Journal of Herbal Drug
journal homepage: www.jhd.iaushk.ac.ir

Analgesic and anti-inflammatory effects of *Chlorophytum alismifolium* extract in murine models

Abdulhakim Abubakar*[1], Amina Hamzat Nauzo[1], Abdullahi Balarabe Nazifi[2], Zahatu Muhammad[1], Idris Mohammed Maje[1], Nuhu Mohammed Danjuma[1]

[1]*Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria-Nigeria;*
***Email:** *abdulhakimevuti@gmail.com*

[2]*Department of Pharmacology and Therapeutics, Bayero University Kano-Nigeria;*

ARTICLE INFO

Type: *Original Research*
Topic: *Medicinal Plants*
Received *April 12th 2020*
Accepted *July 08th 2020*

Key words:
- ✓ *Chlorophytum alismifolium*
- ✓ *GC-MS*
- ✓ *hexane extract*
- ✓ *Inflammation*

ABSTRACT

Background & Aim: Medical conditions associated with pains and inflammation are prevalent in the global population. The tubers of Chlorophytum alismifolium are widely used traditionally in Nigeria for the management of pain and inflammation. This study aims to establish the chemical profile and evaluate the anti-inflammatory and analgesic activities of the hexane extract of C. alismifolium (HECA) tubers in experimental animals.

Experimental: Gas chromatography-mass spectrometry (GC-MS), phytochemical screening and acute toxicity studies were conducted according to standard protocols. Analgesic activity was investigated with graded doses of HECA (250, 500 and 1000 mg/kg) using acetic acid-induced writhing test in mice and formalin-induced pain in rats; while the anti-inflammatory activity was evaluated using carrageenan-induced paw oedema model in rats.

Results: The GC-MS analysis revealed the presence of eighteen compounds covering an area of approximately 100%. Phytochemical screening revealed the presence of flavonoids, alkaloids, steroids and triterpenes while the oral median lethal dose was estimated to be >5000 mg/kg in rats and mice. In the 4th and 5th hour of the carrageenan test, HECA at 500 and 1000 mg/kg significantly (P<0.01) reduced the oedema index, respectively. In the analgesic study, HECA significantly (P<0.001) reduced the mean number of writhes with the highest inhibition (79.67%) obtained at 500 mg/kg. In the formalin test, HECA at 250 mg/kg significantly (P<0.05) reduced the mean pain scores in both phases of the test.

Recommended applications/industries: The findings depict that HECA possesses pharmacologically active compounds that can be applied in the management of inflammation and pain.

1. Introduction

Pain cannot be objectively defined satisfactorily but it is an unpleasant sensation which only the individual can describe and also causes significant discomfort (Satoskar *et al.*, 2015). It is a serious health challenge with harsh consequences and the experienced sensation of discomfort depicts injury to the body (Andersen *et al.*, 2015). Acute pain also referred to as nociceptive

pain is characterized by sudden or excruciating feeling (Tedore *et al.*, 2015) which occurs as a physiological reaction to tissue destruction or traumatic experience (Rauf *et al.*, 2016, Odoma *et al.*, 2017). Chronic pain is very debilitating, persists for several months and attributed to abnormal sensation initiated or caused by a primary lesion or malfunction of the nervous system (Steeds, 2016; Shchegol'kov *et al.*, 2017). Inflammation is a crucial tissue reaction to intrinsic or extrinsic damage (Gallo *et al.*, 2017) and it is linked to a myriad of responses such as enzyme activation, release of mediators, tissue repair or breakdown (Khan *et al.*, 2020). Inflammation is a protective mechanism by the body to eliminate injurious stimuli and initiate the process of healing (Paliwal *et al.*, 2017). Acute inflammation is the initial response of the body which begins immediately after a tissue injury (Paliwal *et al.*, 2017) and it is exacerbated to chronic inflammation if the injurious stimulus remains for a longer time and cannot be removed by the body (Agli *et al.*, 2013).

Non-steroidal anti-inflammatory drugs (NSAIDs) and opioids are the main classes of drugs used in the alleviation and management of pain (Malm and Borisch, 2015). The application of NSAIDs is marred with severe side effects including gastrointestinal, hepatic, renal and cardiovascular complications (Bozimowski, 2015; Patrono, 2016). Similarly, opioid analgesics are grossly associated with dependence, tolerance, addiction and also have the propensity to be abused by individuals (Arora *et al.*, 2014). Despite the application of the aforementioned classes of medications in the treatment of pain and inflammatory diseases, these ailments still represent a significant global health burden (Cirino *et al.*, 2019; Khan *et al.*, 2020).

Medicinal plants have history of wide usage since antiquity and forms the basis of Traditional Medicine Systems across the world (Mondal *et al.*, 2019). They possess several compounds eliciting diverse pharmacological activities with lesser adverse effects compared to conventional therapies (Samani *et al.*, 2017; de olivera *et al.*, 2019) and also widely utilized in the management of inflammation and pain (Solati *et al.*, 2017).

Chlorophytum alismifolium Baker (liliaceae) is a short stem herb with tubers which are abundant in streams (Burkill, 1995). Alimsa-leaved ground lily (Morton) is the common name and the local names are *Rogon makwarwa*, *Cigorodi* and *Ekuce* in Hausa, Fulfulde and Agatu languages of Northern Nigeria respectively. Experimentally, *Chlorophytum alismifolium* has been shown to possess antihyperglycaemic and antinociceptive activities (Abubakar *et al.*, 2018; Abubakar *et al.*, 2020), with appreciable degree of safety following sub-acute administration (Abubakar *et al.*, 2019). This study focused on the chemical composition, anti-inflammatory and analgesic potentials of hexane extract of *C. alismifolium* in experimental animals.

2. Materials and Methods

2.1. Chemicals

Pentazocine (Juhel Nigeria Limited), Ketoprofen (M and B, Lagos Nigeria.), Formalin (Johnson Solomon Export Limited. London, England), Acetic acid, and Carrageenan (Mayer and Baker Limited, Daotham, England) and Hexane (JHD, China).

2.2. Preparation of HECA and phytochemical screening

The powdered material of *C. alismifolium* (1 kg) was extracted intensively with 2.5 L of normal hexane solvent for 2 days using a Soxhlet apparatus (James *et al.*, 2014). This was followed by the concentration of the extract on a laboratory water bath fixed at 45°C and the percentage yield was calculated. The preliminary screening for the presence or absence of secondary metabolites using standard tests was done according to established procedures (Evans, 2009).

2.3. Chemical profiling

Gas chromatography-mass spectrometry (GC-MS) investigation was done using an Agilent gas chromatography system7890B and a 5977A mass spectrum detector, Agilent Technologies, USA. The chromatography was carried out on a HP-5 MS capillary column (30m×250µm×0.25µm). High purity Helium was carrier gas used and the continuous flow rate of helium was 3.6839 ml/min. The Split injection proportion was 5:1. The temperature of the GC started at 50°C for 1 min, raised to 200°C at a rate of 3°C/min, then raised to 300°C at 3°C/min for 15 min and finally held at 325°C (1 min). MS program scanned quality range of 30amu - 600amu, ionization voltage of 70eV, ionization current of 150µA (EI). The quadrupole and

ion source temperatures were fixed at 150°C 230°C, respectively. The Compounds in the HECA were finally detected using NIST 14.L database (Stein, 2014).

2.4. Experimental animals

Male and female Albino mice (18-35 g) and Wistar rats (80-130g) were obtained from the Animal House Facility of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, Nigeria. They were kept under standard laboratory conditions and allowed free access to feed (Grower's pelletized feed, Jos-Nigeria) and water *ad libitum*. The studies were conducted with the approval of the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) with the Ethical Approval Number: ABUCAUC/2020/31.

2.5. Acute toxicity

The estimation of the acute toxicity of HECA in rats and mice was done according Lorke's method (1983). In the first phase of the study, three groups of three animals were administered widely differing doses of HECA (10, 100 and 1000 mg/kg), respectively, and were observed for signs of toxicity and mortality for 24 h. In the second phase, 3 animals were administered 1600, 2900 and 5000 mg/kg of the extract (based on the outcome of the first phase) and then observed for signs of toxicity and mortality for 24 h. The LD_{50} was calculated as the geometric mean of the lowest lethal dose and the highest non-lethal dose.

2.6. Anti-inflammatory study

Carrageenan-induced paw oedema in rats was conducted as described by (Winter *et al.*, 1962). Thirty (30) rats were divided into five (5) groups of six (6) rats each. Rats in the first group were administered 1 ml/kg distilled water and served as the negative control. Those in groups 2, 3 and 4 were given HECA orally at the doses of 250, 500 and 1000 mg/kg, respectively. Those in group 5 served as the positive control and received ketoprofen (20 mg/kg). After 60 minutes of treatment with various agents, localized inflammation was produced by injecting carrageenan (0.1% w/v, 100 µl) into the sub plantar tissues of the left hind paws of the rats. Using a digital vernier caliper, the paw sizes were measured at various time intervals (0, 2, 3, 4 and 5 hours). A change in the paw size of each rat was estimated as the difference in paw diameter before carrageenan was induced and after at each time interval, this was followed by the calculation of percentage inhibition of inflammation for each group with respect to the distilled water group using the following equation:

Percentage Inhibition= $=[(C_t - C_o)_{control} - (C_t - C_o)_{treated}/(C_t - C_o)_{control}] \times 100\%$

Where C_t = mean oedema index for each group at time t and C_o = mean oedema index for each group before carrageenan injection.

2.7. Analgesic studies

2.7.1. Acetic acid- induced pain model in mice

Thirty mice were categorized into 5 groups. Group 1 received 10 ml/kg of distilled water, groups 2, 3, and 4 received 250, 500 and 1000 mg/kg doses of HECA, respectively, while those in group 5 were administered ketoprofen (20 mg/kg) all via the oral route of administration. An hour later, the mice in all groups were administered 10 mg/kg of 0.6 % v/v acetic acid through the intraperitoneal route (Koster *et al.*, 1959). The mice were allowed for 5 minutes and then placed in observation cages after which the number of abdominal constrictions were counted for each mouse over a period of 10 minutes, a waiting period of 5 minutes was allowed. Analgesia was depicted by a decrease in the number of writhes when compared to the mice in control group and was expressed as percentage inhibition using the equation:

% Inhibition= [Mean number of writhes (control) − Mean number of writhes (test)/ Mean number of writhes (control)] ×100

2.7.2 Formalin-induced pain model in rats

The technique explained by (Dubuisson and Dennis, 1977; Tjolsen *et al.*, 1992) was adopted. 30 rats were divided into 5 groups each consisting of 6 animals. Rats in group 1 received 1ml/kg of distilled water, those in groups 2, 3 and 4 were given HECA at the doses of 250, 500 and 1000 mg/kg, respectively. Those in group 5 received 20 mg/kg of pentazocine all through the oral route. After 60 minutes of various treatments, formalin solution (50 µl of 2.5% v/v) was injected subcutaneously under the plantar surface of the left hind paw. The rats were then placed in examination cages and observed for 60 minutes, the gravity of pain responses were noted based on the magnitude: Zero (0) mice walked or stood definitely on injected paw; one

(1) the injected paw was incompletely raised; two (2) the injected paw was noticeably elevated off the floor; three (3) the rat chewed, licked or trembled over the injected paw. The anti-nociceptive effect was evaluated and recorded in two stages; during the initial 5 minutes and the final 45 minutes, with a waiting time of 10 minutes between both phases.

2.8. Statistical analyses

Data obtained from anti-inflammatory and analgesic studies were presented as Mean ± S.E.M. Results of oedema index were analyzed using repeated measure analysis of variance (ANOVA) followed by Bonferroni post hoc test, data from acetic acid-induced pain model were analyzed by one way ANOVA followed by Dunnett post hoc test while data obtained from formalin-induced pain model were analyzed using Kruskal-Wallis test. Values of $P \leq 0.05$ were taken into consideration as significant.

3. Results and discussion

The extraction of 1 kg of the powdered plant material gave 24 g of HECA and the percentage yield was thus calculated to be 2.4%$^{w}/_{w}$. Phyto-constituents found in HECA include flavonoids, steroids, triterpenes and alkaloids. Phyto-constituents or secondary metabolites such as alkaloids, flavonoids, steroids and triterpenoids have been linked to the anti-inflammatory and analgesic activities of medicinal plants (Oliveira *et al.*, 2014; Paliwal *et al.*, 2017). Some of the aforementioned phytochemicals are present in HECA which might be acting alone or synergistically to produce the observed anti-inflammatory and analgesic activities thereby further validating its ethno-medicinal use as earlier reported by Abubakar *et al*. (2020).

GC-MS is a technique which has been widely utilized for the evaluation of chemical composition, identification and quantification of substances (Hage, 2018) and it is valuable for the analysis of many classes of compounds including phyto-constituents (Steimling and Kahler, 2018). The chemical profiling using GC-MS revealed the presence of 18 compounds covering the total area of 100.3 % (Tables 1 and 2). The GC-MS analysis of HECA revealed the presence of compounds some which have been reported to have anti-inflammatory and analgesic activities. They include isoxazolidine derivatives which are compounds with established anti-inflammatory activity (Park *et al.*, 2006); derivatives of thiazoles have been explored in the past for their analgesic and anti-inflammatory activities (Saravanan *et al.*, 2011); isothiazole derivatives have been reported to possess anti-inflammatory property (Clerici *et al.*, 2008); synthetic benzothiazole derivatives have also been evaluated for their anti-inflammatory activity (Mohammed *et al.*, 2009); 1,2,4-dithiazole and their derivatives serve as anti-phlogistic cyclo-oxygenase 2 inhibitors and thereby elicit antipyretic, analgesic and anti-inflammatory activities (Bohme and Ahrens, 1974; Pandeya *et al.*, 1978); 8-nonynoic acid is an amino compound with an established anti-inflammatory activity (Kumaravel *et al.*, 2017); Propanamides and Methyltriazolo (1, 2, 4) triazine and their derivatives also possess anti-inflammatory and analgesic activities (Odin and Onoja, 2015).

Oral administration of HECA neither elicited any clear symptom of toxicity nor death in rats and mice over a period of 24 hr, hence the LD_{50} was estimated to be greater than 5000 mg/kg. This reveals that HECA is practically non-toxic in rats and mice when administered orally.

In the anti-inflammatory study, the injection of carrageenan produced a local oedema in all groups tested with the peak inflammation obtained in the 3rd hour. HECA as well as ketoprofen were able to reduce inflammation induced by carrageenan throughout the period of study. In the 4th hour, HECA produced a significant ($P<0.05$) reduction in the oedema index at 500 and 1000 mg/kg when compared distilled water group. Similarly, in the 5th hour, HECA at the doses investigated elicited a significant ($P<0.05$) reduction the in the oedema index when compared to the distilled water group. On comparison over time, HECA at the doses tested produced a significant ($P<0.05$) reduction in the oedema index when compared to the 3rd hour (peak of inflammation) (Figure 1).

Edema caused by carrageenan is an established model used for screening compounds with anti-inflammatory activity (Paliwal *et al.*, 2017). It produces edema through a biphasic mechanism; the initial or early phase which is obtainable within the first hour of injection and involves the release of bradykinin, serotonin and histamine while the final phase which occurs within the 2nd to 5th hours is attributed to the release of prostaglandins (Yu *et al.*, 2012). The ability

of HECA to reduce the edema index in the 4th and 5th hours (2nd phase) signifies that it possesses anti-inflammatory activity probably linked to the inhibition of inflammatory mediators like prostaglandins (Yu *et al.*, 2009; Rauf *et al.*, 2016).

In the acetic acid-induced writhing test, HECA showed a significant (P<0.001) reduction in the number of writhes caused by acetic acid with the highest percentage inhibition of (79.65%) obtained at 500 mg/kg and the effect of HECA at all the doses under investigation was found to be less than that of the standard (Ketoprofen) at 20 mg/kg (Figure 2). The acetic acid-induced writhing test is a valuable experimental model for evaluating peripheral analgesic activity and the abdominal writhing it produces is attributed to prostaglandins (Sengar *et al.*, 2015). The HECA reduced the abdominal constrictions in mice and the reduction may be attributed to its ability to inhibit cyclooxygenase and thus preventing the synthesis of prostaglandins (Rauf *et al.*, 2015).

In the formalin test, HECA at 250 mg/kg elicited a significant (P<0.05) reduction the mean pain scores in both stages of the formalin test. HECA at 500 mg/kg produced a significant (P<0.05) reduction in mean pain scores during second stage of the study. The positive control (Pentazocine, 20 mg/kg) showed a significant (P<0.05) reduction in the mean pain scores in both the initial and final stages of the formalin test (Figure 3). The use of formalin for evaluating pain and analgesia in rats has long been established (Tjolsen *et al.*, 1992). Formalin-induced pain produces a biphasic response with centrally acting analgesics being active in both phases, whereas and the non-steroidal anti-inflammatory drugs are known to block the final phase. HECA at 250 mg/kg inhibited both phases indicating that it possesses both central and peripheral analgesic activity further validating the acetic acid-induced test and this may be attributed to its propensity to hinder the transfer of painful impulses in the central nervous system or its ability to block peripheral production of prostaglandins (Rauf *et al.*, 2016). Furthermore, HECA and ketoprofen both produced anti-inflammatory and analgesic activities, most of the peripherally acting analgesics have also been reported to possess anti-inflammatory activity and the mechanism of action has been described as the blockade of cyclooxygenase and thus stopping the formation of prostaglandins (Satoskar *et al.*, 2015).

Table 1. Phytochemical constituents of hexane extract of *Chlorophytum alismifolium*.

Phytochemical constituent	Inference
Anthraquinones	-
Glycosides	-
Cardiac glycosides	-
Saponins	-
Steroids	+
Triterpenes	+
Tannins	-
Flavonoids	+
Alkaloids	+

Key: (+) = Present, (–) = Absent

Table 2: Chemical profile of hexane extract of *Chlorophytum alismifolium* using GC-MS.

S/No.	Compounds	Area covered (%)	Retention time (min)
1	Aminoacetonitrile	6.50	5.9
2	Isoxazolidine	14.93	6.5
3	2-Propenal	2.86	7.5
4	Acetonitrile	1.38	9.6
5	Propanamide	7.60	10.3
6	Methyl-1-cyclopropane carboxylate	5.74	12.0
7	N-Ethylformamide	10.76	15.37
8	Sec-Butylamine	5.83	22.5
9	3-Iodothiophene-2-carboxamide	6.11	25.7
10	Isothiazole	1.3	27.1
11	3,6-Bis(N-formamido) carbazole	3.73	27.5
12	5,6-Dihydro-5-methyldithiazine	1.58	27.7
13	3,4-hexanediol	2.36	28.1
14	Benzothiazole	2.29	28.3
15	Formamide	1.17	28.9
16	6-Methyl-triazolo-triazine	16.99	29.9
17	1,2,4-Dithiazole-3-thione	2.11	35.3
18	8-Nonynoic acid	7.11	37.7

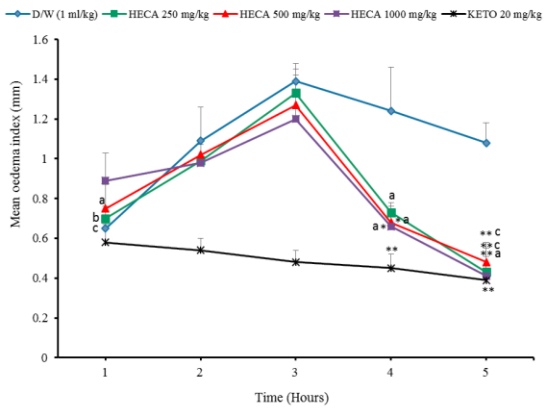

Figure 1. Effect of hexane extract of Chlorophytum alismifolium tubers on carrageenan-induced rat paw oedema. Values are presented as mean ± SEM, * = P<0.05, ** = P<0.01 compared to distilled water (D/W) group; a, b, and c = P<0.05, P<0.01 and P<0.001, respectively, compared to time 3h – Repeated measures ANOVA followed by Bonferroni- test, n = 6, KETOP = Ketoprofen, HECA = Hexane extract of Chlorophytum alismifolium.

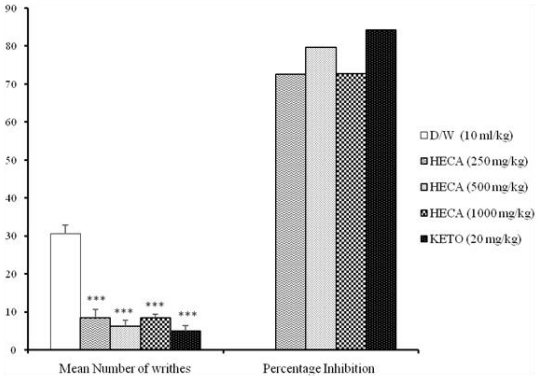

Figure 2. Effect of hexane extract of Chlorophytum alismifolium tubers on acetic acid-induced writhing in mice. Values are Mean ± S.E.M., *** = P<0.001 compared to D/W group – one way ANOVA followed by Dunnett post hoc test; n=6, D/W = distilled water, HECA = Hexane extract of Chlorophytum alismifolium, KETO = Ketoprofen.

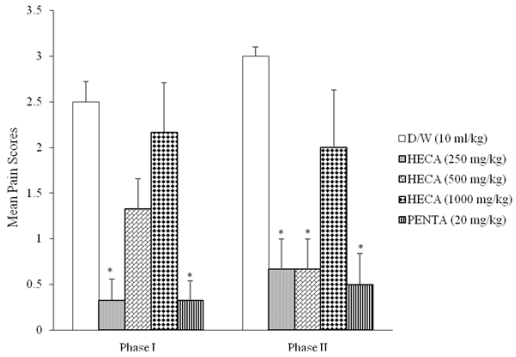

Figure 3. Effect of hexane extract of Chlorophytum alismifolium tubers on formalin-induced pain in rats. Values are Mean ± S.E.M., * =P<0.05 compared to D/W group – Kruskal-Wallis test, n=6, D/W = Distilled water, HECA = Hexane extract of Chlorophytum alismifolium, PENTA = Pentazocine

4. Conclusion

The hexane extract of *C. alismifolium* possesses pharmacologically active compounds with significant anti-inflammatory and analgesic activities in rats and mice.

5. Acknowledgments

The authors would like to acknowledge Dr. Bashir Musa of Multi-User Science Research Laboratory, Ahmadu Bello University Zaria, Nigeria for the GC-MS analysis and Mal. Abubakar Balarabe of the Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria, Nigeria for Technical Assistance in the course of this research.

6. References

Abubakar, A., Danjuma, N.M., Chindo, B.A. and Nazifi, A.B. 2018. Anti-hyperglycaemic activity of tuber extract of *Chlorophytum alismifolium* Baker in streptozotocin-induced hyperglycaemic rats. *Bulletin of Faculty of Pharmacy, Cairo University*, 56 (1): 60-67.

Abubakar, A., Nazifi, A.B., Ismail, H.F., Duke, K.A. and Edoh, T.D. 2019. Safety assessment of *Chlorophytum alismifolium* tuber extract (Liliaceae): Acute and sub-acute toxicity studies in Wistar rats. *Journal of Acute Disease*, 8(1): 21-27.

Abubakar, A., Nazifi, A.B., Odoma, S., Shehu, S. and Danjuma, N.M. 2020. Anti-nociceptive activity of methanol extract of *Chlorophytum alismifolium* tubers in murine model of pain: Possible involvement of α-$_2$adrenergic receptor and K_{ATP} channels. *Journal of Traditional and Complementary Medicine*, 10 (1): 1-6.

Agli, M.D., Di Lorenzo, C., Badea, M., Sangiovanni, E., Dima, L., Bosisio, E. and Restani, P. 2013. Plant food supplements with anti-inflammatory properties: a systematic review. *Critical Reviews in Food Science and Nutrition*, 53 (5): 403-413.

Andersen, L.P.H., Gogenur, I., Fenger, A.Q., Petersen, M.C., Rosenberg, J. and Werner, M.U. 2015. Analgesic and antihyperalgesic effects of melatonin in a human inflammatory pain model: a randomized, double blind, placebo-controlled, three-arm crossover study. *Pain*. 156 (11): 2286-2294.

Arora, N., Cao, M. and Javaheri, S. 2014. Opioids, sedatives, and sleep hypoventilation. *Sleep Medicine Clinics*, 9: 391-398.

Bohme, H. and Ahrens, K.H. 1974. 5-Aaryl-3H-1,2,4-dithiazoles: A new class of highly potent fungicides. *Archives of Pharmacy*, 307 (11): 828-836.

Bozimowski G. 2015. A review of nonsteroidal anti-inflammatory drugs. *American Association of Nurse Anaesthetics Journal*, 83: 425-433.

Burkill, H.M., 1995. *The useful plant of West Tropical Africa*. Royal Botanical Gardens. Vol. 3. pp. 494.

Cirino, T.J., Eans, S.O., Medina, J.M., Wilson, L.L., Mottinelli, M., Intagliata, S., McCurdy, C.R. and McLaughlin J.P. 2019. Characterization of sigma 1 receptor antagonist CM-304 and its analog, AZ-66: novel therapeutics against allodynia and induced pain. *Frontiers in Pharmacology*, 10:1-14.

Clerici, F., Gelmi, M.L. and Pellegrino, S. 2008. *Comprehensive Heterocyclic Chemistry III*, Elsevier, Oxford, United Kingdom.

de Oliveira, J.R., Camargo, S.E.A. and de Oliveira L.D. *2019*. *Rosmarinus officinalis* L. (rosemary) as therapeutic and prophylactic agent. *Journal of Biomedical Science*, 26 (5): 1-22.

Dubuisson, D. and Dennis, S.G. 1977. The formalin test; a quantitative study of the analgesic effect of morphine, meperidine and brain stem stimulation in rats and cats. *Pain*, 4: 161-174.

Evans, W.C., 2009. *Trease and Evans Pharmacognosy*, (16th ed.) Elsevier Health Sciences, London, United Kingdom.

Gallo, J., Ruska, M., Kriegova, E. and Goodman, S.B. 2017. Inflammation and its resolution and the musculoskeletal system. *Journal of Orthopedic Translation*, 10: 52-67.

Hage, D.S. 2018. Small Molecules, Peptides and Pathogens. Principles and Applications of Clinical Mass Spectrometry. 1st edition. Elsevier, USA. pp. 1-32.

James, R., Malcolm, K., Dariel, B. and Joanna, V. 2014. Using Soxhlet ethanol extraction to produce and test plant material [essential oils] for their antimicrobial properties. *Journal of Microbiology and Biology Education*, 15 (1): 45-46.

Khan, H., Pervaiz, A., Intagliata S., Das, N., Kalyan C. Venkata., N., Atanasov, A. G., Najda, A., Nabavi S.M.., Wang,. D., Pittalà, V. and Bishayee, A. 2020. The analgesic potential of glycosides derived from medicinal plants. *DARU Journal of Pharmaceutical Sciences*, 28 (1): 387-401.

Koster, R., Anderson, M. and Debeer, E.J. 1959. Acetic acid for analgesic screening. *Federation Proceedings*, 18:412.

Kumaravel, S., Muthukumaran, P. and Shanmugapriya, K. 2017. Chemical composition of *Trigonella foenum-graecum* through gas chromatography mass spectrometry analysis. *Journal of Medicinal Plants Studies*, 5 (13):1-3.

Lorke, D. 1983. A new approach to acute toxicity testing. *Archives of Toxicology*, 5: 275-287.

Malm, H., Borisch, C., 2015. Analgesics, non-steroidal anti-inflammatory drugs (NSAIDs), muscle relaxants, and anti-gout medication, in Drugs during Pregnancy and Lactation: Treatment Options and Risk Assessment. *Academic Press*. pp. 27-58.

Mohammed, A., Arun, K., Israr, A. and Khan, S.A. 2009. Synthesis of pharmaceutically important 1, 3, 4-thiadiazole and imidazolinone derivatives as antimicrobials. *Indian Journal of Chemistry*, 48: 1288-1293.

Mondal, M., Hossain, S., Das, N., Khalipha, A.B.R., Sarkar, A.P., Islam, T., Smrity, S.Z., Biswas, S. and Kundu S.K. 2019. Phytochemical screening and evaluation of pharmacological activity of leaf methanolic extract of *Colocasia affinis* Schott. *Clinical Phytoscience*, 5 (8): 1-11.

Odin, E.M. and Onoja, P.K. 2015. Synthesis of bridgehead-fused 1,2,3-triazolo [1,5-C]-1,2,4-Triazines: novel anti-inflammatory and analgesic therapeutic systems. *Global Journal of science Frontier Research in Chemistry*, 15:1-17.

Odoma, S., Zezi, A. U., Danjuma N.M. Ahmed, A. and Magaji, M.G. 2017. Elucidation of the possible mechanism of analgesic actions of butanol leaf

fraction of Olax subscorpioidea Oliv. *Journal of Ethnopharmacology*, 199:323–327.

Oliveira, R.G., Mahon, C.P.A.N., Ascêncio, P.G.M., Ascêncio, S.D., Balogun, S.O. and Martins, D.T.O. 2014. Evaluation of anti-inflammatory activity of hydroethanolic extract of *Dilodendron bipinnatum* Radlk. *Journal of Ethnopharmacoly*, 155 (1):387-395.

Paliwal, S.K., Sati, B., Faujdar, S. and Sharma S. 2017. Studies on analgesic, anti-inflammatory activities of stem and roots of *Inula cuspidata* C.B Clarke. *Journal of Traditional and Complementary Medicine*, 7 (4): 532-537.

Pandeya, S.N., Kumar, A., Singh, B.N. and Mishra, D.N. 1978. Synthesis and biological activity of isodithiobiurets, dithiobiurets, and dithiazoles. *Pharmaceutical Research*, 4: 321–326.

Park, K.K., Ko, D.H., You, Z., Khan, M.O.F. and Lee, H.J. 2006. In vitro anti-inflammatory activities of new steroidal antedrugs: [16 alpha, 17 alpha-d] isoxazoline and [16 alpha, 17 alpha-d]-3'-hydroxy-iminoformyl isoxazo- line derivatives of prednisolone and 9 alpha – fluoroprednisolone. *Steroids*, 71: 183-188.

Patrono, C. 2016. Cardiovascular effects of nonsteroidal anti-inflammatory drugs. *Current Cardiology Reports*, 18 (3): 1-8.

Rauf, A., Uddin, G., Khan, H., Siddiqui, B.S. and Arfan, M. 2015. Antihyperalgesic activity of crude extract and 7-methyljuglone of *Diospyros lotus* roots. *Natural Product Research*, 29: 2226-2229.

Rauf, A., Ali, J., Khan, H., Mubarak, M. S. and Patel, S. 2016. Emerging CAM *Ziziphus nummularia* with in vivo sedative-hypnotic, antipyretic and analgesic attributes. *Biotechnology*, 6: 11-20.

Samani, B.H., Lorigooini, Z., Zareiforoush, H. and Jafari, S. 2017. Effect of ultrasound andinfrared drying methods on quantitative and qualitative characteristics of *Satureja bachtiarica* essential oil. *Journal of Essential Oil Bearing Plant*s, 20(5): 1196-1208.

Saravanan, G., Alagarsamy, V., Prakash, C.R., Dinesh, K.P. and Panneer, S.T. 2011. Synthesis of novel Thiazole derivatives as analgesic agents. *Asian Journal of Research in Pharmaceutical Sciences*, 1 (4):134-138.

Satoskar, R.S., Rege, N.N. and Bhandarkar, S.D. 2015. *Pharmacology and Pharmacotherapeutics*. (24th ed.) Popular Prakashan, Mumbai, India.

Sengar, N., Joshi, A., Prasad, S.K. and Hemalatha, S. 2015. Anti-inflammatory, analgesic andanti-pyretic activities of standardized root extract of *Jasminum sambac*. *Journal of Ethnopharmacology*, 160:140-148.

Shchegol'kov, E.V., Shchur I.V., Burgart, Y.V., Saloutin, V.I., Trefilova, A.N., Ljushina, G.A., Solodnikov, S.Y, Markova, L.N., Maslova, V.V., krasnykh ,O.P., Borisevich, S.S. and Khursan, S.L. 2017. Polyfluorinated salicylic acid derivatives as analogs of known drugs: Synthesis, molecular docking and biological evaluation. *Bioorganic and Medicinal Chemistry*, 25 (1): 91-99.

Solati, K., Asadi-Samani, M., and Heidari-Soureshjani, S. 2017. Effects and mechanisms of medicinal plants on dopamine reward system to reduce complications of substance abuse: A systematic review. *Middle East Journal of Family Medicine*, 15: 202-207.

Steeds, C.E. 2016. The anatomy and physiology of pain, *Basic Science*, 34: 55-59.

Steimling, J. and Kahler, T. 2018. Liquid Chromatography's complementary role to gas chromatography in cannabis testing. LC-GC N. *Special Issues*, 36 (s6): 36-40.

Stein, S.E., 2014. *NIST Mass Spectral Search Program and NIST/EPA/NIH Mass Spectral Library version 2.2. National Institute of Standards and Technology*, USA.

Tedore, T., Weinberg, R., Witkin, L., Giambrone, G.P., Faggiani, S.L. and Fleischut, P.M. 2015. Acute pain management/regional anesthesia. *Anesthesiology Clinics*, 33 (4):739–751.

Tjolsen, A., Berge, O.G., Hunskaar, S., Rosland, J.H. andItole, K. 1992. The formalin test: an evaluation of the method. *Pain*, 51: 5-17.

Winter, C.A., Risley, E.A. and Nuss, G.W. 1962. Carrageenan -induced oedema in hind paw of rats as an assay for anti-inflammatory drugs. *Proceedings of the Society for Experimental Biology and Medicine*, 111: 544-547.

Yu, C., Tang, W., Peng, C., Sun, T., Liu, B., Li, M., Xie, X., and Zhang, H. 2012. Diuretic, anti-inflammatory, and analgesic activities of the ethanol extract from *Cynoglossum lanceolatum*. *Journal of Ethnopharmacology*, 139 (1): 149-154.