South Asian Research Journal of Natural Products

3(2): 27-37, 2020; Article no.SARJNP.58860

Working with Bioactive Substances from Medicinal Plants in Animals

Idris Zubairu Sadiq[1*], Fatima Sadiq Abubakar[2], Hassan Aliyu Hassan[3] and Maryam Ibrahim[4]

[1]Department of Biochemistry, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.
[2]National Agricultural Extension and Liaison Services, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.
[3]Department of Biochemistry, Faculty of Sciences, Federal University, Dutse, Jigawa State, Nigeria.
[4]Department of Biochemistry and Molecular Biology, Faculty of Life Sciences, Federal University, Dutsin-Ma, Katsina State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors IZS, FSA, HAH and MI generate the idea, all the authors wrote the first draft of the manuscript. Author IZS managed the literature searches and write the final paper. All authors read and approved the final manuscript.

Article Information

Editor(s):
(1) Dr. Elmarie Van Der Watt, University of the Free State, South Africa.
Reviewers:
(1) Victor Okello Sirama, Rongo University, Kenya.
(2) Shirish H. Sonawane, National Institute of Technology, Warangal, India.
(3) Mohammad Arif, Madhya Pradesh Medical Science University, HSZH Government Unani Medical College, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/58860

Mini-review Article

Received 02 May 2020
Accepted 09 July 2020
Published 20 July 2020

ABSTRACT

Background: Working with bioactive substances from the medicinal plant requires various steps from plant extract preparation to the calculation for scientific evaluation and safe medicinal plant extract administration. The paper aims to discuss procedures involve in evaluating bioactive compounds from medicinal plants.
Methodology: Review of relevant literature.
Results: We have described the preparation of plant extract, toxicological methods of evaluating bioactive substances, vehicles for biological research, evaluating analgesic, neuropharmacological and anti-inflammatory activities from medicinal plants.

*Corresponding author: Email: idrisubalarabe2010@gmail.com;

Conclusion: Medicinal plants are potent stores of bioactive compounds that needed to be carefully extracted, toxicologically and preliminarily evaluated in animals for new drug development.

Keywords: Plant extract; toxicological methods; anti-inflammatory activities; evaluating analgesic activity.

1. INTRODUCTION

Delivery of plant extracts with bioactive pharmacological substances to laboratory animals is an important part of the procedure in experimentations and experimental designs involving animals [1]. It is aimed at investigating clinical parameters, testing of pharmaceutical products, biomedical research, infectious disease agents, vaccinations, anesthetics, and analgesics, electrolytes, and nutraceuticals [1]. This process requires careful planning and consideration while minimizing risk or toxicity that may arise due to the administration of the extracts or pharmaceutical products. The calculation of doses and volumes to be administered to experimental animals is mandatory for acceptable scientific experiments. Toxicity studies are carried out to test the toxicity of either naturally occurring compounds from plant extracts or other synthetic therapeutic compounds.

Medicinal plants are important sources of many therapeutic substances and are considered significance due to their varieties of secondary metabolites [2]. Therapeutic substances from plants and animals have a great impact on health and cancer chemoprevention [2-4]. Ethnobotanical survey shows that preparations from medicinal plants are used for management and as alternative therapy against common community ailments such as malaria, diabetes, sickle cell anemia, hypertension, ulcer, and paralysis, typhoid fever and immune deficiency [5]. Toxicity to medicinal plants has also been reported [6]. The search for natural sources for the formulation of therapeutics, cosmetic products and alternative therapies has increased the demand for medicinal plants [7]. Medicinal plants are explored as food, tea, perfume, pest-control, anti-insects, and as a dyer besides medical and pharmaceutical uses [5]. The paper aims to discuss procedures involve in evaluating bioactive compounds from medicinal plants.

2. COLLECTIONS, IDENTIFICATION, AND PROCESSING OF PLANT SAMPLE

This involves collecting plant parts, which could be leaves, roots, stem bark, fruit, seeds, and flowers. The plant is then identified and deposited at plant herbarium (Fig. 1). The plant material may be collected fresh or dried. Fresh plant material is washed with clean water and air-dried in shade to minimize the loss of secondary plant metabolites. The dried plant sample is then ground to a coarse powder with mortar and pestle then further processed to a fine powder.

3. PREPARATION OF PLANT EXTRACT

Common methods used in the research laboratory include maceration (cold extraction) and soxhlet (hot) extraction. Extraction of water-soluble compounds (hydrophilic) uses a solvent suchas methanol, ethanol, and ethyl acetatewhile that of hydrophobic uses dichloromethane or a mixture of dichloromethane/methanol in the ratio of 1:1 [8,9]. Different solvents are used to carry out plant extraction based on solubility properties (Fig. 2). The solvent to be used largely depends on the target compound to be isolated. The figure below shows the solubility of different phytochemicals in commonly used solvents.

4. EXTRACTION METHODS

Methods used to obtain medicinal plant extracts depend upon the final secondary metabolites targeted. Some of the commonly used methods include decoction, maceration, infusion, percolation, soxhlet extraction, microwave-assisted extraction, accelerated solvent extraction, ultrasound-assisted extraction, accelerated solvent extraction, and superficial fluid extraction (Table 1). These various methods were described in the Table 1.

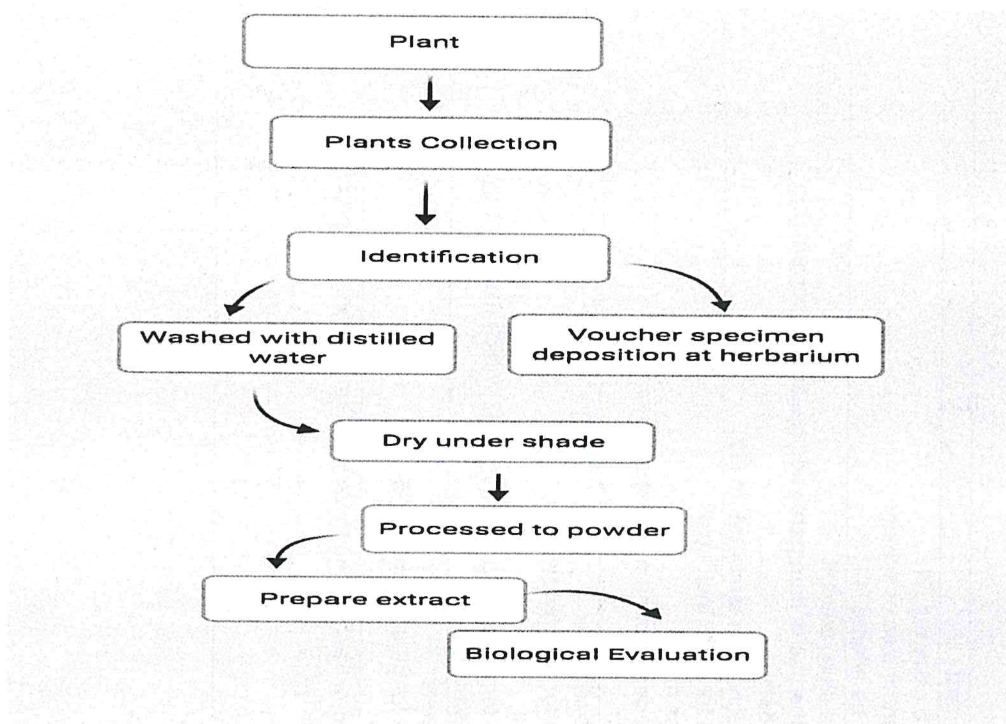

Fig. 1. Steps in plant extraction preparation

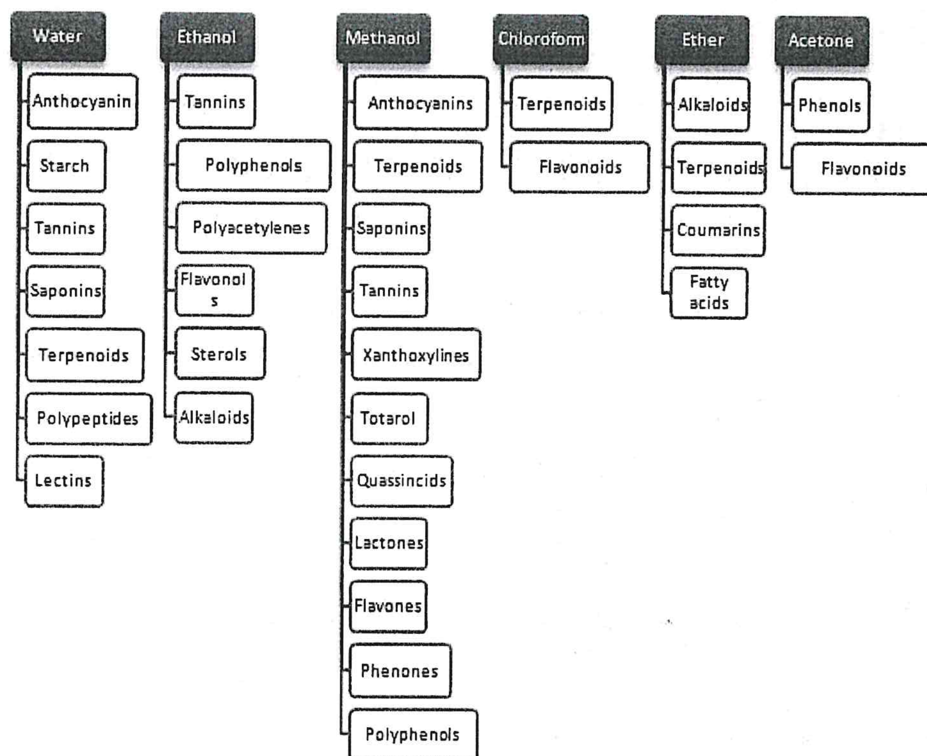

Fig. 2. Solubility of bioactive compound from plants in different solvents

Table 1. Extraction methods

Extraction method	Procedure	Merit and demerit
Decoction, maceration, infusion, Percolation	Soak plant part in solvent and allow to stand for about 1-3days[2,10]	• Simple to carry out [2] • A different choice of solvent • PH may be altered [11]
Soxhlet extraction	The sample is placed in the thimble and placed chamber of soxhlet. Solvent extraction [2] occurs by heating which vaporized and drip	• Required small solvent • Involve the use of flammable and hazardous solvent [2]. • Often costly • It often considers temperature [12]
Microwave-assisted extraction	Interaction of microwave radiation with sample and solvent produce heating on the surface of the material [13,14].	• Suitable for polar molecules • Take less time • Thermal degradation may occur [13].
Ultrasound-assisted extraction	Uses ultrasound (20-2000KHz) [10] and extraction occurs via mechanical effect from the ultrasound, which increases the surface area between sample and solvent [2].	• It is cost-effective • Suitable for large scale • It is rapid • Require temperature and pressure [2] which may destroyed phytochemicals
Accelerated solvent extraction	A sample is placed in the extraction cell and the solvent is added. The cell is pressurized and heated to a specific temperature [14,15].	• Higher yield • Suitable for quality control [15-17].
Superficial Fluid Extraction	Uses pressure and temperature to separate extract from the matrix using superficial fluids for example CO_2 became superficial fluid at 31.1°C and 7380 kPa [2,18].	• Selective extraction due to varying temperature and pressure • Very costly [19].

Table 2. Common vehicles used in animal research

Vehicle	Animal	Route of administration	Reference
Acetate sodium	Rat	Oral	
Acetic acid	Rat, mouse	Intravenous, intraperitoneal	[20-23]
Acetone	Rat, mouse, guinea pig, rabbit	Oral, dermal	[20,21]
Alginic acid	Rat	Intraperitoneal	[20,21]
Benzoic acid	Rat	Oral	[20,21]
Beta-cyclo-dextrin	Rat, primate	Oral/intravenous	[20,21]
Citrate buffer	Dog, rat	Intravenous/oral	[20,21]
Carboxymethylcellulose	Primates, rat	Oral	[20,21]
Cyclohexane	Rat/rabbit	Oral	[20,21]
Dimethyl sulfoxide (DMSO)	Dog, rats, guinea pig, mouse primate	Oral, intravenous, intraperitoneal, subcutaneous	[20,21]
Ethanol	Dog, rat, primate	Oral, intravenous, intraperitoneal	[20,21]
Glycerol	Rat, mouse, guinea pig, rabbit	Oral, intravenous, intraperitoneal	[20,21]
Dextrin	Dog, rat	Intravenous	[20,21]
D-glucose anhydrous	Rat, primate, mouse	Oral, intravenous subcutaneous	[20,21]
Ascorbic acid	Rat	Oral	[20,21]

5. VEHICLES FOR BIOLOGICAL RESEARCH

A vehicle may be defined as any substance used in dissolving experimental compounds thereby increasing its solubility. This also covers substances used for the formulation of pharmaceutical products term as non-active ingredients or excipients. To test whether a vehicle cause-effect to the administered animals, one group is normally treated with the vehicle and compare with the control. Examples of some vehicles used for non-clinical purposes are given in Table 2. [20].

6. TOXICITY TEST IN ANIMALS

Toxicity testing involves the assessment of a substance to ascertain its degree of toxicity. In toxicity testing, information about the toxic properties of a substance is evaluated. The following changes may be evaluated following the administration of plant extracts: behavior patterns, diarrhea, skin, eyes and saliva, and fur (hairs).

7. CALCULATING MEDIAN LETHAL DOSE (LD_{50}) FROM PLANT EXTRACT

There are many methods for calculating LD_{50} for plant extracts in animals, for example, lorke's method, kerbe's method and up and down method.

7.1 Lorke's Method

Lorke's method can be carried out in two phases. Phase one of the lorke's method makes use of nine animals, which are divided into three groups with three animals in each group (Fig. 3). The following doses are administered to animals in each group (10, 100, and 1000 mg/kg) and the animals are observed for mortality and behavioral change for 24 hours. If no mortality occurs the experiment goes into the second phase [24].

In the second phase, three animals may be divided into three groups with each group having one animal (Fig. 3). The following doses are then administered (1600 mg/Kg, 2900 mg/Kg, and 5000 mg/Kg) in the three groups respectively [24].

Calculation of LD_{50} using the formula

$$\mathrm{LD50} = \sqrt{\mathrm{D0} \times \mathrm{D100}}$$

D_0 = Highest dose that gave no mortality,
D_{100} = Lowest dose that produced mortality.

If for example the highest dose that gave no mortality of a leaf extract is 200 mg kg−1 and the lowest dose that produced mortality 400 mg kg−1. We can calculate the LD_{50} as

$$\mathrm{LD50} = \sqrt{200 \times 400}$$

$$\mathrm{LD50} = 282.84\ \mathrm{mg\ kg} - 1$$

7.2 Karber's Method

Karber's method of toxicity test; test toxicity using different amounts of a substance to various groups. In these methods, 5 animals are placed in each group with the first group-receiving vehicle used in dissolving experimental compounds (tab.2). The other groups received different doses in increasing order. The mean of the mortality is reported in each of the groups [25].

Karber's method Calculation of LD_{50} using the formula:

$$\mathrm{LD50} = \mathrm{LD100} - \sum\{(x + Y) / n\}$$

Where,

LD_{50} = Median lethal dose
LD_{100} = Least dose required to kill 100%
x = Dose difference
y = Mean mortality
n = Group population.

7.3 Up and Down Method

This approach involves the serial dosing with plant extract under investigation in animals one at time within 48 hours of time. Once the first dose is given, the next dose is decided by the result of the previous administered dose [25]. When the animal survives the previous dose the dose is increased upward, but it is adjusted downward when mortality is reported at the previous dose. A constant factor is normally maintained as the dose moves up or down hence the name "up and down method". Testing stops when the upper limit (2000-5000 mg / kg) has been reached without mortality or the LD50 has been calculated from the test [25-27].

8. SELECTING ADMINISTRATION DOSES FROM LD_{50}

One tenth of the lethal dose (LD_{50}) of mice is one of the main parameters used to obtain a safe starting dose [28]. Therefore, Once the LD50 of

the extract is known, the dose can be selected by multiplying the LD_{50} with (1/10), (1/20), (1/40), (1/80), (1/160) and so on. Standard form for estimating starting dose for humans as well as in animals is to calculate one 10th of this lethal dose [29]. For example, if LD_{50} of an extract is greater than 5000mg/Kg, we can calculate the doses to be administered to animals as follows:

1. $1/10\ x\ 5000\ mg/Kg \quad = 500\ mg/Kg$
2. $1/20\ X\ 5000\ mg/Kg = 250\ mg/Kg$
3. $1/40\ X\ 5000\ mg/Kg = 125\ mg/Kg$
4. $1/80\ X\ 5000\ mg/Kg = 62.5\ mg/Kg$
5. $1/120\ X\ 5000\ mg/Kg = 31.3\ mg/Kg$

9. PARENTERAL FORMULATIONS DOSE CALCULATION FOR DELIVERY INTO THE ANIMAL BODY

The injection volumes of parenteral formulations are calculated by the equation presented below [30]. Animal dose (mg/Kg) is normally selected based on the calculated LD_{50} for example 125, 250, and 500 mg/Kg can be selected from the plant extract with LD_{50} greater than 5000 mg/Kg. concentration (mg/ml) represents the final concentration of the prepared extract or the pharmacological agent under investigation.

$$Injection\ volume\ (ml) = \frac{Animal\ Weight\ (Kg)}{Concerntratiom\ \left(\frac{mg}{ml}\right)} \times Animal\ dose\ \left(\frac{mg}{Kg}\right)$$

For an animal of 120 g = 0.12 Kg at concentration of plant extract of 40 mg/ml and animal dose of 500 mg/Kg, we can calculate injection volume using the above equation.

$$Injection\ volume\ (ml) = \frac{0{,}12Kg}{4\ 0mg/ml} \times 500mg/Kg$$

$$Injection\ volume\ (ml) = 1.5ml$$

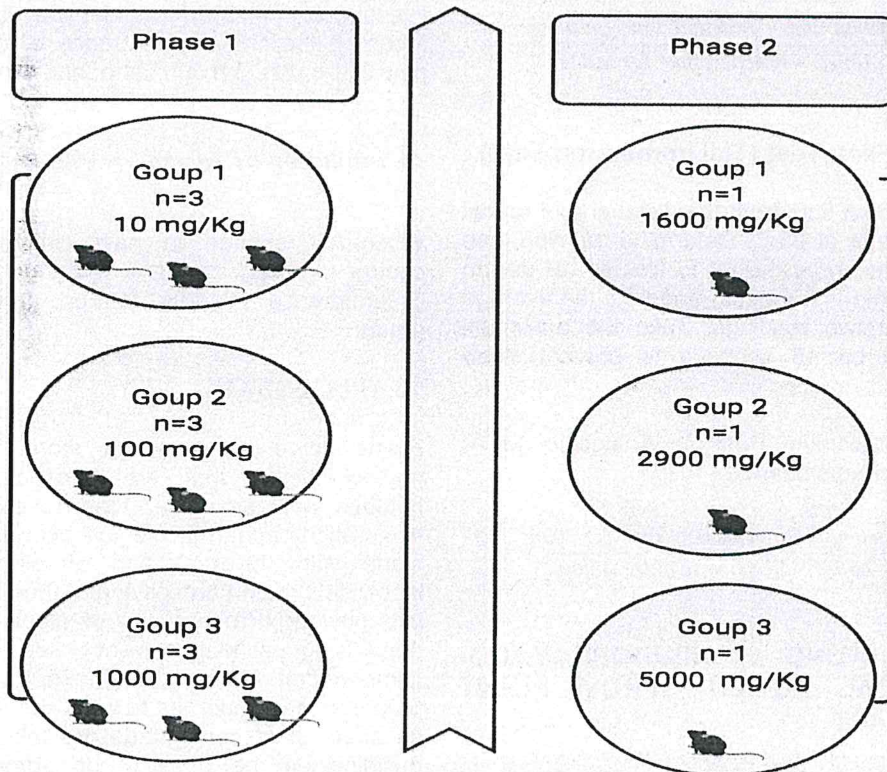

Fig. 3. Lorke's method of LD_{50} determination

10. EVALUATION OF ANALGESIC ACTIVITY FROM PLANT EXTRACT

10.1 Hot Plate Method

Animals to be evaluated are normally treated according to the experimental design by the investigator and then placed on a hot plate at 55°C within the restrainer of the plate. The reaction/latency time (seconds) for a rat to react for example 0,15,30,45 and 60 seconds is determined. It is recommended that maximum time should be 45 seconds after treatment to avoid injury [31,32].

Calculate Maximum Possible Analgesic (MPA) using the formula below.

$$MPA = \frac{Reaction\ time - Reaction\ time\ for\ Saline}{15Sec - Reaction\ time\ for\ saline}$$

10.2 Tail-Flick Test (Tail immersion Test)

Immerse about 5cm from the distal end of rat-tail in warm water at 55°C. Determine reaction time (seconds) taken by the rat to flick its tail due to pain. Omit the first reading and take the average of the next two readings. Take the maximum reaction to be 15 seconds to prevent injury [32,33].

Calculate Maximum Possible Analgesic (MPA) using the formula below

$$MPA = \frac{Reaction\ time - Reaction\ time\ for\ Saline}{45Sec - Reaction\ time\ for\ saline}$$

11. EVALUATING NEUROPHAMACO-LOGICAL ACTIVITY FROM PLANT EXTRACT

11.1 Thiopental Sodium-induced Sleeping Time

Rats may be divided into groups according to experimental design with each group containing 5 rats. Each group should be placed in a separate cage and treatments should be administered including negative, experimental groups, and standard drugs. Administer thiopental sodium 40 mg/kg intraperitoneally to induce sleep and place mice in an inverted position. Determine the time taken for the mice to turn into normal position. Take the hypnotic index as the interval between loss and recovery of the right reflex. Take latency as the time between injections of thiopental sodium to sedation [34].

12. EVALUATION OF ANTI-INFLAMMATORY ACTIVITY FROM PLANT EXTRACT

Rats may be divided into groups depending on the experimental design including negative, positive, and test groups. For example (1%(v/v) tween-80 in distilled water 10 mg/kg may be used as negative control and indomethacin as oral suspension 10 mg/kg as a positive control [34]. Administer test groups according to experimental design and Induce sub-acute inflammation in all groups by subcutaneous by injecting 0.1 ml 2% formaldehyde at right paw. Measure the line circumference of the injected paw at 1 h, 2 h, 3 h, 4 h, 24 h, and 48 h [34].

$$\%\ inhibition\ of\ edema = 100 \times \left(lo - \frac{l1}{lo} \right)$$

Where l_0 = change in paw circumference in control group and l_1 = change in paw circumference in drug treated group or test group.

13. DISCUSSION

Plants serve as a potent store of different phytochemicals that are beneficial to both humans and animals. Good research in the exploration of pharmacological activities of plants starts with the collection of plant material, identification, and processing of the plant sample into powder. Preparations of plant extract are done using a suitable solvent based on the target compound(s). Once plant extract has been prepared, it is important to carry out toxicity tests as some plant compounds are toxic [6] before deciding on the dose to be administered to animals for pharmacological evaluations. Toxicity testing helps to provide information on the relative safety of drug(s) on animal studies for example in preclinical studies before clinical studies are performed. Tests are usually carried out according to Lorke's method developed in 1983 or other methods based on the kind of experiment.

Analgesic activities are widely determined from medicinal plants as plants are a rich source of pharmacological substances. Analgesics acts on

the central nervous system and other pain mediators without affecting consciousness essentially interfering with the activities of key important enzymes and metabolic pathways. In this paper, we described two methods for analgesic activity testing: Hot plate and tail-flick tests. These tests are considered to be selective for different animal species and testing opioid-like compounds [35,36]. A hot pate method is also suitable for determining neurologic pain and seeks to measure acute and non-inflammatory pain [37]. Neuropharmacological activities from plants are also evaluated to discover a new drug for treating neuropsychiatric disorders such as depression and anxiety. Natural compounds from plants prevent the inflammation process, a complex process that serves as a defense mechanism for the host [38]. Many plants exhibit anti-inflammatory effects though acting as inhibitors of enzymes and biochemical pathways involve information of cytokines and eicosanoids [38].

14. CONCLUSION

Medicinal plants are potent stores of bioactive compounds that needed to be carefully extracted, toxicologically and preliminarily evaluated in animals for new drug development.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Turner PV, Pekow C, Vasbinder MA, Brabb T. Administration of substances to laboratory animals: Equipment considerations, vehicle selection, and solute preparation. J. Am. Assoc. Lab. Anim. Sci. 2011;50(5):614–627.
2. Azwanida NN. A review on the extraction methods uses in medicinal plants, principle, strength and limitation. Medicinal and Aromatic Plants. 2015;4:196.
3. Venugopal R, Liu RH. Phytochemicals in diets for breast cancer prevention: The importance of resveratrol and ursolic acid. Food Sci Hum Well. 2012;1:1-13.
4. Khan AR, Sadiq IZ, Abdullahi LI, Danlami D, Taneja P. Chemoprotective role of bovine lactoferricin against 7, 12 dimethylbenz (a) anthracene induced skin cancer in female swiss albino mice. int J pharm pharm sci. 2016;8(8):215-222.
5. Sadiq IZ, Abubakar FS, Ibrahim B, Usman MA, Kudan ZB. Medicinal plants for management and alternative therapy of common ailments in Dutsin-Ma (Katsina State) in Nigeria. Herba Pol. 2019;65(4): 45-55.
6. Bussmann RW, Malca G, Glenn A, Sharon D, Nilsen B, Parris B, et al. Toxicity of medicinal plants used in traditional medicine in Northern Peru. J Ethnopharmacol. 2011;137(1):121–140
7. Mukherjee PK, Maity N, Nema NK, Sarkar BK. Bioactive compounds from natural resources against skin aging. Phytomedicine. 2011;19: 64-73.
8. Cosa P, Vlietinck AJ, Berghe DV, Maes, L. Anti-infective potential of natural products: How to develop a stronger *in vitro* 'proof-of-concept'. J ethnopharmacol. 2006;106: 290–302.
9. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. Extraction, isolation and characterization of bioactive compounds from plants' extracts. Afr J Trad Complem. 2001;8(1):1–10.
10. Handa SS, Khanuja SPS, Longo G, Rakesh DD. Extraction technologies for medicinal and aromatic plants, (1st edn). Italy: United Nations Industrial Development Organization and the International Centre for Science and High Technology. 2008;66.
11. Yung OH, Maskat MY, Wan Mustapha WA. Effect of extraction on polyphenol content, antioxidant activity and pH in pegaga (*Centella asiatica*). Sains Malaysiana. 2010;39:747-752.
12. Amid, Salim RJ, Adenan M. The factors affecting the extraction conditions for newroprotective activity of *Centella asiatica* evaluated by metal chelating activity assay. J Appl Sci. 2010;10:837-842.
13. Kaufmann B, Christen P. Recent extraction techniques for natural products: Microwave-assisted extraction and pressurized solvent extraction. Phytochem Anal. 2002;13:105-113.
14. Rahmalia W, Fabre JF, Mouloungui Z. Effects of cyclohexane/acetone ratio on bixin extraction yield by accelerated solvent extraction method. Procedia Chem. 2015;14:455-464.

15. Methods optimization in accelerated solvent extraction in technical note. 2013; 208: 1-4.
16. Trusheva B, Trunkova D, Bankova V. Different extraction methods of biologically active components from propolis: A preliminary study. Chem Cent J. 2007;13.
17. Tan Ji T, Jiang G, Hu F. Simultaneous identification and quantification of five flavonoids in the seeds of Rheum palmatum L. by using accelerated solvent extraction and HPLC-PDA-ESI/MSn. Arab J Chem. 2014;12:1345-1352.
18. Patil, Sachin BS, Wakte PS, Shinde DB. Optimization of supercritical fluid extraction and HPLC identification of wedelolactone from Wedelia calendulacea by orthogonal array design. J Adv Res. 2013;5:629-635.
19. Naudé Y, De Beer WHJ, Jooste S, Van Der Merwe L, Van Rensburg SJ. Comparison of supercritical fluid extraction and Soxhlet extraction for the determination of DDT, DDD and DDE in sediment. Water SA.1998;24:205-214.
20. Gad S, Cassidy C, Aubert N, Spainhour B, Robbe H. Nonclinical Vehicle Use in Studies by Multiple Routes in Multiple Species. Inter J Toxicol. 2006;25(6):499-521.
21. Gad SC. Routes in toxicology: An overview. J. Am. Coll. Toxicol. 1994;13: 34–39.
22. Frutuoso Vda S, Monteiro MM, Amendoeira FC, Almeida AL, do Nascimento DD, Bérenger AL, Kaplan MA, Figueiredo MR, Bozza PT, Castro-Faria-Neto HC. Analgesic and anti-inflammatory activity of the aqueous extract of *Rheedia longifolia* Planch & Triana Mem Inst Oswaldo Cruz. 2007; 102(1):91-6.
23. Martinez RM, Zarpelon AC, Domiciano TP, Georgetti SR, Baracat MM, Moreira IC, Andrei CC, Verri WA Jr, Casagrande R. Antinociceptive effect of *Tephrosia sinapou* extract in the acetic acid, phenyl-p-benzoquinone, formalin, and complete freund's adjuvant models of overt pain-like behavior in mice. Scientifica (Cairo). 2016; 2016:8656397
24. Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol. 1983; 54(4):275-287.
25. Chinedu E, Arome D, Ameh FS. A new method for determining acute toxicity in animal models. Toxicol Inter. 2013;20(3): 224–226.
26. Bruce RD. An up-and-down procedure for acute toxicity testing. Fundam Appl Toxicol. 1985;5:151–7.
27. Organization for Economic Co-operation and Development (OECD). Guideline for the testing of chemicals 423. Documentation on acute oral toxicity and acute class method; 2001.
[Last accessed on 6 June 2020]
Available:https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl423.pdf
28. Le Tourneau C, Stathis A, Vidal L, Moore MJ, Siu LL. Choice of starting dose for molecularly targeted agents evaluated in first-in-human phase I cancer clinical trials. J Clin Oncol. 2010;28(8):1401-1407.
29. Hosseini A, Shorofi SA, Davoodi A, Azadbakht M. Starting dose calculation for medicinal plants in animal studies; recommendation of a simple and reliable method. Res J Pharmacogn. 2018;5(2):1-7.
30. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharma. 2016;7:27-31.
31. Eddy NB, Leimback D. Synthetic analgesic II. Diethlenyl butenyl and dithienyl butylamines. J Pharmacol Exp Ther. 1953; 107:385–393.
32. Fan S, Ali N, Basri D. Evaluation of analgesic activity of the methanol extract from the galls of *Quercus infectoria* (olivier) in rats. Evid Based Complementary Alter Med. 2014;1-6.
33. Sewell R. Antinociceptive activity of narcotic agonist and partial agonist analgesics and other agents in the tail-immersion test in mice and rats. Neuro Pharmacology. 1976;15(11):683-688.
34. Anisuzzman M, Hasan MM, Acharzo AK, Das AK, Rahman S. *In vivo* and *in vitro* evaluation of pharmacological potentials of secondary bioactive metabolites of *Dalbergia candenatensis* leaves. Evid Based Complementary Alter med: eCAM. 2017;5034827.
35. Johnson P, Besselsen D. Practical aspects of experimental design in animal research. ILAR Journal. 2002;43(4):202-206.
36. Malairajan P, Gopalakrishnan G, Narasimhan S, Jessi Kala Veni K. Analgesic activity of some Indian medicinal

plants. J Ethnopharmacol. 2006;106(3): 425-428.

37. Zihad S, Bhowmick N, Uddin S, Sifat N, Rahman M, Rouf R. et al. Analgesic activity, chemical profiling and computational study on *Chrysopogon aciculatus*. Front pharmacol. 2018;15(9): 1164.

38. Oguntibeju OO. Medicinal plants with anti-inflammatory activities from selected countries and regions of Africa. J Inflamm Res. 2018;11:307-317

© 2020 Sadiq et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/58860