin Ouédraogo.

Ethics

Ethics Committee Approval: The animal study protocol was submitted to the ICCBS/Karachi University Ethical Committee (date: 20.02.2019, approval number: 2019-006).

Informed Consent: Not necessary.

Authorship Contributions

Concept: R.J.O., L.O., S.U.S., F.S., Design: R.J.O., L.O., S.U.S., F.S., G.A.O., Data Collection or Processing: R.J.O., L.O., S.U.S., F.S., M.J., F.K., M.N.U.H., Analysis or Interpretation: R.J.O., L.O., S.U.S., F.S., M.J., F.K., Literature Search: R.J.O., L.O., S.U.S., F.S., M.J., F.K., Writing: R.J.O.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

REFERENCES

1. Yuen AWC, Keezer MR, Sander JW. Epilepsy is a neurological and a systemic disorder. Epilepsy Behav. 2018;78:57-61.
2. Banerjee PN, Filippi D, Allen Hauser W. The descriptive epidemiology of epilepsy-a review. Epilepsy Res. 2009;85:31-45.
3. Moshé SL, Perucca E, Ryvlin P, Tomson T. Epilepsy: new advances. Lancet. 2015;385:884-898.
4. Macdonald RL. Antiepileptic drug actions. Epilepsia. 1989;30 Suppl 1:S19-28.
5. Kobayashi K, Endoh F, Ohmori I, Akiyama T. Action of antiepileptic drugs on neurons. Brain Dev. 2020;42:2-5.
6. Copmans D, Orellana-Paucar AM, Steurs G, Zhang Y, Ny A, Foubert K, Exarchou V, Siekierska A, Kim Y, De Borggraeve W, Dehaen W, Pieters L, de Witte PAM. Methylated flavonoids as anti-seizure agents: Naringenin 4',7-dimethyl ether attenuates epileptic seizures in zebrafish and mouse models. Neurochem Int. 2018;112:124-133.
7. Devinsky O, Vezzani A, O'Brien TJ, Jette N, Scheffer IE, de Curtis M, Perucca P. Epilepsy. Nat Rev Dis Primers. 2018;4:18024.
8. Shorvon S, Perucca E, Engel J. The treatment of epilepsy. Third Edition, Blackwell Publishing Ltd. 2009:1075.
9. Huang RQ, Bell-Horner CL, Dibas MI, Covey DF, Drewe JA, Dillon GH. Pentylenetetrazole-induced inhibition of recombinant gamma-aminobutyric acid type A [GABA(A)] receptors: mechanism and site of action. J Pharmacol Exp Ther. 2001;298:986-995.
10. Cragg GM, Newman DJ, Snader KM. Natural products in drug discovery and development. J Nat Prod. 1997 ;60:52-60.
11. Arbonnier M. Arbres, arbustes et lianes des zones sèches d'Afrique de l'Ouest. 2002;2-87.
12. Nacoulma OG. Plantes médicinales et pratiques médicinales traditionnelles: cas du plateau central. Ph.D. Thesis, Universite de Ouagadougou. 1996;1-328.
13. Ouédraogo S, Yoda J, Traore TK, Nitiema M, Sombie BC, Diawara HZ, Yameogo JBG, Djande A, Belemnaba L, Kini FB, Ouédraogo S, Semde R. Production de matières premières et fabrication des médicaments à base de plantes médicinales. Int J Biol Chem Sci. 2021;15:750-772.
14. Timothy SY, Wazis CH, Helga BI, Maina A, Bomai HI. Anticonvulsant screening of the aqueous and ethanol extracts of *Mitragyna inermis* bark in pentylenetetrazole and strychnine induced seizures in albino rats. Int J Pharma Ther. 2014;5:358-363.
15. Atinga V. Evaluation of *Mitragyna inermis* (Wild) leaf extract as an anticonvulsant agent in pentylenetetrazole induced seizures in mice. Adv Biomed Pharm. 2015;2:205-210.
16. Shellard EJ, Sarpong K. The alkaloids of the leaves of *Mitragyna inermis* (Willd.) O. Kuntze. J Pharm Pharmacol. 1969;21:Suppl:113S+.
17. Shellard EJ, Sarpong K. The alkaloidal pattern in the leaves, stem-bark and root-bark of Mitragyna species from Ghana. J Pharm Pharmacol. 1970:Suppl:34S+.
18. Toklo PM, Eléonore YL, Amoussatou S, Fidèle MA, Géorcelin GA, Mathias AA, Sylvie HA, Joachim DG. Phytochemistry and pharmacological review of *Mitragyna inermis* (Willd.) Kuntze (*Rubiaceae*). J Pharmacogn Phytochem. 2020;9:22-30.
19. Ouédraogo RJ, Aleem U, Ouattara L, Nadeem-Ul-Haque M, Ouédraogo GA, Jahan H, Shaheen F. Inhibition of advanced glycation end-products by *Tamarindus indica* and *Mitragyna inermis* extracts and effects on human hepatocyte and fibroblast viability. Molecules. 2023;28:393.
20. Jäger AK, Saaby L. Flavonoids and the CNS. Molecules. 2011;16:1471-1485.
21. Zhu HL, Wan JB, Wang YT, Li BC, Xiang C, He J, Li P. Medicinal compounds with antiepileptic/anticonvulsant activities. Epilepsia. 2014;55:3-16.
22. Yuan H, Ma Q, Cui H, Liu G, Zhao X, Li W, Piao G. How Can synergism of traditional medicines benefit from network pharmacology? Molecules. 2017;22:1135.
23. Ouédraogo RJ, Ouattara L, Kabre P, Sanou Y, Somda MB, Ouoba P, Anicet G, Draogo Q. Season and ecotype effects on soluble phenolic compounds content and antioxidant potential of *Tamarindus indica* and *Mitragyna inermis*. J Pharm Pharmacol. 2022;10:143-156.
24. Checkouri E, Reignier F, Robert-Da Silva C, Meilhac O. Evaluation of polyphenol content and antioxidant capacity of aqueous extracts from eight medicinal plants from reunion island: protection against oxidative stress in red blood cells and preadipocytes. Antioxidants (Basel). 2020;9:959.
25. Ranilla LG, Kwon YI, Apostolidis E, Shetty K. Phenolic compounds, antioxidant activity and *in vitro* inhibitory potential against key enzymes relevant for hyperglycemia and hypertension of commonly used medicinal plants, herbs and spices in Latin America. Bioresour Technol. 2010;101:4676-4689.
26. Silva LMP, Alves JSF, da Silva Siqueira EM, de Souza Neto MA, Abreu LS, Tavares JF, Porto DL, de Santis Ferreira L, Demarque DP, Lopes NP, Aragão CFS, Zucolotto SM. Isolation and identification of the five novel flavonoids from genipa americana leaves. Molecules. 2018;23:2521.

27. Beckett AH, Shellard EJ, Phillipson JD, Lee CM. Alkaloids from *Mitragyna speciosa* (Korth). J Pharm Pharmacol. 1965;17:753-755.

28. Paradowska K, Wolniak M, Pisklak M, Gliński JA, Davey MH, Wawer I. (13)C, (15)N CPMAS NMR and GIAO DFT calculations of stereoisomeric oxindole alkaloids from Cat's Claw (*Uncaria tomentosa*). Solid State Nucl Magn Reson. 2008;34:202-209.

29. Toure H, Babadjamian A, Balansard G, Faure R, Houghton PJ. Complete ^{1}H and ^{13}C NMR chemical shift assignments for some pentacyclic oxindole alkaloids. Spectrosc Lett. 1992;25:293-300.

30. García Giménez D, García Prado E, Sáenz Rodríguez T, Fernández Arche A, De la Puerta R. Cytotoxic effect of the pentacyclic oxindole alkaloid mitraphylline isolated from *Uncaria tomentosa* bark on human Ewing's sarcoma and breast cancer cell lines. Planta Med. 2010;76:133-136.

31. Salim F, Ahmad R. Isopteropodic acid from Malaysian *Uncaria longiflora* var. pteropoda. World Appl Sci J. 2010;10:1333-1337.

32. Flores-Bocanegra L, Raja HA, Graf TN, Augustinović M, Wallace ED, Hematian S, Kellogg JJ, Todd DA, Cech NB, Oberlies NH. The Chemistry of Kratom [*Mitragyna speciosa*]: updated characterization data and methods to elucidate indole and oxindole alkaloids. J Nat Prod. 2020;83:2165-2177.

33. Ouédraogo RJ, Somda MB, Ouattara L, Kagambega W, Ouoba P, Ouédraogo GA. Evaluation of the antioxidant and α-amylase inhibitory activities of *Mitragyna inermis* (Willd) O. Kuntze and *Tamarindus indica* Linn. J Exp Biol Agric Sci. 2020;8:676-682.

34. Monjanel-Mouterde S, Traoré F, Gasquet M, Dodero F, Delmas F, Ikoli JF, Lorec AM, Chamlian V, Portugal H, Balansard G, Pisano P. Lack of toxicity of hydroethanolic extract from *Mitragyna inermis* (Willd.) O. Kuntze by gavage in the rat. J Ethnopharmacol. 2006;103:319-326.

35. Ouedraogo Y, Guissou IP, Nacoulma OG. Biological and toxicological study of aqueous root extract from *Mitragyna inermis* (Willd Oktze) *Rubiaceae*. Int J Pharmacol. 2007;3:80-85.

36. Traore F, Gasquet M, Laget M, Guiraud H, Di Giorgio C, Azas N, Doumbo O, Timon-David P. Toxicity and genotoxicity of antimalarial alkaloid rich extracts derived from *Mitragyna inermis* O. Kuntze and *Nauclea latifolia*. Phytother Res. 2000;14:608-611.

37. Lee JS, Yang MY, Yeo H, Kim J, Lee HS, Ahn JS. Uncarinic acids: phospholipase Cgamma1 inhibitors from hooks of *Uncaria rhynchophylla*. Bioorg Med Chem Lett. 1999;9:1429-1432.

38. Aponte JC, Vaisberg AJ, Rojas R, Sauvain M, Lewis WH, Lamas G, Sarasara C, Gilman RH, Hammond GB. A multipronged approach to the study of peruvian ethnomedicinal plants: a legacy of the ICBG-Peru Project. J Nat Prod. 2009;72:524-526.

39. Ahmad R, Salim F. Oxindole alkaloids of *Uncaria (Rubiaceae,* subfamily *Cinchonoideae*): a review on its structure, properties, and bioactivities. Stud Nat Prod Chem. 2015;45:485-525.

40. Toure H, Balansard G, Pauli AM, Scotto AM. Pharmacological investigation of alkaloids from leaves of *Mitragyna inermis* (*Rubiaceae*). J Ethnopharmacol. 1996;54:59-62.

41. Nelsen JL, Lapoint J, Hodgman MJ, Aldous KM. Seizure and coma following Kratom (*Mitragynina speciosa* Korth) exposure. J Med Toxicol. 2010;6:424-426.

42. Suhaimi FW, Yusoff NH, Hassan R, Mansor SM, Navaratnam V, Müller CP, Hassan Z. Neurobiology of Kratom and its main alkaloid mitragynine. Brain Res Bull. 2016;126:29-40.