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Anti-nociceptive and Anti-inflammatory Effects of *Gmelina arborea* Roxb. e x. Sm.

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Abstract

Background: The aim of the present study was to evaluate the anti-inflammatory and anti-nociceptive activities of the methanolic extract of *Gmelina arborea* fruit (GAF) and stem bark (GASB) on egg-albumin induced inflammation and heated plate metal respectively.

Methods: 500 g of each of the pulverised samples was extracted with 90% methanol using soxhlet extractor to produce dense mass of methanolic extracts after distillation and kept in a refrigerator at 4^{o}C until further analysis. The anti-inflammatory activity was determined on fresh egg albumins over 4 h by measurement of rat paw oedema according to established procedure. The anti-nociceptive was evaluated by placing each rat on the heated metal plate (Hot plate) maintained at the temperature of about 50-55^{0}C within the restraining plastic cylinder while animals responses were taken over 90 min.

Results: Methanolic extracts of *G. arborea* yielded blackish thick mass and greenish paste viscous extract for the GASB and GAF respectively with percentage yields of 28% and 30% respectively. The yield revealed more extractives in the GAF than GASB. The anti-nociceptive properties of both GAF and GASB shows a relatively time dependent activity as rate of inhibition was high at the 30th min (at the peak of p<0.001) for all doses except for 100 mg/kg per oral for the GAF. At increased time, the activity of most of the extracts reduced considerably. The egg-albumin induced inflammation was inhibited by the extracts as well in different doses. There were considerable inhibition for the GAF as well as the GASB at a minimum significant value of p<0.05 from the 1st h to the 4th h with maximum percentage inhibition (PI) values ranging between 30% to a peak of 70% when compared to the control. The methanolic extract of *G. arborea* fruit and stem bark showed significant (p<0.05) anti-inflammatory activity.

Conclusions: This study has shown that GASB and GAF displayed significant anti-inflammatory effect on paw oedema induced by egg-albumin by inhibiting the release of mediators for the entire 4 h experimental period. The anti-nociceptive actions were also significant when compared with the control.

Keywords: *Gmelina arborea; methanol extract;* anti-inflammatory activity; anti-nociceptive action.

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Background

The plant, *Gmelina arborea* Roxb. (family (Verbenaceae) is a deciduous large sized bush or shrub, commonly growing to about 4m to 8m tall and much branched. The tree form is fair to good, with 6-9 m of branchless, often crooked trunk and a large, low-branched crown. The colour of bark is gray and bark is thin. The plant leaves structure is simple, opposite, less heart-heart shaped, length is 10-25 cm and width 5-18 cm. Flowers are brown in colour and arranged in penciled cymes 15-30 cm and it can appears after leaf- fall. Fruit size is drup 2.25 cm long and contains 1-4 seeds. Literature information has shown extracts of *G. arborea* exhibited anti-inflammation [1], antihypertensive effect [2], anti-oxidative stress [3], antibacterial, antioxidant [4,5] and antidiabetic [5,6] activities. In addition, the immunostimulant potential of extract of *G. arborea* has been reported [7]. The anti-inflammatory effect of methanol extract of *G. arborea* and its fractions can be attributed to the presence of the flavonoid [8] present therein. Moreover, the insecticidal activity of *G. arborea* extracts against *Megalurothrips sjostedti* [9] and *Callosobruchus chinensis* and *Sitophilus oryzae* [10], would healing effects [11], anti-ulcer potentials [12] and antiangiogenic activity [13] have been reported. It was also reported that an ethanolic leaf extract of *G. arborea* has potent *in vitro* cytotoxic activity against colon cancer (COLO 201), gastric cancer (HT-29) and human oesophagel cancer (TE-2) cell lines [14]. Information on the analgesic [15,16], antipyretic [16] activities of *G. arborea* are also available in the literature. Several compounds were isolated and characterized from *G. arborea*. These included hentriacontanol, ceryl alcohol, β-sitosterol, n-octacosanol and gmelinol from the roots [17], ethyl α-D-glucopyranoside, hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-(R*,R*-(E)], 9,12,15-octadecatrienoic acid and pentadecanoic acid from the leaves [18]. The aerial parts of *G. arborea* consisted of 6-*O*-*α*-L-(2″,3″-di-*O*-acetyl-4″-*O*-*cis*-cinnamoyl)rhamnopyranosylcatalpol and 6-*O*-α-l-(2″,4″-di-*O*-*trans*-cinnamoyl)rhamnopyranosylcatalpol [19].

The heartwood of *G. arborea* afforded 6-*O*-(3″-*O*-benzoyl)-α-l-rhamnopyranosylcatalpol, 6-*O*-(3″-*O*-*trans*-cinnamoyl)-α-l-rhamnopyranosylcatalpol, 6-*O*-(3″-*O*-*cis*-cinnamoyl)-α-l-rhamnopyranosylcatalpol and 6-*O*-(3″,4″-*O*-dibenzoyl)-α-l-rhamnopyranosylcatalpol [20]. Umbelliferone 7-apiosylglucoside has been characterised from the root of *G. arborea* [21]. The lignans isolated from have been determined. They are 6“-bromo-isoarboreol, 4-hydroxysesamin, 4,8-dihydroxysesamin, 1,4-dihydroxysesamin (gummadiol), 2-piperonyl-3-hydroxymethyl-4-(α-hydroxy-3,4-methylenedioxybenzyl)-4-hydroxytetrahydrofuran and 4-O-glucoside of 4–epigummadiol [22]. The other compounds from the heartwood *G. arborea* were (+)-7′-O-ethyl arboreol, (+)-paulownin, (+)-gmelinol, (+)-epieudesmin and (−)-β-sitosterol, show antifungal activity against *Trametes versicolor* [23]. An alkaloid, premnazole, was reported to be responsible for the anti-inflammatory activity of the leaves of *Gmelina arborea* [24].The major chemical compositions of the fruit essential oil of *Gmelina arborea* [25] were identified as (Z)-3-hexenol (17.9%), nonanal (8.7%), 1-octen-3-ol (8.4%) and hexanol (6.1%). The aim of the present paper was to report the results of our investigation into the anti-nociceptive and anti-inflammatory activities of methanol extracts of the stem bark and fruits of *G. arborea* grown in Nigeria, in continuation of our research on the biological activities of Nigerian medicinal plants and herbs [26].

Methods

Chemicals

Chemicals and reagents: Unless otherwise stated, all chemicals and reagents were obtained from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). All the chemicals used including the solvents, were of analytical grade. Diclofenac injection and Acetylsalicyclic salicylate injection (RX, Nigeria Ltd) were purchased from Lagos State University Pharmacy manufactured by May and Baker.

Animals

Eight weeks Wistar rats of average weight of 150 to 200 g of either sex were bought and kept in the animal house of the Department of Biochemistry, Lagos State University, Nigeria. Standard conditions of temperature (23 ± 2^{o}C), light accessibility (12 h light and darkness cycle) with free access to standard pellet feed, tidy environment and water ad libitum. All experimental procedures were approved under the Lagos State University Research Ethical Clearance Committee (RECC) of the University (Approval no: 012/2016/LASU/BCH).

Preparation of plant materials

The stem bark and fruit of *G. arborea* were collected from allocation in Ore, Ondo State, Nigeria, in January 2017. Botanical identification was carried out

by Mr. Ademoriyo of the herbarium, Botany Department, Obafemi Awolowo University, Nigeria. A voucher specimen, OAU-23, was deposited at the herbarium. The stem bark and fruit of *G. arborea* were properly separated from the stock and kept in a black polythene bag. The samples were air-dried for 15 days and pulverised with a laboratory blender.

Extraction of crude extracts

500 g of each of the pulverised samples was extracted with 90% methanol using soxhlet extractor to produce a dense mass of methanolic extracts after distillation and kept in a refrigerator at 4 ^{o}C until further analysis.

Anti-inflammatory test: Egg-albumin induced rat paw oedema

A modified form of the method for the measurement of rat paw oedema was used [26]. Wistar rats were assigned to one of 5 groups consisting of 5 animals each as follows: group 1-control (treated with 1 mL NaCl (saline) solution), group 2- standard (treated with Diclofenac Sodium injection 100 mg/kg, orally), while the tested groups (3, 4, 5) were treated with 1 mL of 100 mg, 200 mg and 400 mg of methanolic extracts dissolve in 1% DMSO respectively. Diclofenac drug is a potent non-steroidal anti-inflammatory drug (NSAID's) and inhibits COX and other inflammatory mediators.

Preceding analysis, animals were starved overnight to allow for proper sample absorption into the blood stream through the stomach cavity and to empty part of gastrointestinal tract [27]. Thirty minutes after drug administration, 1.0 mL of 50% (v/v) of fresh egg albumin was injected subcutaneously into the subplantar surface of the right hind paw. Rat paw oedema was assessed by changes in paw sizes, method measured with a vernier caliper before and after egg-albumin injection at 30 min, 1, 2, 3, and 4 h. The changes in paw sizes were then evaluated. From the mean oedema volume, the percent inhibition was calculated by using following formula earlier described [26];

$$\% \text{ Inhibition of oedema} = 100 * (Vc-Vt/Vc)$$

Where, Vc =Mean paw oedema volume of control group
Vt =Mean paw oedema volume of treated group

Hot Plate test for anti-nociceptive

The experiment was carried out using the method earlier described [28]. Twenty-five (25) mature Wistar rats of both sexes were randomly divided into 5 groups of 5 rats per group. The grouping was used as above. The animals were fasted for 12 h with provision of clean water *ad libitum*. Each rat was placed upon the heated metal plate (Hot plate) maintained at the temperature of about 50-55^{0} C within the restraining plastic cylinder. Group 1 rat received 10 mL of distilled water and served as control. Group 2 rat received acetylsalicylic acid 400 mg/kg (ASA) (standard control) and groups 3, 4 and 5 received 100, 200 and 400 mg/kg of *G. arborea* extract respectively per 0s. Acetylsalicylic acid, though an NSAID's possesses both anti-nociceptive and anti-inflammatory property: It has been shown that aspirin-like drugs reduce the enhanced nociceptor activity in damaged tissue, probably as a result of prostaglandin synthesis inhibition and thus are perfect candidate for nociceptive activities.

Animal response to the heat varies and such changes includes; kicking of hind foot and jumping about, licking of foot, raising the foot, holding the foot tightly to its body or shaking of the foot. Readings were taken 30, 60 and 90 min after administration.

Statistical analysis

Repeated Measures One way ANOVA Analysis using Tukey's multiple comparisons Test was performed using GraphPad Prism (version 7.02), San Diego CA, USA, www.graphPad.com) to compare activity between treatment group, control and the standard. The P value was significant for P > 0.05 and above values. The Tukey confidence limits allows for multiple comparison of sets of data. Results were expressed as mean ± standard error of the mean [26].

Results

G. arborea methanolic extract yielded a blackish thick mass and greenish paste viscous extract for the stem bark (GASB) and fruit (GAF) respectively. Percentage yields of the extracts were 28% and 30% respectively. The polar solvent was used to its ability to absorb phenolic compounds which had been shown to be prevalent in the *G. arborea* [20,21]. The yield revealed more extractives in the GAF than GASB.

Anti-nociceptive activity

The result of the anti-nociceptive effects of GAF in single doses of 100, 200 and 400 mg/kg body weight, p.o., showed an increase in the hot plate reaction time. As shown in Figure 1, there was a significant increase in activity as expected for the standard (acetylsalicylate) through the experiment duration. The activity of the 100 mg/kg p.o. reduces considerably

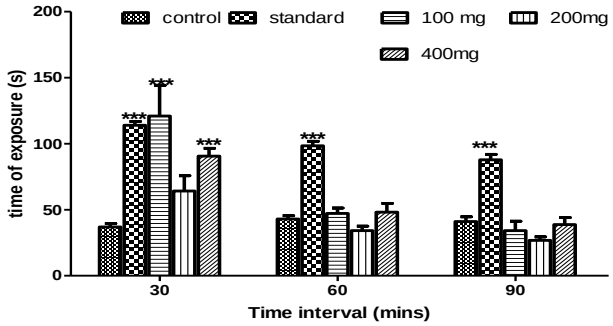

Figure 1. Effect of GAF on heat induced pain. Control, Standard, and GAF represent 1 mL saline solution, 100 mg/kg of Diclofenac injection and 1 mL of 100, 200 and 400 mg of GAF respectively. **p*<0.05, ***p*<0.01, *** *p*<0.001 statistically compared to control from a high activity significance (*p*<0.001) at the 30th min to a non-significant level as the reaction time increases (i.e from *p*<0.001 to *p*>0.05). The same pattern of activity was observed for the 400 mg/kg dose. The animal response to heat reduced as the reaction time reduced but were non-significant statistically for the doses at the 60th to the 90th min when compared to the control.

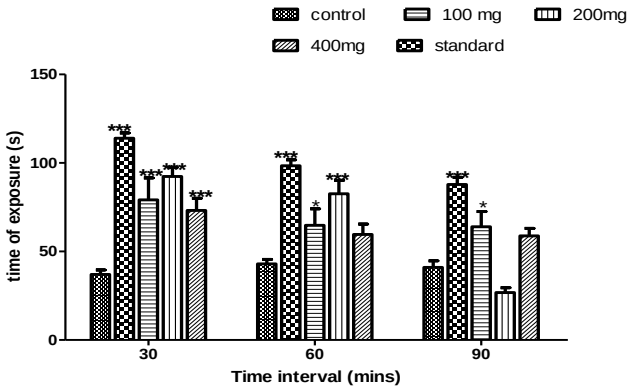

Figure 2. Effect of GASB on heat induced pain. Control, Standard, and GASB represent 1 mL saline solution, 100 mg/kg of Diclofenac injection and 100, 200 and 400 mg of GASB respectively. **p*<0.05, ***p*<0.01, *** *p*<0.001 statistically compared to control

Figure 2 shows the anti-nociceptive properties of the GASB using the hot plate analysis. The extract at 100 mg dose levels shows an increased level of pain response time (PRT) at the 30th min at a confidence level of *p*<0.001. However the activity reduced as observation time increases. The high absorption of the phytochemicals could have resulted in this early inhibition. Rate of extracts absorption and types of phytochemicals present affects the activity of plant extracts. In the study, the assumption was made using rate of absorption as probable mechanism of inhibition. The anti-nociceptive effects of the extract at 200 mg/kg p.o. were highly significant in the 30th and the 60th min but showed a sharp reduction to *p*>0.05 at the 90th min. Dose dependent activity of the extracts at 400 mg/kg p.o. however showed a reduction of activity at the 60th and 90th min (*p*<0.001 to *p*>0.05) which could be attributed to the low level of absorption and activity of the extracts (The effect was only significant at the 30th min).

Anti-inflammatory activity

The anti-inflammatory activities show a relative level of inhibition by both the GAF and GASB extracts. The egg-albumin mediated phlogistic anti-inflammatory activities were dose dependent and activities of GAF extracts (Figure 3) were constant at *p*<0.05 up to the 2nd h (30-40%). However, at the 3rd and 4th h, the 200 and 400 mg/kg shows a higher inhibition at *p*<0.01 at 60% and 70% respectively, while the activity of the 100 mg/kg became insignificant (*p*>0.05). Such characteristics behaviour can be predicted on the rate of absorption and activity of the plant extracts. On the other hand, GASB extracts (Figure 4) showed a constant inhibition (*p*<0.05) throughout the experiment duration irrespective of the dose concentration.

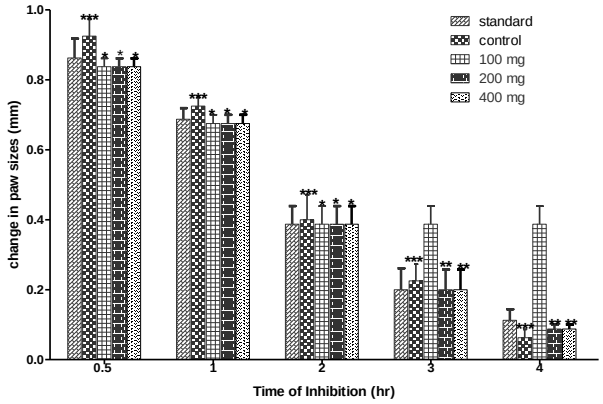

Figure 3. Effect of GAF on egg-albumin induced inflammation. Control, Standard, 100 mg/kg of Diclofenac injection and 100, 200 and 400 mg/kg p.o. of GAF respectively. **p*<0.05, ***p*<0.01,*** *p*<0.001 statistically compared to control.

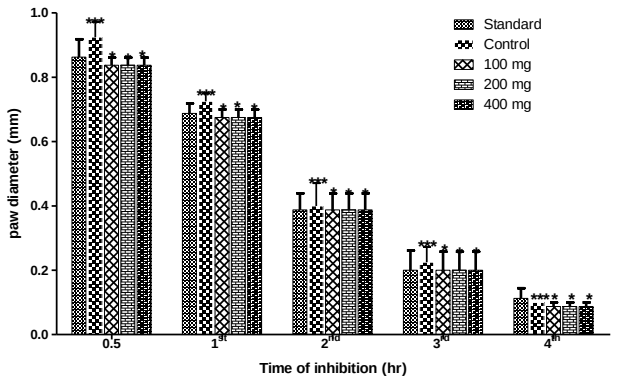

Figure 4. Effect of GASB on egg-albumin induced inflammation. Control, Standard, 100 mg/kg of Diclofenac injection and 100, 200 and 400 mg of GASB respectively. **p*<0.05, ***p*<0.01, *** *p*<0.001 statistically compared to control.

The anti-inflammatory activities show a relative level of inhibition by both the GAF and GASB extracts. The egg-albumin mediated phlogistic anti-inflammatory activities of the doses were significant in inflammation inhibition. The GASB considerably reduced inflammation through-out the time of analysis to about 75% to 80% for all the doses. The GAF exhibited same activity except for the 100 mg p.o whose activity within the hours of analysis remains constant for the 2nd to the 4th h. The reduction in the size of the rat hind paw volume also reveals a considerable activity which is time dependent.

Discussion

Pain is known to be a response to an external or internal stimuli which could be caused by thermal, mechanical or chemical influence [29]. In the hot plate model, increase in the pain reaction time (latency period) indicates the level of analgesia induced by the drug or extract [30]. The hot plate test is valuable as it examines central analgesic activity due to sensitivity to strong analgesics and partial tissue damage [1]. *G. arborea* methanolic extracts produced a dose dependent and significant (*p*<0.001) increase in pain threshold in the rats mice in this models that was comparable to the reference drug ASA. In these model, increase in stress tolerance capacity of the animals indicates the possible involvement of a centrally mediated opiods [31]. The ability of the extracts to increase latency is due to its effect on the peripheral or central nervous system and they acts by raising the threshold for pain and as well by altering physiological response to pain [32]. The inactivity of the 100 mg and 200 mg observed in both extracts could be attributed to their uncharacteristic inactivity at the supraspinal level which is suggested to be common for the paw licking [32]. Earlier study has shown that *G. arborea* bark ethyl acetate and methanol extracts at a dose of 500 mg/kg body displayed anti-nociceptive activity from which also agrees with our present study at the dose of 400 mg/kg body weight [1]. This study suggests that the analgesic activity of GAF and GASB at 100, 200 and 400 mg may be involved in the the peripheral pain mechanism or may be through inhibition of prostaglandin activities or synthesis.

The anti-inflammation activity is a study of intensed pain reaction as compared to the peripheral stimuli in nociceptive activities. In this model, oedema is induced with an egg albumin which is rich in protein but gradualy agglutinates as soon as its embedded within the right hind paw of a wistar rat. Oedema development is a three-phase event [26]; release of histamine and serotonin in the initial phase (0–2 h), cytokines in the second phase (3 h) and prostaglandin in the third phase (4 h). The anti-inflammatory effects of *G. arborea* show high potency from the 1st to 4th h. Figures 3 and 4 reveals such activities and could suggests that the GASB extract had a more inhihibiting properties than the GAF extract and thus hinder inflammation considerably at every hour of analysis. Inflammation mediators such as histammines, prostagladins. Nf-KB,COX-1 and leukotrienes and others can thus be hindered by the methanolic extracts.

Earlier study from India have shown that *G. arborea* bark extract and its fractions at different dose of body weight shows anti-inflammatory activity [1,8,24] which has attributed to the presence of the flavonoid [8]. These results also agree with the present study from Nigeria on the extracts from the fruits and stem bark. It may be postulated the anti-nociceptive and anti-inflammatory activities of *G. arborea* may be universal. This shows *G. arborea* extracts contains potential anti-nociceptive and anti-inflammatory agent. In addition, these phytochemicals had also been reported to be present in the leaves and stem bark of *G. arborrea* from Nigeria and could be attributed to the pharmacological effect observed in this study [26, 33].

Conclusions

The study evaluates both the analgesic and anti-inflammatory properties of the methanolic extracts of the fruits and stem bark of *G. arborea*. The activity shows that the extract is both a CNS stimulant and the other way applicable as a non-steroidal anti-inflammatory drug (NSAID) due to its ability to hinder at both phases (anti-nociceptive and anti-inflammatory). The study confirms the traditional use

of the plant and the best plant parts for each activity. Further research on the isolation of the active ingredients and the molecular study of pain inhibition is ongoing.

Authors' Contribution

Both authors ONA and IAO designed the study, performed the statistical analysis, wrote the protocol as well as the first and final draft of the manuscript. Author IOO carried out the research. Authors ONA and IAO supervised IOO in the extraction process and in the anti-inflammatory/anti-nociceptive studies. Author OAL managed the literature searches. All authors read and approved the final manuscript.

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Conflict of interest

Authors declared that there are no competing interests.

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References

1. Kulkarni YA, Ritesh P, Vishvas P, Aditi T, Alok G. 2013. Effect of *Gmelina arborea* Roxb in experimentally induced inflammation and nociception. *J Ayurveda Integr Med*. 4(3): 152-157.
2. Awede B, Osseni R, Lagnika L, Adjagba M, Kpadonou C, Darboux RB. Laleye A. 2015. Antihypertensive effects of *Gmelina arborea* Roxb (Verbenaceae) leaves crude aqueous extract fractions in Wistar rats. *J Phys Pharm Adv*. 5(12): 803-810.
3. Sinha S, Priyanjali D, Sujata B, Devasagayam, TPA, Saroj G. 2006. Bark and fruit extracts of *Gmelina arborea* protect liver cells from oxidative stress. *Pharm Biol*. 44(4): 237-243.
4. Audipudi AV, Chakicherla BVS. 2010. Antioxidative and antimicrobial activity of methanol and chloroform extracts of *Gmelina arborea* Roxb. *Int J Biotechnol Biochem*. 6(1): 139-144.
5. Nayak BS, Ellaiah P, Dinda SC. 2012. Antibacterial, antioxidant and antidiabetic activities of *Gmelina arborea* Roxb fruit extracts. *Int J Green Pharm*. 6(3): 224-230.
6. Kulkarni YA, Addepalli V. 2013. Effects of *Gmelina arborea* extract on experimentally induced diabetes. *Asian Pac J Trop Med*. 6(8): 602-608.
7. Shukla SH, Saluja AK, Pandya SS. 2010. Modulating effect of *Gmelina arborea* Linn. on immunosuppressed albino rats. *Pharmacog Res*. 2(6): 359-36.3
8. Kaur S, Preet M, Singh B, Navreet K. 2017. Anti-inflammatory effect of methanolic extract of *Gmelina arborea* bark and its fractions against carrageenan induced paw oedema in rats. *Nat Prod Res*. (in press).
9. Oparaeke AM. 2006. Studies on insecticidal potential of extracts of *Gmelina arborea* L. products for insect pests control on cowpea. 1. The legume flower bud thrips, *Megalurothrips sjostedti* Trybom. *Arch Phytopathol Plant Prot*. 39(3): 209-214.
10. Choudhury PP. 2012. Toxicity of heart wood extract of *Gmelina arborea* against stored grain pests. *Agric Sci Res J*. 2(3): 131-133.
11. Bhagyalakshmi CP, Deepthi V, Saju G, Sameer K, Suresh NN, Ajithkumar KG, Juliet SB, Ravindran R. 2017. Wound healing and anti-inflammatory activity of methanolic extract of *Gmelina arborea* and *Hemigraphis colorata* in rats. *Int J Curr Microbiol App Sci*. 6(8): 3116-3122.
12. Lawrence L, Seema M, Sheka V, Sivaram VP, Jose P. 2016. Radical scavenging and gastroprotective activity of methanolic extract of *Gmelina arborea* stem bark. *J Ayurveda Int Med*. 7(2): 78-82.
13. Fajardo WT, Cancino LT, Dudang EB, Suratos SCM, Macayana FB. 2015. Phytochemical analysis and antiangiogenic potential of *Gmelina arborea* Roxb. (Paper Tree) fruit exocarp using duck chorioallantoic membrane (Cam) assay. *Asia Pac J Multidiscip Res*. 3(5): 72-79.
14. Punitha D, Angamuthu T, Karuppanan A, Sreenivasapuram NS. 2012. Potent *in vitro* cytotoxic effect of *Gmelina arborea* Roxb. (Verbenaceae) on three human cancer cell lines. *Int J Pharma Sci Res*. 3(4): 357-363.
15. Gangwar A, Ghosh AK, Hoque M, Saxena V. 2013. Analgesic activity of *Gmelina arborea* Roxb in colony bred Swiss mice and Wister rats. *Int J Pharmacog Phytochem Res*. 5(1): 66-67
16. Parhi PK, Pattanayak P, Mishra P, Pani MK. 2011. An *in-vivo* study on analgesic and antipyretic activity of bark extracts of *Gmelina arborea*. *Int J Pharm Sci Rev Res*. 10(2): 78-81.
17. Joshi KC, Singh P, Pardasani RT. 1977. Chemical components of the roots of *Tectona grandis* and *Gmelina arborea*. *Planta Med*. 32(5): 71-75
18. Hosny M, Rosazza JPN. 1998. Gmelinosides A-L, twelve acylated iridoid glycosides from *Gmelina arborea. J Nat Prod*. 61(6): 734-742.
19. Tiwari N, Yadav AK, Srivastava P, Shanker K, Verma RK, Gupta MM. 2008. Iridoid glycosides from *Gmelina arborea.* Phytochem. 69(12): 2387-2390.
20. Satyanarayana P, Subrahmanyam P, Kasai R, Tanaka O. 1985. An apiose-containing coumarin glycoside from *Gmelina arborea* root. *Phytochem*. 24(8): 1862-1863.
21. Anjaneyulu ASR, Madhusudhanarao A, Kameswara V, Rao L, Ramachandra R, Andrew P. Robert SW. 1977. Novel hydroxy lignans from the heartwood of *Gmelina arborea*. *Tetrahed*. 33(1): 133-143.
22. Anjaneyulu ASR, Rao KJ, Rao VK, Row LR, Subrahmanyam C, Pelter A, Ward RS. 1975. The structures of from *Gmelina arborea* Linn. *Tetrahed*. 31(10): 1277-1285.
23. Kawamura F, Ohara S, Nishida A. 2005. Antifungal activity of constituents from the heartwood of *Gmelina arborea*: Part 1. Sensitive antifungal assay against *Basidiomycetes*. *Holzforsch*. 58(2): 189-192.
24. Barik BR, Bhowmik AK, Dey AK, Patra A, Chatterjee A, Joy S, Susan T, Alam M, Kundu AB. 1992. Premnazole, an isoxazole alkaloid of *Premna integrifolia* and *Gmelina arborea* with antiinflammatory activity. *Fitoterapia*. 63(4): 295-299.
25. Moronkola DO, Ogunwande IA, Başer KHC, Ozek T, Ozek G. 2009. Essential oil composition of *Gmelina arborea* Roxb., Verbenaceae, from Nigeria. *J Essent Oil Res*. 21(3): 264-266.
26. Avoseh NO, Ogunbajo LO, Ogunwande IA, Ogundajo LA, Lawal OA. 2018. Anti-inflammatory activity of hexane and ethyl acetate extracts of *Hura crepitans* L. *European Journal of Medicinal Plants.* 24(1): 1-6
27. Nebendahl K. 2000. In *The Laboratory Rat*. pp. 463-483.
28. Shethy SN, Anika SM. 1982. *Laboratory manual of Pharmacology and Toxicology* (1st ed). Fourth Dimension Pub: Enugu, Nigeria. pp. 78-81.
29. Rang HP, Dale MM, Ritter JM, Moore JS. 2003. *Pharmacology* (5th ed). Churchill Livingstone Pub: New York. pp. 201-204.

30. Ramadran K, Basinath L. 1986. A critical analysis of the experimental evaluation of nociceptive reactions in animals. *Pharm Res*. 4(5): 263-270.
31. Vogel H. 2007. *Drug discovery and evaluation*: Pharmacological assays. Springer. pp. 234-275.
32. Fan S, Ali NA, Basri DF. 2014. Evaluation of analgesic activity of the methanol extract from the galls of *Quercus infectoria* (Olivier) in rats. *Evidence-Based Complem Alter Med.* 1-6 http://dx.doi.org/10.1155/2014/976764.
33. Ogbonnaya EA , Awah FM, Mounmbegna PE 2012 Phytochemical screening, and assessment of ameliorating effect of aqueous and ethanolic extracts of *Gmelina arborea* on drug induced hepatic and renal insufficiency in rats. *Pak. J. Pharm. Sci.,* 25(2): 457-461.