ejpmr, 2024, 11(1), 272-278

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

NEUROPHARMACOLOGICAL & NEUROPROTECTIVE EFFECTS OF METHANOLIC EXTRACT OF LEAVES OF SESBANIA GRANDIFLORA IN RATS

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Article Received on 30/10/2023 **Article Revised on 20/11/2023** **Article Accepted on 10/12/2023**

ABSTRACT

The methanolic extract of leaves of *Sesbania Grandiflora* was evaluated for its neuroprotective activity. Preliminary phytochemical analysis revealed the presence of alkaloids, carbohydrates, saponins, glycosides, protein and amino acids, phenolic compounds. The present investigation of the methanolic extract of leaf of *Sesbania Grandiflora* produced central inhibitory effect in mice and rats. Behavioural pharmacology field makes the concepts that are derived from pharmacology and psychology to study behaviour in animal models. The present study shows that the methanolic extract of leaf of Sesbania Grandiflora protected some of the animals against seizures induced by maximal electroshock. Antiepileptic drugs which inhibit voltage-dependent Na+ channels, such as phenytoin can prevent MES-induced tonic extension. In MES-induced convulsion animals represents grandmal type of epilepsy. It has often been stated that antiepileptic drugs that block MES-induced tonic extension phase act by blocking seizure spread. Moreover, MES-induced tonic extension phase can be prevented either by drugs that inhibit voltage-dependent Na+ channels, such as phenytoin, valproate, felbamate and lamotrigine or by drugs that block glutaminergic excitation mediated by the N- methyl- D-Aspartate (NMDA) receptor such as felbamate. Our conclusion, investigation suggests that methanolic extract of powdered leaves of *Sesbania Grandiflora*. Which possess potent neuroprotective effects in rats & mice.

KEYWORDS: Neuropharmacological Effects, Methanolic Extract, Leaves Of Sesbania Grandiflora, Rats & Mice.

INTRODUCTION

A variety of natural products have been used as medicines and have been associated with traditional medicine for thousands of years.[1] Compounds derived from natural products have gained substantial market share or serve as biochemical tools for demonstrating the role of specific pathways in disease and their potential as drugs.[2] Thus, natural products have historically consisted of the most successful sources of new medicines. Compounds derived from natural products serve as both drugs and templates for drugs directly, as well as leading to the discovery of novel aspects of physiology or biology that assist in better understanding disease targets and pathways.[1,3] According to the World Health Organization, natural products are considered to be important sources of medicine because of their traditional uses or remedies, and through a few systematic approaches to exploring naturally used products or compounds that can be developed as drug leads, the world has recognized the importance of natural products as medicines.[4] To find drugs from natural products, it is important to know which active compounds accurately target the pathways of disorders.

An indigenous Southeast Asian plant with specific medicinal benefits, Mitragyna speciosa Korth. (Rubiaceae) Havil. is also known as kratom, kakuam, ithang, thom in Thailand; ketum or biak-biak in Malaysia; or krypton when combined with O-demethyltramado.[5,6] Thailand, Indonesia, Malaysia, Myanmar, and Papua New Guinea are among the countries that originated it.[7,8] Different formulations are available for kratom, including raw leaves, tea, capsules, tablets, powders, and concentrated extracts. It is widely used for anxiety, depression, pain relievers, talkativeness, sociability, sedation, mood enhancement,

constipation, increase energy, appetite, sexual desire, wound healer as a local anesthetic, and other ailments/conditions as a traditional medicine. The Ketum solution is recommended to ease opiate withdrawal symptoms by taking three 3 × 250 mL daily.[9,10,11,12] Due to addiction concerns and an increase in the number of young people utilizing the leaf material and developing a "hook", kratom was formerly outlawed in Thailand and the adjacent country of Malaysia.[13] By using a quick and reliable PCR-reverse dot blot (RDB) hybridization experiment, kratom demonstrated the presence of several narcotic specimens, such as ground leaves and kratom's cocktail, particularly in matK sequences, a diagnostic barcode.[14] In a different investigation, DNA barcoding in conjunction with high-resolution melting (Bar-HRM) analysis was used to confirm the validity of kratom as a species of narcotic plant for law enforcement.[15] Since at least the eighteenth century, it has been employed in herbal medicine, with various therapeutic benefits, including antioxidant,[16,17,18] antimicrobial, antibacterial,[16,19] antinociceptive,[20] anti-inflammatory,[21] cytotoxic,[20] weight reduction,[22] analgesic,[23,24,25,26] antipyretic, sedative, stimulant, antidiabetic, anxiolytic and anti-depressant,[27,28,29,30] antidopaminergic, and antidiarrheal. Very recently, Salleh et al. reported the potential neuroprotective role of kratom on aging brains.

Very less pharmacological studies have been carried out on the plant *Sesbania Grandiflora.* As per the literature review, so far no scientific study has been carried on this plant *Sesbania Grandiflora* to explore the neuroprotective activity. So, the present study has been under taken to explore the neuropharmacological effect of leaves of methanolic extract of leaves of *Sesbania Grandiflora* which includes studies on pharmacological, Preliminary phytochemical and its pharmacological evaluation.

MATERIALS AND METHODS

Collection, identification and authentication of plant material

The plant of *Sesbania Grandiflora* inn was collected in the month of January, 2017 from the house garden, Nasiyanur, Erode district, Tamil nadu, India. The plant material was identified and authenticated by Dr. A. Balasubramaniam, Research Consultant, ABS Botanical Garden, Kaaripatti, Salem District, Tamil nadu.

Shade drying, Granulation and Extraction

The plant leaf was taken and dried in shade. Then the shade dried leaf were coarsely powdered by means mix grinder and was through sieve no 60 to get the coarse powder. Then the coarsely powdered materials were weighed, packed in an airtight container and used for extraction with solvent, phytochemical studies and pharmacological studies.

Preparation of plant extract

About 350 gm of coarsely powdered leaf was packed in 1000ml soxhlet apparatus and extract with methanol for 72 hours by continuous hot percolation. After extraction, the solvent was distilled off and extract was concentrated to at room temperature and the percentage yield was calculated.

Pharmacological studies

Animal studies

Source and Maintenance of Experimental Animals

Healthy male and female adult rat & mice, weighing between 20-25 g & 150-250 g were obtained from the Animal House of the KMCH College of pharmacy, Coimbatore. The animals were housed under standard environmental conditions, with feed and water provided *ad libitum*. Proper handling and using of the animals were in accordance with the guidelines and regulations, monitored and approved by the Ethical Committee on Animal use, Department of Pharmacology, KMCH College of pharmacy, Coimbatore.

Animal Model of Convulsion

Maximal Electro Shock (MES) induced Convulsion Principle

The electroshock assay in mice is used primarily as an indication for compounds which are effective in grand mal epilepsy. Tonic hind limb extensions are evoked by electric stimuli which are suppressed by anti-epileptics but also by other centrally active drugs.

Procedure

The maximum electrical shock (MES) induced convulsion in animals represented grand mal type of epilepsy. These are type of procedures use to studies convulsions and to test to anticonvulsant drugs in laboratory animals. In MES convulsions electric shock is applied through the corneal electrode, through optic stimulation cortical excitation are produced .The MES – convulsion are divided into five phase such as Tonic flexion, Tonic extensor, Clonic convulsions, stupor, recovery or death. A substance is known to possess anticonvulsant property if it reduces or abolished the extensor phase of MES convulsions. This procedure may be used to produce convulsions in rat. In this method place corneal electrodes on the cornea and apply the prescribed current and different stages of conclusions are noted as in previous paragraph. Note the time (sec) spent by the animal in each phase of the conclusions. Inject phenytoin (i.p) in rats. Wait for 30 min and subject the animals to electro-convulsions as described. Note the reduction in time or abolition of tonic extensor phase of MES convulsions.

Experimental Design

Wistar albino rats weighed around 150-250g were used for the study. Rats weredivided into four groups of 5 animals each,

Group-1: Received, normal saline (0.1% solution)

Group-2: Received standard drug, Phenytoin (20mg/kg *i.p.*)

Group-3: Received methanol extract of leaves of AV

(250mg/kg; *p.o.* Low dose)
Group-4: Received methanol extract of leaves of AV (500mg/kg; *p.o*. High dose)

Animal Models of Anti-Depressant

Antidepressant activity was indicated the mood elevating due to various mechanism of the antidepressant drugs, such as inhibition of the enzyme of monoamine oxidase,inhibition of reuptake bioamines and enhancement of the concentration of 5-HT e) ct. Lateron, inhibition of reuptake of bioamines was found to be main mechanism of action todowenregulation of β receptor. Several lines of preclinical and clinical evidence indicates that enhancement of 5-HT mediated neurotransmission might underline the therapeutic effect of most of the antidepressant This behavioural effect very similar to that found by other author after treating mice with classical antidepressant drugs as IMI[74] various models are like, Forced swimming Test (FST).

Principle

Behavioural despair was proposed as a model to test for antidepressant activity. It wassuggested that mice or rats forced to swim in a restricted space from which they cannot escape are induced to a characteristic behaviour of immobility. This behaviour reflects a state of despair which can reduce by several agents which are therapeutically effective inhumandepression.

Procedure

The procedures for Forced swim test or Despair swim test were similar to those first described by Porsolt., *et al*. (1977). Male albino mice weighing 20-22 g are used. They are brought to the laboratory at least one day before the experiment and are housed separately in Makrolon® cages with free access to food and water. This test is sensitive to all major classes of antidepressant drugs, hence used as an animal model of depression like behaviour. Mice were grouped into three of five animals each and were trained individually for three consecutive days (pre-test session). In the "test-session" The animals were made to swim individually in an open cylindrical container of diameter 20cm and height 50cm, containing 25cm of water at 25˚C, for a test period of 6 minutes. After a brief period of vigorous activity for about two minutes, mice maintain a typical immobile posture. The animals were considered immobile when they float in an upright position, making only negligible movements to maintain their head above water. The total duration of immobility was recorded and changes in the same were studied for various treatment groups.[75] Experimental Design:

Swiss albino mice weighed around 20-25g were used for the study. Mice were divided into five groups of 5 animals each,
Group-1: Received normal saline (0.1% solution)
Group-2: Received standard drug, imipramine (60mg/kg.*i.p)*
Group-3: Received methanol extract of leaves of AV (250mg/kg; *p.o.* Low dose)
Group-4: Received methanol extract of leaves of AV (500mg/kg; *p.o*. High dose)

Tail suspension Test (TST)

Experimental Design

Swiss albino mice weighed around 20-25g were used for the study. Mice were divided into five groups of 5 animals each,
Group-1: Received normal saline (0.1% solution)
Group-2: Received standard drug, imipramine (60mg/kg.*i.p)*
Group-3: Received methanol extract of leaves of AV (250mg/kg; *p.o.* Low dose)
Group-4: Received methanol extract of leaves of AV (500mg/kg; *p.o*. High dose)

Animal Models for Anti-Anxiety

Elevated Plus-Maze Test (EPM)

Experimental Design

Swiss albino mice weighed around 20-25g were used for the study. Mice were divided into five groups of 5 animals each
Group-1: Received normal saline (0.1% solution).
Group-2: Received standard drug, diazepam (4mg/kg.*i.p).*
Group-3: Received methanol extract of leaves of AV (250mg/kg; *p.o.* Low dose).
Group-4: Received methanol extract of leaves of AV (500mg/kg; *p.o*. High dose)Light-Dark model.

Experimental Design

Swiss albino mice weighed around 20-25g were used for the study. Mice were divided into five groups of 5 animals each
Group-1: Received normal saline (0.1%)
Group-2: Received standard drug, imipramine (60mg/kg. *i.p)*
Group-3: Received methanol extract of leaves of AV (250mg/kg; *p.o.* Low dose)
Group-4: Received methanol extract of leaves of AV (500mg/kg; *p.o*. High dose)

Statistical Analysis

All the data were expressed as the mean ± standard error of the mean (SEM). The statistical significance of the difference between the groups was analyzed by using Graphpad 5.0 software (Graphpad, San Diego, USA) by applying one was analysis of variance (ANOVA) followed by Dunnett‟s test as post hoc and also student‟s paired T-test. The value of $P<0.05$ was considered to be statistically significant.

RESULT AND DISCUSSION

Table 1: Data showing colour, Consistency and yields of methanolic extract of powdered leaf of *Sesbania Grandiflora.*

S. NO.	EXTRACT	COLOUR	CONSISTENCY	%YIELD (W/W)
1	Methanolic Extract	Dark Green	Sticky Mass	12 % (w/w)

Table 2: Results of the phytochemical constituents of Methanolic Extract of *Sesbania Grandiflora.*

S. No.	Constituents	Methanolic extract of Leaves of Sesbania Grandiflora .
1	CARBOHYDRATES	+ Ve
2	FIXED OILS AND FATS	-Ve
3	PROTEIN AND AMINO ACID	+ Ve
4	SAPONINS	+ Ve
5	STEROIDS	+Ve
6	ALKALOIDS	+Ve
7	GLYCOSIDES	+Ve
8	FLAVONOIDS	+Ve
9	TANNINS	+Ve
10	GUM & MUCILAGE	-Ve
11	TRITERPENOIDS	+Ve
12	PHENOLIC COMPOUNDS	+Ve

(+) Presence (-) Absence

Anti-Convulsant Activity

Table 3: Effect of Methanolic extracts of *Sesbania Grandiflora.* On Maximalelectroshock induced in rats.

Group	Dose (mg/kg)	Flexion	Extension	Clonus	Stupor	Recovery/ Death
Control	Normalsaline	15.35±0.157	13.5±0.025	14.13±0.035	6.09±0.039	Recovery
Phenytoin	20 mg/kg	3.14±0.049***	0	3.17±0.036***	1.07±0.023***	Recovery
MEAV	250 mg/kg	4.20±0.067***	1.05±0.010***	5.73±0.108***	3.63±0.025***	Recovery
MEAV	500 mg/kg	3.29±0.049***	0.72±0.014***	4.53±0.028***	1.10±0.026***	Recovery

Anti-Depressant Activity

Forced Swimming Test (TST)

Table 4: Effect of Methanolic Extracts of *Sesbania Grandiflora* . on forced swimmingtest in mice.

Group	Dose (mg/kg)	Duration of immobilitytime (sec)
Control	Normal saline	105.5±1.658
Imipramine	60 mg/kg	76.25±1.377***
MEAV	250 mg/kg	92.25±1.887***
MEAV	500 mg/kg	81.5±0.866***

Tail Suspension Test (TST)

Table 5: Effect of Methanolic Extracts of *Sesbania Grandiflora*. on Tail suspensiontest in mice.

Group	Dose (mg/kg)	Duration of immobilitytime (sec)
Control	Normal saline	178±3.24
Imipramine	60 mg/kg	109.8±1.75***
MEAV	250 mg/kg	131.3±3.119***
MEAV	500 mg/kg	118.8±1.887***

Anti-Anxiety Activity

Elevated Plus Maze Test

Table 6: Effect of Methanolic Extract of *Sesbania Grandiflora*. on Elevated plus maze(EPM) test in mice.

Group	Dose (mg/kg)	Time spent in closed arm (sec)	Time spent in open arm (sec)
Control	Normal saline	429.4±0.1586	100.4±0.1304
Diazepam	4 mg/kg	331.4±0.0312 ns	260.4±0.1493***
MEAV	250 mg/kg	305.5±0.04131 ns	205.5±0.1602**
MEAV	500 mg/kg	351.4±0.1035 ns	220.4±0.2121***

Light- Dark Model

Table 7: Effect of Methanolic Extract of *Sesbania Grandiflora.* on Light Dark Modelin mice.

Group	Dose (mg/kg)	Time spent in the lightchamber (sec)
Control	Normal saline	64.25±1.702
Imipramine	60 mg/kg	130.5±0.866***
MEAV	250 mg/kg	108.3±1.436***
MEAV	500 mg/kg	102±1.08***

DISCUSSION

The methanolic extract of leaves of *Sesbania Grandiflora* was evaluated for its neuroprotective activity. Preliminary phytochemical analysis revealed the presence of alkaloids, carbohydrates, saponins, glycosides, protein and amino acids, phenolic compounds. The present investigation of the methanolic extract of leaf of *Sesbania Grandiflora* produced central inhibitory effect in mice and rats. Behavioural pharmacology field makes the concepts that are derived from pharmacology and psychology to study behaviour in animal models. The discovery of new compounds which act on CNS process (either CNS depressant or CNS stimulant) will-provide clinical useful information for validation of animals. This will also new insight to researcher to understand the physio-pathological and neurochemical process involved in investigation of new compounds.

The present study shows that the methanolic extract of leaf of *Sesbania Grandiflora* protected some of the animals against seizures induced by maximal electroshock. Antiepileptic drugs which inhibit voltage-dependent Na+ channels, such as phenytoin can prevent MES-induced tonic extension.

In MES-induced convulsion animals represents grandmal type of epilepsy. It has oftenbeen stated that antiepileptic drugs that block MES-induced tonic extension phase act by blocking seizure spread. Moreover, MES-induced tonic extension phase can be prevented either by drugs that inhibit voltage-dependent Na+ channels, such as phenytoin, valproate, felbamate and lamotrigine or by drugs that block glutaminergic excitation mediated by the N- methyl- D-Aspartate (NMDA) receptor such as felbamate. The MEAV showed anticonvulsant activity against MES-induced convulsion, it was abolished tonic extension phase due to it might be either inhibit voltage-dependent Na+ channels or act as a NMDA antagonists.

In the result of present study, MEAV (250-500 mg/kg) produced significant antidepressant effect in FST & TST. These models of depression are widely used to screen new antidepressant drugs. The tests are quite sensitive and relatively specific to all major classes of antidepressant drugs including TCAs, SSRIs, MAOI, Atypical antidepressants. The forced swimming test is the most widely used tool for assessing antidepressant activity pre- clinically. The widespread use of this simple model is mainly due to its ability to detect a broad spectrum of antidepressant agents. It has been argued that TST (Tail SuspensionTest) is less stressful than FST (Forced swim test) and has greater pharmacological sensitivity. The results obtained from TST are in concordance with the validated FST by Porsolt et al. Environmental factors and hereditary factors play a major role in producing deficient monoaminergic transmission in central nervous system thereby producing symptoms of depression.

The EPM is one of the most widely validated tests and is highly sensitive to the influence of both anxiolytic and anxiogenic drugs acting at the gamma amino-butyric acid type A ($GABA_A$) - benzodiazepine complex. In EPM, normal mice will normally prefer to spend much of their allotted time in the closed arms. This preference appears to reflect an aversion towards open arms that is generated by the fears of the open spaces. Drug like diazepam that increases open arm exploration are considered as anxiolytic and the reverse holds true for anxiogenics. In this study, we observed that the administration of different doses (250 and 500 mg/ kg body weight) of methanolic extract of *Sesbania Grandiflora*. induced an anxiolytic-like effect in mice, as it increased open arm entries and the time spentin the open arms of the EPM when compared to the control animals.

The light –dark test were used to access the anxiolytics potentials of *Sesbania Grandiflora*. In the light-dark test (250-500mh/kg) dose showed the most prominent anxiolytic – like properties by spending the highest time in the light chamber. This corroborate the suggestions that the time mice spent in the illuminated side of the light dark chamber is the most useful and consistent parameter of anxiety.

From the present study, it can be concluded that *Sesbania Grandiflora*. (MEAV) shows significant psychotherapeutics effects as antidepressants and anxiolytics agent. This research work has eliminated the involvement of neurotoxicity in the use of *Sesbania Grandiflora* for pharmacotherapy in anxiety and depression. Detailed laboratory analysis is required for a definitive conclusion and isolation of major active secondary metabolites responsible for these therapeutic actions. So, a potent antidepressants and anxiolytics may emerge from *Amaranthus viridis.* Since the extract shows potent anxiolytic and depressant effects nearly at same dose range hence it could also be used in treatment of mixed anxiety and depression syndrome.

Results of the present investigation suggest that the extract of *Sesbania Grandiflora*. possesses

neuropharmacological activity and provide the scientific basis for the use of the plant in traditional system of medicine in the treatment of nervous disorders.

SUMMARY AND CONCLUSION

The present study was undertaken to determine the effect of neuropharmacological effects of leaves of methanolic extract of *Sesbania Grandiflora.* The pharmacognostical studies made on the *Sesbania Grandiflora* determines the various parameters for pharmacognostical standards. The present investigation deals with the report on microscopically, transverse section of *A.viridis* leaves of (petiole, midrib, lamina, venation pattern) and different chemical parameters have been determined. These findings will be towards establishing pharmacognostic standards on identification, purity, quality, classification of the plant, which is gaining relevance in plant drug research. The powdered leaves like ash values, extractive values and loss of drying gave valuable information. This helped for correct identification of plant The preliminary phytochemical investigation showed the presence of carbohydrates, alkaloids, steroids and sterols, glycosides, saponins, tannins, flavonoids and phenolic compounds Our conclusion, investigation suggests that methanolic extract of powdered leaves of *Sesbania Grandiflora*. Which possess potent neuroprotective effects in rats & mice.

REFERENCES

1. Butler, M.S. The role of natural product chemistry in drug discovery. *J. Nat. Prod.*, 2004; *67*: 2141–2153. [Google Scholar] [CrossRef]
2. Newman, D.J.; Cragg, G.M. Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J. Nat. Prod*, 2012; *75*: 311–335. [Google Scholar] [CrossRef] [PubMed]
3. Koehn, F.E.; Carter, G.T. The evolving role of natural products in drug discovery. *Nat. Rev. Drug Discov*, 2005; *4*: 206–220. [Google Scholar] [CrossRef] [PubMed]
4. Patwardhan, B.; Mashelkar, R.A. Traditional medicine-inspired approaches to drug discovery: Can Ayurveda show the way forward? *Drug Discov. Today*, 2009; *14*: 804–811. [Google Scholar] [CrossRef] [PubMed]
5. Arndt, T.; Claussen, U.; Güssregen, B.; Schröfel, S.; Stürzer, B.; Werle, A.; Wolf, G. Kratom alkaloids and O-desmethyltramadol in urine of a "Krypton" herbal mixture consumer. *Forensic Sci. Int.*, 2011; *208*: 47–52. [Google Scholar] [CrossRef] [PubMed]
6. White, C.M. Pharmacologic and clinical assessment of kratom. *Bull. Am. Soc. Hosp. Pharm*, 2018; *75*: 261–267. [Google Scholar] [CrossRef]
7. Gong, F.; Gu, H.P.; Xu, Q.T.; Kang, W.Y. Genus *Mitragyna*: Ethnomedicinal uses and pharmacological studies. *Phytopharmacology*, 2012; *3*: 263–272. [Google Scholar]
8. Rech, M.A.; Donahey, E.; Dziedzic, J.M.C.; Oh, L.; Greenhalgh, E. New drugs of abuse. Pharmacotherapy. *J. Hum. Pharmacol. Drug Ther*, 2015; *35*: 189–197. [Google Scholar] [CrossRef]
9. Vicknasingam, B.; Narayanan, S.; Beng, G.T.; Mansor, S.M. The informal use of ketum (*Mitragyna speciosa*) for opioid withdrawal in the northern states of peninsular Malaysia and implications for drug substitution therapy. *Int. J. Drug Policy*, 2010; *21*: 283–288. [Google Scholar] [CrossRef]
10. Hassan, Z.; Muzaimi, M.; Navaratnam, V.; Yusoff, N.H.; Suhaimi, F.W.; Vadivelu, R.; Vicknasingam, B.K.; Amato, D.; von Hörsten, S.; Ismail, N.I.; et al. From Kratom to mitragynine and its derivatives: Physiological and behavioural effects related to use, abuse, and addiction. *Neurosci. Biobehav. Rev.*, 2013; *37*: 138–151. [Google Scholar] [CrossRef]
11. Eisenman, S.W. The botany of *Mitragyna speciosa* (Korth.) Havil. and related species. In *Kratom and Other Mitragynines: The Chemistry and Pharmacology of Opioids from a Non-Opium Source*; CRC Press: Boca Raton, FL, USA, 2014; 57: 57–76. [Google Scholar]
12. Warner, M.L.; Kaufman, N.C.; Grundmann, O. The pharmacology and toxicology of kratom: From traditional herb to drug of abuse. *Int. J. Leg. Med.*, 2016; *130*; 127–138. [Google Scholar] [CrossRef] [PubMed]
13. Adkins, J.E.; Boyer, E.W.; McCurdy, C.R. *Mitragyna speciosa*, a psychoactive tree from Southeast Asia with opioid activity. *Curr. Top. Med. Chem*, 2011; *11*: 1165–1175. [Google Scholar] [CrossRef] [PubMed]
14. Jaipaew, J.; Padungchareon, T.; Sukrong, S. PCR-reverse dot blot of the nucleotide signature sequences of matK for the identification of *Mitragyna speciosa*, a narcotic species. *Plant Gene*, 2018; *14*: 46–54. [Google Scholar] [CrossRef]
15. Tungphatthong, C.; Urumarudappa, S.K.J.; Awachai, S.; Sooksawate, T.; Sukrong, S. Differentiation of *Mitragyna speciosa*, a narcotic plant, from allied Mitragyna species using DNA barcoding-high-resolution melting (Bar-HRM) analysis. *Sci. Rep*, 2021; *11*: 6738. [Google Scholar] [CrossRef]
16. Parthasarathy, S.; Bin Azizi, J.; Ramanathan, S.; Ismail, S.; Sasidharan, S.; Said, M.I.M.; Mansor, S.M. Evaluation of antioxidant and antibacterial activities of aqueous, methanolic and alkaloid extracts from *Mitragyna speciosa* (Rubiaceae family) leaves. *Molecules*, 2009; *14*: 3964–3974. [Google Scholar] [CrossRef] [PubMed]
17. Meireles, V.; Rosado, T.; Barroso, M.; Soares, S.; Gonçalves, J.; Luís, A.; Caramelo, D.; Simão, A.Y.; Fernández, N.; Duarte, A.P.; et al. *Mitragyna speciosa*: Clinical, toxicological aspects and analysis in biological and non-biological samples. *Medicines*, 2019; *6*: 35. [Google Scholar] [CrossRef]

18. Yuniarti, R.; Nadia, S.; Alamanda, A.; Zubir, M.; Syahputra, R.A.; Nizam, M. Characterization, phytochemical screenings and antioxidant activity test of kratom leaf ethanol extract (*Mitragyna speciosa* Korth) using DPPH method. *J. Phys. Conf. Ser. IOP Publ.*, 2020; *1462*: 012026. [Google Scholar] [CrossRef]
19. Srichana, K.; Janchawee, B.; Prutipanlai, S.; Raungrut, P.; Keawpradub, N. Effects of mitragynine and a crude alkaloid extract derived from *Mitragyna speciosa* Korth. on permethrin elimination in rats. *Pharmaceutics*, 2015; *7*: 10–26. [Google Scholar] [CrossRef]
20. Goh, Y.S.; Karunakaran, T.; Murugaiyah, V.; Santhanam, R.; Abu Bakar, M.H.; Ramanathan, S. Accelerated solvent extractions (ASE) of *Mitragyna speciosa* Korth. (Kratom) leaves: Evaluation of its cytotoxicity and antinociceptive activity. *Molecules*, 2021; *26*: 3704. [Google Scholar] [CrossRef]
21. Mossadeq, W.S.; Sulaiman, M.; Mohamad, T.T.; Chiong, H.; Zakaria, Z.; Jabit, M.; Baharuldin, M.; Israf, D. Anti-inflammatory and antinociceptive effects of *Mitragyna speciosa* Korth methanolic extract. *Med. Princ. Pract*, 2009; *18*: 378–384. [Google Scholar] [CrossRef]
22. Kumarnsit, E.; Keawpradub, N.; Nuankaew, W. Acute and long-term effects of alkaloid extract of *Mitragyna speciosa* on food and water intake and body weight in rats. *Fitoterapia*, 2006; *77*: 339–345. [Google Scholar] [CrossRef]
23. Watanabe, K.; Yano, S.; Horie, S.; Yamamoto, L.T. Inhibitory effect of mitragynine, an alkaloid with analgesic effect from Thai medicinal plant *Mitragyna speciosa*, on electrically stimulated contraction of isolated guinea-pig ileum through the opioid receptor. *Life Sci.*, 1997; *60*: 933–942. [Google Scholar] [CrossRef]
24. Pathak, L.; Agrawal, Y.; Dhir, A. Natural polyphenols in the management of major depression. *Expert Opin. Investig. Drugs*, 2013; *22*: 863–880. [Google Scholar] [CrossRef] [PubMed]
25. Carpenter, J.M.; Criddle, C.A.; Craig, H.K.; Ali, Z.; Zhang, Z.; Khan, I.A.; Sufka, K.J. Comparative effects of *Mitragyna speciosa* extract, mitragynine, and opioid agonists on thermal nociception in rats. *Fitoterapia*, 2016; *109*: 87–90. [Google Scholar] [CrossRef]
26. Kruegel, A.C.; Uprety, R.; Grinnell, S.G.; Langreck, C.; Pekarskaya, E.A.; Le Rouzic, V.; Ansonoff, M.; Gassaway, M.M.; Pintar, J.E.; Pasternak, G.W.; et al. 7-Hydroxymitragynine is an active metabolite of mitragynine and a key mediator of its analgesic effects. *ACS Cent. Sci.*, 2019; *5*: 992–1001. [Google Scholar] [CrossRef] [PubMed]
27. Kumarnsit, E.; Keawpradub, N.; Nuankaew, W. Effect of *Mitragyna speciosa* aqueous extract on ethanol withdrawal symptoms in mice. *Fitoterapia*, 2007; *78*: 182–185. [Google Scholar] [CrossRef]
28. Apryani, E.; Hidayat, M.T.; Moklas, M.; Fakurazi, S.; Idayu, N.F. Effects of mitragynine from *Mitragyna speciosa* Korth leaves on working memory. *J. Ethnopharmacol*, 2010; *129*: 357–360. [Google Scholar] [CrossRef] [PubMed]
29. Idayu, N.F.; Hidayat, M.T.; Moklas, M.; Sharida, F.; Raudzah, A.N.; Shamima, A.; Apryani, E. Antidepressant-like effect of mitragynine isolated from *Mitragyna speciosa* Korth in mice model of depression. *Phytomedicine*, 2011; *18*: 402–407. [Google Scholar] [CrossRef]
30. Obeng, S.; Kamble, S.H.; Reeves, M.E.; Restrepo, L.F.; Patel, A.; Behnke, M.; Chear, N.J.Y.; Ramanathan, S.; Sharma, A.; León, F.; et al. Investigation of the adrenergic and opioid binding affinities, metabolic stability, plasma protein binding properties, and functional effects of selected indole-based kratom alkaloids. *J. Med. Chem*, 2019; *63*: 433–439. [Google Scholar] [CrossRef]