MEDWIN PUBLISHERS
Committed to Create Value for researchers

Journal of Natural & Ayurvedic Medicine
ISSN: 2578-4986

# Phytochemistry and Antimicrobial Properties of *Gmelina arborea* (Verberaceae) Ethanolic Leaf Extract and its Secondary Metabolites

**Ijezie MKL, Ezirim S, Azih I, Ogukwe CE, and Akalezi CO***

Department of Chemistry, Federal University of Technology, Owerri, Nigeria

***Corresponding author:** Christogonus Akalezi, Department of Chemistry, Federal University of Technology, Owerri, Nigeria, Email: chrisakalezi@yahoo.com

**Research Article**

**Volume 4 Issue 4**

**Received Date:** June 06, 2020

**Published Date:** December 04, 2020

DOI: 10.23880/jonam-16000283

## Abstract

The aim of this study was to determine the antimicrobial potential of *Gmelina arborea* ethanolic leaf extract which is used in traditional medicine for treating different ailments such as hallucinations, piles, abdominal pains, burning sensation, diabetes and fever. It is also intended to study its acidic, basic and neutral metabolites with a view to identify the phytochemical compounds responsible for the observed antimicrobial properties. Phytochemical screening was carried out on the air-dried ethanolic leaf extract and its secondary metabolites in the Research Laboratory of Phytochemistry/ Chromatography, N4 Alaenyi Street, Owerri, Imo State, Nigeria. The antimicrobial study was carried out using the Agar disc diffusion method and determination of Minimal Inhibitory Concentration (MIC) in the department of Microbiology, Federal Medical Centre, Owerri, Imo State, Nigeria. The ethanol leaf extract and its secondary metabolites (acidic, basic and neutral) were prepared and assayed for antimicrobial activities. The test microorganisms used were Streptococcus spp, Staphylococcus aureus, Salmonella spp, Escherichia coli and Coliform bacilli. Inhibition zone diameter was used as a measure of the antimicrobial activity. The results of the antimicrobial screening of the crude extract and metabolites showed that the crude extract, acidic and neutral metabolites possessed antimicrobial activity to various extents against the microorganisms tested whereas the basic metabolite showed no activity at all. The results showed that the crude extract exhibited its greatest activity against Streptococcus spp, Staphylococcus aureus and Escherichia coli with inhibition diameter of 20 mm at 1.0 mg/ml and MIC of 0.5 mg/ml. The least activity was against Salmonella spp with inhibition zone diameter of 15 mm and MIC of 0.5 mg/ml. The acidic metabolite exhibited its greatest activity against Escherichia coli with inhibition zone diameter of 35 mm at 1.0 mg/ml and MIC of 0.25 mg/ml. The least activity was against Staphylococcus aureus and Salmonella spp with inhibition zone diameter of 25 mm at 1.0 mg/ml and MIC of 0.5 mg/ml respectively. The neutral metabolite exhibited its greatest activity against Salmonella spp and Coliform bacilli with inhibition zone diameter of 30 mm at 1.0 mg/ml and MIC of 0.5 mg/ml. The least activity was against Staphylococcus aureus with inhibition zone diameter of 22 mm at 1.0 mg/ml and MIC of 0.5 mg/ml. The phytochemical screening results showed the presence of alkaloids, flavonoids, tannins, saponins, cyanogenic glycosides, steroids, carbonyl compounds and carbohydrates. Saponins, carbonyl compounds and carbohydrates were shown to be much higher in concentration than other phytochemicals. The results obtained in this study confirm the antimicrobial properties of *Gmelina arborea* leaf extract and suggest that this property resides in the acidic and neutral metabolites since the basic metabolite showed no activity at all. The study supports the use of this plant in folk medicine for treatment of ailments such as stomach disorders, healing of wounds, burning sensation and fever.

**Keywords:** Phytochemistry; Antimicrobial potential; *Gmelina arborea*; Secondary metabolites

## Introduction

*Gmelina arborea* (Verbenaceae) is a well-known medicinal plant in Africa and in the Ayurveda, an ancient Indian system of medicine. The roots, leaves, flowers, fruits and bark are used for treating different ailments in traditional medicine. The plant has been suggested for use in the treatment of scorpion sting, snake-bites, [1] and diabetes [2]. The plant is anthelmintic and used for treating hallucinations, excess thirst, piles, abdominal pains, burning sensations and fever [3]. There are reports on phytoconstituents present in different parts of the plant. The isolation of luteolin, indole and lignans from the leaves has been reported to be arboreol, isoarboreol, methyl arboreal, arborone, gmelanone, gummediol, and 7-oxodihydrogmelinol have been isolated from the heartwood of the plant [4-7]. Few coumarin glycosides and iridoid glycosides have been reported in roots and leaves, respectively [8,9]. There are iridoid glycosides 6-o-(3”-o-benzoyl)-cc-l-rhamnopyranosylcatalpol,6-o-(3”-o-trans-cinnanoyl-cc-l-rhamnopyranosylcatalpol were isolated from aerial parts of G. arborea and structures were elucidated by spectral analysis [10]. The bark of the plant indicated presence of tyrosol [2-(4-hydroxyphenyl) ethanol];(+)-balanophonin, an 8-50 neolignan, gmelinol, phenylethanoid glycoside {(o)-p-hydroxyphenylethyl [5”o-o-(3,4 dimethoxy-cinnamoyl)-b-D-apiofuranosyl (1”’ 6’)]-b-D-glucopyranoside}, 2,6-dimethoxy-p-benzoquinone and 3,4,5-trimethoxyphenol[11]. Crude extract of the plant are reported to possess wound-healing properties, antidiarrheal activity, antioxidant activity, antidiabetic activity and antiulcer activity [12-16].

Toxicity studies of aqueous extracts and Methanol extracts have been reported to be minimal [17]. However, little information is available on the secondary metabolites of ethanolic leaf extract of G. arborea. Therefore, the present study was designed to determine the antimicrobial potential of the G. arborea ethanolic leaf extract and its secondary metabolites, which is used in traditional medicine with a view to identify the phytochemical compounds responsible for the observed antimicrobial properties.

## Materials and Methods

### Preparation of *Gmelina arborea* Leaf Extract

Seventy (70) g of sun dried leaves of *G. arborea* were ground and put in a soxhlet extractor fitted with a reflux condenser and extracted with 250 ml of ethanol for 6 hours. The ethanol extract was allowed to evaporate completely at room temperature to give a gel, which was dissolved in ethanol/water mixture (4:1) and filtered. The filtrate was used without further purification for the phytochemical screening, antimicrobial experiments and preparation of acidic, basic and neutral metabolites.

### Preparation of Basic Metabolite

This metabolite was prepared as follows; the filtrate of *G. arborea* leaf extract was treated with dilute HCl and extracted with chloroform in a separatory funnel. The lower chloroform layer was removed and reserved for the preparation of neutral metabolite. The HCl layer was treated with $NaHCO_3$ until the mixture became basic. The precipitate from the resultant solution was filtered out and washed well with distilled water. The ppt was dissolved in 40 ml of 95% ethanol and filtered. The filtrate was used for antimicrobial experiments without further purification [18].

### Preparation of Neutral Metabolite

The chloroform layer obtained above was placed in a separatory funnel and treated with dilute NaOH solution. After equilibrating, the aqueous NaOH layer was removed and reserved for preparation of acidic metabolite. The chloroform layer was removed and allowed to evaporate completely at remove temperature to produce a gel, which was dissolved in 40 ml of chloroform and filtered. The filtrate was used without further purification for antimicrobial experiments [18].

### Preparation of Acidic Metabolite

The aqueous alkaline layer obtained above was treated with dilute HCl until the solution became acidic. The precipitate of the mixture was filtered out and washed well with distilled water. The ppt was dissolved in 40 ml of 95% ethanol and filtered. The filtrate was used for antimicrobial experiments without further purification [18].

### Phytochemical Screening of Extract and Metabolites

Preliminary phytochemical screening of the extract and metabolites was performed using standard methods [19] which showed that the crude extract contained alkaloids, flavonoids, tannins, saponins, cyanogenic glycoside, steroids, carbonyl compounds and carbohydrates whereas the basic metabolite showed presence of alkaloids, cyanogenic glycosides and saponins. The acidic metabolite showed the presence of flavonoids, saponins and tannins while the neutral metabolite gave positive results for presence of steroids, esters, carbonyl compounds and carbohydrates.

### Antimicrobial Tests

The antimicrobial experiments were carried out in microbiology Department, Federal Medical Centre Owerri,

Imo State of Nigeria. Test microorganisms used were Streptococcus spp, Staphylococcus aureus, Salmonella spp, Escherichia coli and Coliform bacilli and the method used was Agar disc diffusion method. An inoculating loop was touched on five isolated colonies of the pathogen on an agar plate and used to inoculate a tube of culture broth, which was incubated at 35-37°Ç until it became slightly turbid and was diluted to march the turbidity standard. Then a sterile cotton swab was dipped into the standardized bacterial test suspension and used to evenly inoculate the entire surface of the agar plate. After 5minutes, the appropriate antibiotic test disks were placed with a multiple applicator device, and the agar plate was incubated at 35-37°c for 16-18 hours. The inhibition zone diameter (areas showing little or no microbial growth) were measured to the nearest mm [20] and taken as a measure of the antimicrobial activity.

### Determination of Minimum Inhibitory Concentration (MIC)

The determination of the minimum inhibitory concentration (MIC) was carried out to obtain an idea of the antibacterial activities of the plant extracts, since agar disc diffusion assay is a quantitative method based on the European Society of Clinical Microbiology and Infectious Diseases (2000) for evaluation of antimicrobial potentials. Standard solutions of the metabolites were prepared: 1.0 mg/ml, 0.5 mg/ml, 0.25 mg/ml, 0.125 mg/ml and 0.0625 mg/ml, in agar nutrient and distributed into sterile test tubes. 1ml of the extract or metabolite dilution was separately added into the agar plate for the bacteria and poured into Petri-dishes. The test microorganism was spotted onto the surface of the solidified extract-agar mixture and the plates were inoculated, starting from the lowest concentration to the highest concentration. After inoculation the plates were allowed to dry for 30 minutes and incubated at 37°C for 18 hours, after which the samples were examined for microbial growth. The lowest concentration of the extract or metabolite which showed little or no visible growth of the microorganism was taken as the MIC [20].

## Results and Discussion

### Phytochemical Screening of Extract and Metabolites

Results of phytochemical screening of the extract and various metabolites are presented (Table 1).

| Phytochemical | Crude extract | Acidic metabolite | Basic metabolite | Neutral metabolite |
|---|---|---|---|---|
| Tannins | ++ | ++ | - | - |
| Saponins | +++ | ++ | +++ | - |
| Flavonoids | +++ | +++ | - | - |
| Alkaloids | ++ | - | ++ | - |
| Steroids & triterpenoids | ++ | - | - | ++ |
| Cyanogenic glycosides | ++ | - | ++ | - |
| Aldehydes/ ketones/ | +++ | - | - | +++ |
| carbohydrates | +++ | - | - | +++ |
| Anthraquinones | + | - | - | + |
| Phenols | ++ | ++ | - | - |
| Carboxylic acids | + | + | - | - |
| Esters | + | - | - | + |

**Table 1:** Phytochemical Screening of Extract and Metabolites.
+++= Strongly Positive; ++=Positive; +=Fair; -=not detected

The crude extract showed presence of tannins, saponins, flavonoids, alkaloids, glycosides, Aldehydes, ketones, carbohydrates, Anthraquinones, phenols, carboxylic acids and esters. The acidic metabolite showed presence of tannins, saponins, flavonoids, phenols and carboxylic acids while the basic metabolite showed presence of saponins, alkaloids and glycosides. The neutral metabolite contained steroids/ terpenoids, Aldehydes, ketones, carbohydrate, Anthraquinones and esters.

These phytochemicals present are in accordance with previous work done by other researchers [21]. Luteolin (flavonoid) and gmelinoside (iridoid glycoside) have been found in the leaves of G.arborea which is responsible for its anti-inflammatory and anti-carcinogenic effect [9,22]. Flavonoids and tannins compounds have been attributed to

have an anti-oxidiant property which is why the plant is used as anti-cancer [23]. Quercetin (flavonoid), anthracene and tannins which are obtained from the *G.arborea* plant is used to treat hypertension [21]. These features can be attributed to the plant been used as a medicinal plant since the result obtained from these analysis contain these phytochemicals.

The presence of triterpenoids in natural products have been proved to possess anti-inflammatory and antimicrobial effect [24] also, medicinal plant containing saponins, glycosides, flavonoids, tannins and phenolics have demonstrated antidiabetic activities [25]. Anthraquinones in plant have been discovered to have therapeutic effects which include constipation, arthritis and multiple sclerosis [26] and can be trace to the application of *Gmelina arborea* as anti-inflammatory, antimicrobial, antidiabetic and therapeutic effect.

These phytochemicals contained in the metabolites are substances that control cell growth and division, reduce inflammation, promote formation of blood cells and fight infections [27]. We cannot say (for now) which of these bioactive compounds was responsible for the observed antimicrobial properties of the crude extract, acidic metabolite and/or neutral metabolite. However, five principal bioactive compounds such as alkaloids, saponins, steroids, flavonoids and glycosides were investigated in methanol and chloroform extracts of *Gmelina arborea* [10]. Thus, it may be speculated that the flavonoids, saponins, tannins, steroids/terpenoids and Anthraquinones which were found to dominate in the acidic and neutral metabolites may be responsible for the observed antimicrobial activity/ potential because they have been shown to possess bactericidal, fungicidal and medicinal properties [13].

## Preliminary Antimicrobial Screening

The results of preliminary antimicrobial screening of the crude extract and various metabolites of *Gmelina arborea* are shown (Table 2) from which it was observed that only the crude extract, acidic and neutral metabolites possessed antimicrobial activity against the microorganisms tested to various extents whereas the basic metabolite showed no activity against the microorganisms.

| Microorganism | Crude extract | Acidic Metabolite | Basic metabolite | Neutral Metabolite |
|---|---|---|---|---|
| Streptococcus spp | ++ | +++ | - | ++ |
| Staphylococcus aureus | ++ | ++ | - | ++ |
| Salmonella spp | ++ | ++ | - | ++ |
| Escherichia coli | ++ | +++ | - | ++ |
| Coliform bacilli | ++ | +++ | - | ++ |

**Table 2:** Preliminary Antimicrobial Screening of the Crude Extract and Various Metabolites.
+++=Strongly positive, ++=Positive, +=weakly positive, -=Very weak

## Antimicrobial Activities of Extract and Metabolites

The antimicrobial activities of the crude extract and metabolites were presented (Table 3) from which it was seen that the antimicrobial activities of the extract and metabolites against the organisms were close to each other and their inhibition zone diameters ranged from 15-20 mm (for crude extract), 25-35 mm (for acidic metabolite) and 22-30 mm (for neutral metabolite) at 1.0 mg/ml. The data showed that the crude extract exhibited its greatest activity against Streptococcus spp, *Staphylococcus aureus* and Escherichia coli with inhibition zone diameter of 20 mm at 1.0 mg/ml. The least activity was against Salmonella spp with inhibition zone diameter of 15 mm. The acidic metabolite exhibited its greatest activity against Escherichia coli with inhibition zone diameter of 35 mm at 1.0 mg/ml. The least activity was against *Staphylococcus aureus* and Salmonella spp with inhibition zone diameter of 25 mm at 1.0 mg/ml respectively. The neutral metabolite exhibited its greatest activity against Salmonella spp and Coliform bacilli with inhibition zone diameter of 30 mm at 1.0 mg/ml. the least activity was against *Staphylococcus aureus* with inhibition zone diameter of 22 mm at 1.0 mg/ml. the data presented showed that the acidic metabolite and the neutral metabolite exhibited almost the same activity against the test microorganisms especially against Coliform bacilli where they have the same inhibition zone diameter of 30 mm at 1.0 mg/ml respectively. It was also observed that the basic metabolite showed no activity against the microorganisms tested. These differences in bioactivity arose probably from differences in phytochemical composition of the various metabolites [18,28,29]. The result of the control experiment with the following drugs Augmentin, Ciprofloxacin, Gentamycin, Cefuroxine and Ceftriaxon against the test microorganisms were presented (Table 3b) showing their different inhibition zone diameters.

| Microorganism | Crude extract | Acidic metabolite | Basic metabolite | Neutral metabolite |
|---|---|---|---|---|
| Streptococcus spp | 20 mm | 30 mm | 0 mm | 25 mm |
| Staphylococcus aureus | 20 mm | 25 mm | 0 mm | 22 mm |
| Salmonella spp | 15 mm | 25 mm | 0 mm | 30 mm |
| Escherichia coli | 20 mm | 35 mm | 0 mm | 25 mm |
| Coliform bacilli | 18 mm | 30 mm | 0 mm | 30 mm |

**Table 3:** Antimicrobial Activities of Extract and Metabolites at 1.0 mg/ml.

| Microorganism | Augmentin | Ciprofloxacin | Gentamycin | Cefuroxine | Ceftriaxon |
|---|---|---|---|---|---|
| Streptococcus spp | 10 mm | 25 mm | 15 mm | 12 mm | 28 mm |
| Staphylococcus aureus | 12 mm | 20 mm | 14 mm | 15 mm | 22 mm |
| Salmonella spp | 16 mm | 27 mm | 23 mm | 10 mm | 20 mm |
| Escherichia coli | 0 mm | 28 mm | 25 mm | 14 mm | 18 mm |
| Coliform bacilli | 0 mm | 24 mm | 15 mm | 13 mm | 24 mm |

**Table 3b:** Control Drugs.

### Minimum Inhibitory Concentration of Extract and Metabolites

The MIC of the extract and metabolites were presented (Table 4) from which it was observed that the crude extract and the neutral metabolite exhibited MIC of 0.5 mg/ml respectively against the entire test microorganism. The acidic metabolite exhibited MIC of 0.5 mg/ml against *Staphylococcus aureus* and Salmonella spp while the MIC against Streptococcus spp, Escherichia coli and Coliform bacilli was 0.25 mg/ml respectively.

| Microorganism | Crude extract (Mg/ml) | Neutral metabolite (Mg/ml) | Acidic metabolite (Mg/ml) | Basic metabolite (Mg/ml) |
|---|---|---|---|---|
| Streptococcus spp | 0.5 | 0.5 | 0.25 | - |
| Staphylococcus aureus | 0.5 | 0.5 | 0.5 | - |
| Salmonella spp | 0.5 | 0.5l | 0.5 | - |
| Escherichia coli | 0.5 | 0.5 | 0.25 | - |
| Coliform bacilli | 0.5 | 0.5 | 0.25 | - |

**Table 4:** MIC of Extract and Metabolites.

Over the centuries, man made use of medicinal plants/herbs even though he was unable to find a rational explanation for their effect, it wasn't until the 19th century and the rapid development of organic chemistry and pharmacology, that man determine which active principle are responsible for a given therapeutic effect. The outcomes of this, is that there has been a marked renewal of medicinal interest in classic physiotherapy [30]. Man has also searched for remedies to problems of infections and diseases by the use of chemical substances that can eradicate or at least minimize the effects of the microorganisms without harming the host. Many antibiotics have also been used as preservatives for raw foods, especially protein foods like meat, fish, poultry, etc but microorganisms soon became resistant to these antibiotics and new strains of these organisms developed WHO Report. Hence, the use of medicinal plant extracts and metabolites became necessary [31].

The crude leaf and stem bark extracts of G. arborea have being reported to show significant antimicrobial activities against Gram positive and Gram negative organisms and the activity is due to presence of bioactive compounds such as alkaloids, saponins, carbohydrates, phenolics, tannins and anthraquinone in leaf while the stem back possessed saponins, carbohydrates, alkaloids, tannins and anthraquinone but no phenolics [32]. In vitro studies of both extract of leaf and stem bark shown significant activity against *E. coli, K. pnemoniae, P. dysentria* and *S. typhi*. It have been suggested that the presence of saponins, tannins, flavonoids, phenolics, alkaloids and anthraquinone in the leaf

extract of *G. arborea* are responsible for the antimicrobial action of the metabolites[33-36]. The crude extract had been reported to facilitate wound- healing due to the presence of tannins in the extract because tannins and tannic acids owe their astringent action to their ability to precipitate proteins and this forms a protective coating over the wound, prevents external irritation and promotes healing [12].

Hence, the observed antimicrobial activities of the crude extract, acidic metabolite and neutral metabolite against the tested microorganisms may be due to tannins or other phenolics, saponins, flavonoids, steroids and anthraquinone found in this study to be present in the metabolites or due to alkaloids present in the basic metabolite that was found in this study to lack antimicrobial activity against the tested microorganisms. Nevertheless, more work is to be done to isolate and characterize the active principle responsible for the observed antimicrobial potential of the ethanolic leaf extract.

## Conclusion

The results obtained in this study confirmed the antimicrobial activities/ properties of *Gmelina arborea* leaf extract and suggest that this property resides in the acidic metabolite and/or neutral metabolite since the basic metabolite showed no activity at all against the microorganisms tested. The study supports the use of *G. arborea* in folk medicine for treatment of disease conditions such as stomach disorders, healing of wounds and fever. Meanwhile, there is need for more research to ascertain the bioactive compound responsible for the observed antimicrobial activities.

## References

1. Nadkadkarni KM (2000) Indian Materia Medica. India: popular pratcashan, pp: 584-585.

2. Khan IA, Khanum A (2005) Herbal Therapy for Diabetes. India: Ukaaz Publications, pp: 34-35.

3. Kirtikar KR, Basu BD (1999) Indian Medicinal plants. India: India: International Book Distributors, pp: 1932-1933.

4. Rao DV, Rao EV, Viswanathan N (1967) Occurrence of Luteolin in the leaves of *Gmelina arborea* Linn. curr sci 3: 714.

5. Bhattacharjee AK, Das AK (1969) Phytochemical survey of few mysorc plants. Econ Bot.23: 274-276.

6. Joshi KC, Prakash L, Singh LB (1971) Extractives from heartwoods: Isolation of Hentriacontanol from *Gmelina arborea*. J Ind Chem Soc 48: 11756.

7. Anjaneyulu As, Jaganmohan RK, Kameswara RV, Ramachandra RL, Subrahmanyam C, et al. (1975). The structures of Lignans from *Gmelina arborea*. Tetrahedron 31(10): 1277-1285.

8. Satyanarayana P, Koteswara R, Ward RS, Pelter A (1986) Arborone and 7-oxodihydrogmelinol: Two new keto-lignans from *Gmelina arborea*. J Net prod 49(6): 1061-1014.

9. Hosny M, Rosazza JP (1998) Gmelinosides AL, twelve acylated iridoid glycosides from *Gmelina arborea*. J Nat prod 61(6): 734-742.

10. Tiwari N, Yadav AK, Srivastava P, Shankar K, Verma RK, et al. (2008) Iridoid glycosides from *Gmelina arborea*. Phytochemistry 69(12): 2387-2390.

11. Syamsul F, Takashi K, Toshisada S (2008) Chemical constituents from *Gmelina arborea* bark and their antioxidant activity. J wood sci 54: 483-489.

12. Shirwaikar A, Ghosh S, Rao PG (2003) Effects of *Gmelina arborea* Roxb leaves on wound healing in rats. J Nat Rem 3(1): 45-48.

13. Aguwa A, Yusuf S, Andrew GO, Zezi Au, Abdurahman EM (2005) Evaluation of five medicinal plants used in diarrhea treatment in Nigeria. J Ethnopharmacol 101(1-3): 27-30.

14. Sinha S, Dixit P, Bhargava S, Devasagayam TP, Ghaskadbi S (2006) Bark and fruit extracts of *Gmelina arborea* protect liver cells from oxidative stress. Pharm Biol 44(4): 237-243.

15. Nurkarni YA, Addepalli V (2011) Effect of *Gmelina arborea* extract in STZ induced type 1 diabetic rats. FASER J 25: 805-809.

16. Giri M, Divakar K, Goli D, Dighe SB (2009) Antiulcer activity of leaves of *Gmelina arborea* plant in experimentally induced ulcer in Wister rats. Pharmacol 1: 102-110.

17. Kulkarni YA, Addepalli V (2010) Toxicological studies on aqueous extract of *Gmelina arborea* in rodents. Pharm Biol 48(12): 1413-1420.

18. Ejele AE, Alinnor JI (2010) Anti-Sickling Potentials of Aloe vera extract IV. Effects of Acidic, Basic and Neutral Metabolites on the Gelling and Sickling of human HbSS erythrocytes. Inter J Nat Appl Sci 6(2): 155-160.

19. Ejele AE, Njoku PC (2008) Anti-Sickling Potentials of Aloe vera extract. J Sci food and Agri 88(8):1482-1485.

20. Garred LP, O-Graddy F (1983) Antibiotic and Chemotherapy. 4th (Edn.), Churchill Livingstone Publishers, London, pp: 189.

21. Awede B, Osseni R, Lagnika L, Adjagba M, Kpadonou C, et al. (2015) Antihypertensive effects of *Gmelina arborea* Roxb(Verbenaceae) Leaves Crude Aqueous Extract Fraction in Wistar Rats. J Phys Pharm Adv 5(12): 803-810.

22. Seelinger G, Merfort I, Woelfle U, Schempp C (2008) Anti-carcinogenic Effects of Flavonoid Luteolin. Molecules 13(10): 2628-2651.

23. N'gaman KCC, Mamyrbekova-Bekro JA, Bekro YA (2011) Effects of flavonoids of *Gmelina arborea* Roxb. (verbenaceae) from cote d'ivoire on the antioxidant activity and osmotic stability of erythrocytes. J Appl Biosci 39: 2626-2634.

24. Dzubak P, Hajduch M, Vydra D, Hustova A, Kvasnica M, et al. (2006) Pharmacological activities of natural triterpenoids and their therapeutic implications. Nat Prod Rep 23(3): 394-411.

25. Yogesh AK, Addepalli V (2013) Effects of *Gmelina arborea* extract on experimentally induced diabetes. Asian pacific journal of tropical medicine 6(8): 602-608.

26. Malik E, Mueller C (2016) Anthraquinones as pharmacological tool and drugs. Med Res Rev 36(4): 705-478.

27. Frazier WC, Westhoff DC (1995) Impact of technological Diagram on Biochemical and Microbiological Quality of *Borassus akeassli* Wine produced traditionally in Burkina Faso. Food Microbiology, 4th (Edn.), pp: 384-396.

28. Alinnor JI, Ejele AE (2009) Phytochemical Analysis and Antimicrobial Activity Screening of crude extract of leaves of Gongronema latifolium. Indian J Botanic Res 5(2): 161-168.

29. Ejele AE, Akujobi CO (2011) Effect of secondary metabolite of garcina kola on the microbial spoilage of cajanus cajan extract. Inter J Trop Agri Food Sci 5(1): 8-14.

30. Stary F (1991) The Natural Guide to Medicinal Herbs and Plants.

31. Levy JS (1998) The causes of war and the conditions of peace. Annual review of political science 1: 139-165.

32. Kulkarni YA, Vearanjaneyulu A (2012) Toxicological Evaluation of the methanol Extract of *Gmelina arborea* Roxbera. Bark in Mice and Rats. Toxicol Int 19(2): 125-131.

33. Kulkarni YA, Addepalli V (2009) Antidiabetic activity of aqueous extract of *Gmelina arborea* bark in rats. Altern Tuer Health med 15: 183-1834.

34. Satyanarayana P, Subrahmanyam P, Kasai R, Tanka O (1985) An apiosecontaining coumarin glycoside from *Gmelina arborea* root. Phytochem 24(8): 1862-1863.

35. Govindachari TR, Parthasarathy PC, Dasai HK (1972) Arboreal, a new lignan from *Gmelina arborea*. Ind J Chem 10: 1120-1122.

36. Evans WC (2002) Trease and Evans Pharmacognosy. W.B Saunders Company Limited. Harcourt Publishers, pp: 221-225.