IJCIM

International Journal of Complementary and Internal Medicine

RESEARCH ARTICLE

Phytochemical Content and Anti-Inflammatory Potential of Solanum americanum Mill. (Solanaceae) Methanol Leaf Extract in Wistar Rats

Cletus Anes Ukwubile

Department of Pharmacognosy, Faculty of Pharmacy, University of Maiduguri, Maidugur

Corresponding Author: Cletus Anes Ukwubile, Department of Pharmacognosy, Faculty of Pharmacy, University of Maiduguri, Maiduguri, Nigeria. E-mail: doccletus@yahoo.com.

Received: May 21, 2024 **Published:** June 03, 2024

Citation: Cletus Anes Ukwubile. Phytochemical Content and Anti-Inflammatory Potential of Solanum americanum Mill. (Solanaceae) Methanol Leaf Extract in Wistar Rats. Int J Complement Intern Med. 2024;6(1):259–267. DOI: 10.58349/IJCIM.1.6.2024.00140

Abstract

Background: The leaf of *Solanum americanum* Mill. has been used by some communities in Northeast Nigeria for the treatment of pain, inflammation, convulsion, diabetes, and other diseases for many decades.

Aim: This present study was carried out to evaluate the phytochemical contents as well as the anti-inflammatory activity of *S. americanum* methanol leaf extract in Wistar rat models.

Methods: The preliminary phytochemical screening of *S. americanum* methanol leaf extract (SAME) was determined following standard methods while the acute oral toxicity test of SAME was determined in five Wistar rats of opposite sex using the OECD guideline 425. The anti-inflammatory activity of the extract was evaluated using carrageenan and formalin-induced rat paw oedema.

Results: The results of the phytochemical screening showed the presence of flavonoids, alkaloids, tannins, saponins, and phytosterols. The total phenolics and total flavonoid contents were 345.12 ± 2.04 mg GAE and 88.24 ± mg QE/g respectively. Oral acute toxicity of SAME showed that extract was well tolerated by the animals at the dose of 5000 mg/kg body weight after one week. Neither signs of toxicity nor mortality were witnessed in the animals, especially within the first 24 hours of oral toxicity testing. The anti-inflammatory evaluation of extract at the doses of 300, 600 and 1200 mg/kg body weight (i.p.) showed a dose-dependent decrease in rat's paw oedema in both carrageenan and formalin-

induced rat paw oedema. These decreases were significant ($p < 0.05$) when compared to that of the standard drug (Diclofenac sodium 10 mg/kg; i.p.).

Conclusion: The results from the study showed the *S. americanum* methanol leaf extract possessed anti-inflammatory activity by suppressing the production of pro-inflammation mediators. It further justifies the acclaimed use of *S. americanum* in traditional medicine for the treatment of inflammation.

Keywords: Solanum americanum, Phytochemicals, Anti-inflammatory, Carrageenan

Introduction

Traditional medicine is known as indigenous, folk medicine or home remedy practices that were developed over generations within various societies before the era of modern medicine. It is the combination of the knowledge, skills, and practices based on the beliefs and experiences indigenous to different cultures used in the maintenance of wealth, prevention, diagnosis and treatment of external and internal illness.[1] A plant becomes medicinal when its biological activity has been ethnomedical reported or scientifically established.[2] These plants synthesize many chemical compounds for biological functions, including defense against insects, fungi and herbivorous mammals. Over 12,000 active compounds have been isolated and identified.[3] These phytochemicals work similarly to pharmaceutical drugs, so herbal medicines can be beneficial and have harmful side effects just like conventional drugs. Herbs, such as culinary herbs and spices, are widely used to treat diseases in non-industrialized societies. Angiosperms (flowering plants) were the source of most plant medicines.[4]

Solanum americanum Mill. (Plate I) belongs to the family Solanaceae. It has been used medicinally for a long period, dating back to ancient times. *Solanum americanum* is also known as American black nightshade, glossy nightshade, popolo, and popolohua. it is locally called "Gautan kadìi or Gautan kaajíí'' in Hausa, "Oju ologbo'' in Yoruba and "Anya nwora'' in the Igbo language. The plant has been used in the treatment of diarrhoea and inflammation in some parts of North-Eastern Nigeria and thus, the quest for scientific validation of this traditional claim. It is an important ingredient in traditional medicine for the treatment of dysentery, and fever and it helps in reducing gas formation in the stomach and is well used as a strong analgesic and sedative with powerful narcotic properties. It is also used topically for the treatment of herpes zoster, measles, itching, inflammation, and eczema, etc. It has been reported to contain high amounts of protein, calories, fibre, calcium, iron and vitamins B and C.[5]

Inflammation is a highly complex reaction in the vascularized connective tissue to exogenous or endogenous stimuli that can potentially cause cell injury.[6] At the cellular level, the interaction between leukocytes and endothelial cells plays a key role in acute inflammation, ultimately leading to the accumulation of fluid and leukocytes in the extravascular tissue. Inflammation is a vital part of the immune system which signals the immune system to neutralize injurious agents, and heal or repair damaged tissue.[7] However, without inflammation as a physiological response, the wound would fester and infections deteriorate and become deadly. Chronic inflammation has been linked to certain diseases such as heart disease or stroke and may also lead to autoimmune disorders such as Bronchial asthma, rheumatoid arthritis and lupus. But healthy diet and lifestyle can help keep inflammation under control.[8]

This present was carried out to evaluate the phytochemical contents and anti-inflammatory activity of *S. americanum* methanol leaf extract in the *in vivo* Wistar rat model.

Plate I: Image of S. americanum in its habitat.

Methods

Collection, identification, and preparation of plant

Fresh leaves of *S. americanum* were collected in the morning hours in June 2022 at Fori, Maiduguri. The plant was identified by Dr. C. A. Ukwubile of the Department of Pharmacognosy, Faculty of Pharmacy, University of Maiduguri, Nigeria. A voucher specimen number of UMM/FPH/SON/003 was deposited for the plant at the herbarium of the Department of Pharmacognosy. The leaves were thoroughly washed with running tap water to remove dirt. They were then air-dried under shade until constant weight was obtained for two weeks. The leaves were then pulverized into fine powder, weighed and stored in a clean sample bottle as plant material with proper labelling.

Extraction of plant material

Exactly 800 g of the powdered leaves were extracted by cold maceration technique using 1.5 L of 100 % (v/v) methanol (Sigma Aldrich, St Lous Mo, USA). The filtrate was concentrated *in a vacuum* using a water bath to obtain a dark-green extract tagged *S. americanum* methanol extract (SAME). The percentage (%) yield of extract was calculated regarding the initial weight of powdered leaves (800 g) using the formula below:

$$\% \, yield = \frac{Weight \, of \, powdered \, leaves}{The \, final \, weight \, of \, the \, extract} \; x \; 100$$

Evaluation of qualitative phytochemical contents of extract

The preliminary phytochemical contents of the extract were carried out to detect the presence of some secondary metabolites such as alkaloids, flavonoids, saponins, tannins, triterpenes and phenolics following standard procedures.[9,10]

Quantitative phytochemical analysis

Determination of total phenolic content

The total phenolic content was determined using Folin–Ciocalteu colorimetric assay as was previously described.[11] Briefly, 2 mL of *S. americanum* methanol leaf extract (SAME) was mixed with 3.5 mL Folin-Ciocalteu reagent (JoeChem Nig., Ltd). Five minutes later, 6 % of 2 mL $NaCO_3$ was added to the mixture, and allowed to stand for one hour. Thereafter, the absorbance of the solution was measured at 765 nm using the SPECTRONIC 200 UV-Vis spectrophotometer (ThermoFisher Scientific, UK). The gallic acid calibration curve was prepared likewise from concentrations 20 to 80 µg/mL, and the results were expressed in mg equivalent to gallic acid (GAE) per gram.[12]

Determination of total flavonoid content

The total flavonoid content was determined using the $AlCl_3$ (aluminium chloride) colourimetric assay as previously described with slight modification.[13] Briefly, 2 mL each of SAME and quercetin (standard) were separately placed in test tubes. Thereafter, 10 % $AlCl_3$ (w/v), 0.1 mL potassium acetate, 1.5 mL of 80 % methanol (v/v) and 5 mL of distilled water were added to each test tube and mixed thoroughly. The quercetin calibration curve was prepared from a concentration of 20 to 80 µg/mL. The blank was prepared similarly but distilled water (0.5 mL) was used instead of the extract or quercetin. All test tubes were then incubated in a dark cupboard for 30 min at room temperature. The absorbance of the solutions was measured at 415 nm using the UV-Vis spectrophotometer. Flavonoid content was then expressed in mg equivalent to quercetin (QE) per gram. Each experiment was carried out in triplicate.[14]

Experimental Animals

Thirty-five (35) Wistar rats of opposite sex weighing between 80 g and 140 g were purchased from the Department of Pharmacology, Bayero University, Kano. The animals were housed separately in aluminium cages and were allowed to acclimatise for 7 days. They were fed with vital feed (finishers) and water *ad libitum*. All experimental protocols guiding the use of laboratory animals were strictly observed.[15]

Animal Groupings

In the determination of acute oral toxicity (LD_{50}) of SAME, the animals were grouped into five groups of one rat per group following OECD methods,[16] while in anti-inflammatory evaluation, the animals were grouped into five groups of five rats per group.[17]

Acute Oral Toxicity (LD_{50}) Testing of SAME

In determining the effect of short administration of *S. americanum* methanol leaf extract (SAME) on the rats, the OECD guidelines paragraph 425 was used. In this case, five Wistar rats of the opposite sex were administered 5000 mg/kg body weight maximum dose (i.p.) and observed for signs of toxicity, especially within the first 4 hours. The observation was continued for 14 days and the study was terminated since no signs of toxicity or mortality were witnessed.[18]

Evaluation of Anti-inflammatory Activity of SAME

In evaluating the anti-inflammatory effect of the extract, the rats were grouped into five groups of five rats per group as follows:
Group I was the normal control (NC) and received 10 mL distilled via oral gavage,
Group II received 50 mg/kg diclofenac sodium (standard) orally,
Group III received 300 mg/kg body weight (b.w.) SAME orally,
Group IV received 600 mg/kg b.w. SAME orally,
Group V received 1200 mg/kg b.w. SAME orally.

Carrageenan-induction Paw Oedema in Rats Model

Rat paw oedema was induced by sub-plantar injection of 0.1 mL of 1 % w/v carrageenan which was suspended in 1 % carboxyl methyl cellulose (CMC) (JoeChem Nig., Ltd) into rats' tissue. Paw oedema volume or thickness was measured before carrageenan injection, as well as the durations of 0, 60, 120, 180, and 240 min. using the Vernier caliper.[19] The anti-inflammatory activity of SAME was then calculated using the formula below:

$$\%\ paw\ oedema\ inhibition = \frac{PVc - PVt}{PVc} \times 100$$

Where PVc denotes the paw volume of the control group, and PVt denotes the paw volume of rats given the extract doses.

Statistical Analysis

The data obtained were expressed as mean ± SD (n = 5). Comparison between means was analyzed using one-way and split plot ANOVA followed by Dunnett's post hoc test. The values of $p < 0.05$ were considered statistically significant when compared with the control group. Analysis was done using SPSS statistical software version 23.

Results and Discussion

The qualitative phytochemical analysis showed the presence of flavonoids, alkaloids, tannins, terpenoids, steroids and saponins (Table 1). It has been reported that these metabolites play crucial roles in anti-inflammatory effects in animals. For instance, alkaloids and flavonoids have shown anti-inflammatory effects in rats like non-steroidal anti-inflammatory drugs (Kasolo et al., 2011).

The total phenolic content of SAME was 345.12 ± 2.04 mg GAE/g while the total flavonoid content was 88.24 ± 1.11 mg QE/g (Table 2). Previous studies have shown that the extract contains anthraquinones and terpenoids while cardiac glycosides were absent similar to the result of the current study.[20] These secondary metabolites have been reported to illicit arrays of pharmacological potentials in humans. For instance, flavonoids and alkaloids have been reported to possess anti-inflammatory, anticancer and antioxidant activities in humans.[10] Their roles in this current research were not different from the results obtained in anti-inflammatory activity discussed below. Phenolic compounds have numerous medicinal uses in the human healthcare system. This is because, some of them are therapeutically used as anti-inflammatory, analgesic, antioxidant, anticancer and antipyretic agents.[9] It is not surprising, therefore, their roles in the current study with a significant concentration of phenolics obtained in extract equivalent to gallic acid per gram (Table 2 and Figure 1).

The acute oral toxicity assessment of SAME (Figure 2), further confirms the safety of the extract as an ethnomedicinal prescription for inflammation in traditional medicine in North-East Nigeria as there were no signs of toxicity or mortality after fourteen days of acute intoxication. This is because, it was reported that an LD_{50} > 5000 mg/kg body weight is biologically important.[21] Although it was reported that *S. americanum* extract contains toxic compounds like solanine alkaloid,[20] which may be lethal to some organs in the body, the dose (5000 mg/kg b.w.) did not produce any toxicity in the animals within the periods of this investigation.

Table 1. Phytochemical constituents of *S. americanum* leaf methanol extract

Constituent	Tests	Observation	Inference
Carbohydrate	Molisch	Blue-black	+
	Barfoed's	Blue	+
Saponin	Frothing	30 min	+
	Haemolysis	Bursting RBC	+
Anthraquinones	Bontrager	Pink-red	-
	Mod. Borntrager	Bright-pink	-
Cardiac glycosides	Keller- Kilian	No brown ring	-
	Kedde's	No colour	-
Flavonoids	Shinoda	Red colour	+
	NaOH	Yellow colour	+
Tannins	Ferric chloride	No colour	+
	Lead subacetate	No ppt	+
	Goldbeater's	No colour	+
Alkaloids	Dragendorff's	Orange ppt.	+
	Mayer's	Creamy ppt.	+
	Wagner's Test	Cloudy ppt.	+
Steroids/Triterpenes	Salkowski	Reddish-brown	+
	Lieberman-Burchard	Pink colour	+

Note: + means present or detected, and - means absent or not detected.

Table 2. Total phenolics and flavonoid content in the SAME

Plant extract	Total phenolics (GAE/g)	Total flavonoids (QE/g)
SAME	345.12 ± 2.04 mg	88.24 ± 1.11 mg

Results are mean ± SD (n = 3).

Table 3. Anti-inflammatory effect of *S. americanum* methanol leaf extract

Group		Diameter of Paw (±SD mm)				
		0 min	60 min	90 min	120 min	240 min
Normal control	5.0	5.12 ±0.04	5.36±0.01	5.44±0.04	5.67±0.02	
Standard drug	5.0	4.12±0.03*	4.10±0.03*	2.14±0.02*	1.03±0.02*	
300 mg/kg SAME	5.0	4.88±0.01*	4.62±0.02*	4.22±0.01*	4.08±0.01*	
600 mg/kg SAME	5.0	4.12±0.02*	4.04±0.01*	4.00±0.01*	3.14±0.02*	
1200 mg/kg SAME	5.0	2.46±0.01*	2.24±0.01*	1.46±0.01*	1.01±0.02*	

Results are mean ± SD (n = 5), * Statistically significant ($p \leq 0.05$; split-plot ANOVA followed by Dunnett's post hoc).

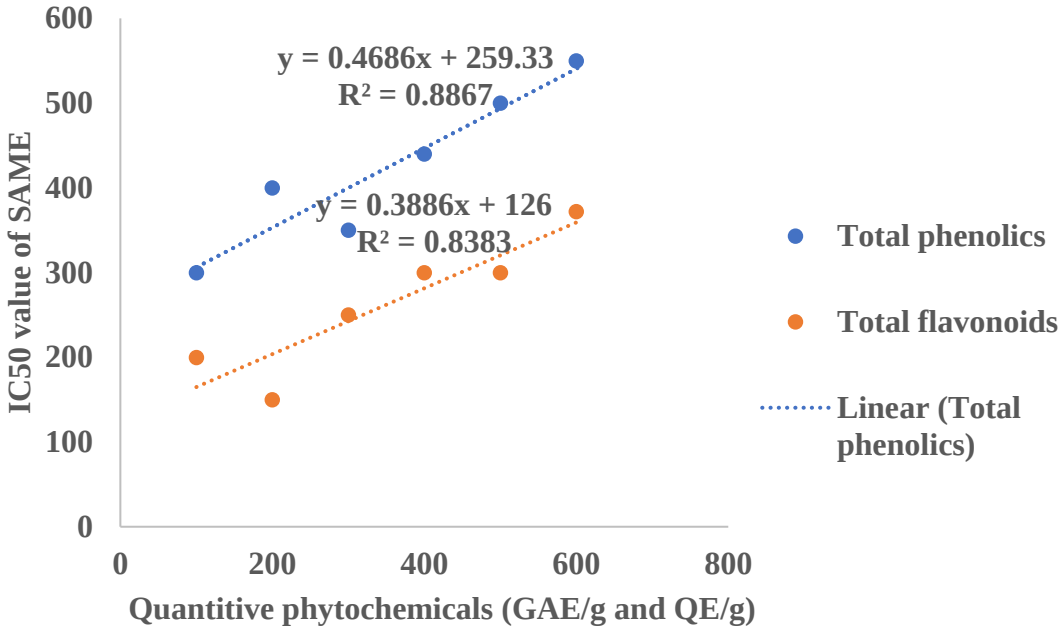

Figure 1. Linear correlation curve of the total phenolics and total flavonoid contents versus anti-inflammatory activity of *S. americanum* methanol leaf extract (SAME).

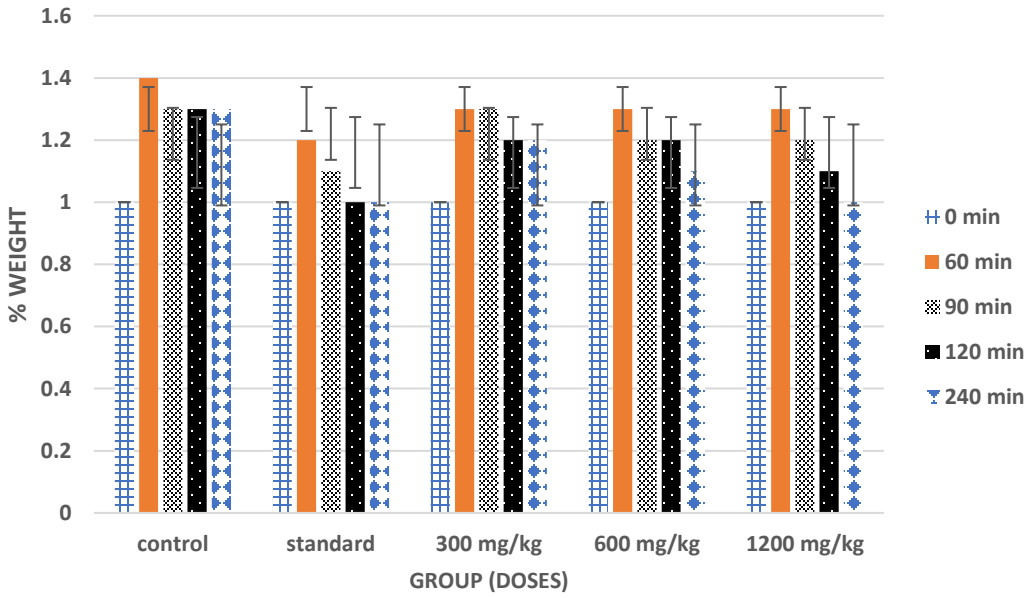

Figure 2. Effect of *S. americanum* methanol leaf extract on rats' percentage weight gain after 240 min. Results are mean ±SD (n = 5). *P < 0.05 is considered statistically significant when compared with the control group using split plot ANOVA (SPANOVA) followed by Dunnett's post hoc test.

In the anti-inflammatory evaluation of SAME, the extract showed a dose-dependent activity in the reduction of paw oedema volume in rats (Table 3). Similarly, the extract did not affect the weights of the animals within the study period because there was no significant reduction in weight in the treated groups when compared with the control ($p < 0.05$) (Figure 2). It has been reported that carrageenan-induced paw oedema model in rats was sensitive to COX (cyclo-oxygenase) inhibitors that synthesize pro-inflammatory agents such as prostaglandins, histamine and serotonin used to evaluate the effect the anti-inflammatory drugs.[22] The extract significantly decreased the diameter or volume of paw oedema in rats induced by carrageenan when compared with the standard drug (diclofenac). Finally, it can be affirmed that the paw oedema inhibitory effect of the SAME was due to inhibition of pro-inflammatory markers such as cyclo-oxygenase enzyme which prevented the production of prostaglandin (PGA1). The anti-inflammatory activity of *S. americanum* observed in this study was due to the presence of certain secondary metabolites especially total phenolics and flavonoid contents (Figure 2) revealed in the phytochemical analysis.

Conclusion

The study showed that methanol leaf extract of *S. americanum* (SAME) possessed potential anti-inflammatory activity due to the presence of certain secondary metabolites. This finding justifies the acclaimed use of *S. americanum* extract for treating inflammation in traditional medicine in North-East Nigeria.

Acknowledgement

The author is grateful to Mr. Yusuf of the Pharmacology Laboratory, University of Maiduguri, Nigeria for his technical assistance in some aspects of this work.

Funding

None

Conflicts of Interest

The authors have no conflicts of interest to declare.

Ethics statement

The animals used in this study follows the guidelines relating to the use of animals in research ARRIVE.

References

1. Alamgir ANM. Introduction BT - Therapeutic Use of Medicinal Plants and Their Extracts: Volume 1: Pharmacognosy. in (ed. Alamgir, A. N. M.) 1–17; Springer International Publishing, 2017.
2. Dermane A. Differential constituents in roots, stems and leaves of Newbouldia laevis Thunb. screened by LC/ESI-Q-TOF-MS. *Results Chem.* 2020;2:100052.
3. Abere TA, Onwukaeme DN, Eboka CJ. Pharmacognostic evaluation of the leaves of dissotis rotundifolia Triana (Melastomataceae). *African J Biotechnol.* 2009;8:113–115.
4. Badal S, Delgoda R. *Pharmacognosy Edited by*. 2017.
5. Ukwubile CA, Ahmed A, Katsayal UA, et al. Acute and subchronic toxicity assessment of Melastomastrum capitatum Fern. leaf methanol extract in Wistar albino rats. *Int Biol Biomed J.* 2019;5.
6. Lee YM, Son E, Kim SH, et al. Anti-inflammatory and anti-osteoarthritis effect of Mollugo pentaphylla extract. *Pharm Biol.* 2019;57:74–81.
7. Fyad K, Belboukhari N, Hadj-Khelil AO, et al. Analgesic and anti-inflammatory activity of aqueous extract of Bubonium graveolens. *Biomed Res Ther.* 2020;7:4002–4009.
8. Sharma VC. Analgesic, anti-inflammatory and antipyretic activities of ethanolic extract of stem bark of Anogeissus latifolia Roxb. *Clin Phytoscience* 2020;6.
9. Dodiya T, JainV. Pharmacognostic, physicochemical and phytochemical investigation of Grangea maderaspatana. *J Phytopharm.* 2017;6:359–363.
10. Kasolo JN, GSB, Ojok L, Ogwal-okeng JW. Phytochemicals and acute toxicity of Moringa oleifera roots in mice. *J Pharmacogn Phyther.* 2011;3:38–42.
11. Onoja. In Vitro Anti-Cholinesterase and Antioxidant Activity of Extracts of Moringa oleifera Plants from Rivers State, Niger Delta, Nigeria. *Medicines.* 2018;5:71.
12. Alamgeer Iman S, Asif H, Saleem M. Evaluation of antihypertensive potential of Ficus carica fruit. *Pharm Biol.* 2017;55;1047–1053.
13. Borquaye LS. Anti-inflammatory and antioxidant activities of extracts of Reissantia indica, Cissus cornifolia and Grosseria vignei. *Cogent Biol.* 2020;6;1785755.
14. Rahmawati DS, Elya B, Noviani A. Inhibitory effects of sangketan (Achyranthes Aspera L.) root extracts on arginase activity. *Int J Appl Pharm.* 2020;12:248–251.
15. Andargie Y, Sisay W, Molla M, et al. Evaluation of the Antiulcer Activity of Methanolic Extract and Solvent Fractions of the Leaves of Calpurnia aurea (Ait.) Benth. (Fabaceae) in Rats. *Evidence-based Complement Altern Med.* 2022.

16. Guidelines O. Test Guideline No . 425 Acute Oral Toxicity : Up-and-Down Procedure. 2022.
17. Khalifa MA. Original Article Acute and Subacute Toxicity Studies of. 2022;8:279–290.
18. Shelar MK, Patil MJ, Bhujbal SS, et al. Evaluation of anticonvulsant activity of the ethanolic extracts from leaves of Excoecaria agallocha. *Futur J Pharm Sci.* 2018;4:215–219.
19. Nakalembe L, Kasolo JN, Nyatia E, et al. Analgesic and Anti-Inflammatory Activity of Total Crude Leaf Extract of <i>Phytolacca dodecandra</i> in Wistar Albino Rats. *Neurosci Med.* 2019;10:259–271.
20. Usman H, Victor V, Waziri I. Qualitative Phytochemical Screening and in Vitro Antimicrobial Activities of Solanum Americanum Mill. *J Eng Technol Environ.* 2018;14: 104–110.
21. Al Batran R. In Vivo Antioxidant and Antiulcer Activity of Parkia speciosa Ethanolic Leaf Extract against Ethanol-Induced Gastric Ulcer in Rats. *PLoS One.* 2013;8:2–12.
22. Pandey BP, Adhikari K, Pradhan SP, et al. inflammatory activities of selected medicinal plants from western Nepal. *Futur J Pharm Sci.* 2020;6:1–12.