GC-MS Analysis and Antibacterial Effects of *Vernonia glaberrima* n-Hexane Extracts alone and in Combination with Standard Antibiotics

P. Gangas, A.B. Aliyu*, A.O. Oyewale
Department of Chemistry, Faculty of Physical Sciences, Ahmadu Bello University, Zaria, Nigeria
*Corresponding author’s e-mail: aliyubabando@gmail.com Phone: +234 9098655974
Received 5 March 2021; accepted 18 March 2021, published online 30 March 2021

ABSTRACT:
The occurrence of drug resistant bacteria warrant investigation on herbal plants for effective antibacterial agents. *Vernonia glaberrima* leaf (VGL) and stem (VGS) hexane extracts were subjected to analysis by gas chromatography-mass spectrometry (GC-MS) and subsequently evaluated for antibacterial activity alone and in combination each with Sparfloxacin (SPX) and Ciprofloxacin (CPX) on selected bacteria including resistant species. GC-MS analysis revealed fatty acid esters, triterpenoids and aromatic derivatives largely identified as responsible for the broad-spectrum antibacterial activity. Both the VGL and VGS demonstrated potent antibacterial activities on *P. aeruginosa* (29 mm and 27 mm), respectively. However, combination of SPX with VGL potentiated the effects on *E. coli* and *S. typhi* by synergistic interaction. Similarly, the efficacy of CFX in combination with VGS on MRSA (30 mm) was significantly enhanced by additive action. It was observed that VGS potentiation of CFX on *P. aeruginosa* (32 mm) was the most effective antibacterial inhibition recorded in the study. Thus, combination of SPX and CFX each with the extracts has revealed remarkable properties for alternative or complementary therapeutic strategy. Our findings elicit enormous potentials of *V. glaberrima* hexane extracts as treatment adjuncts for combating drug resistant bacteria. It will be interesting to evaluate in vivo effects of extracts in combination with antibiotics against drug resistant bacteria.

Keywords: *Vernonia glaberrima*, hexene extract, GC-MS, MRSA, VRE, drug resistant bacteria

INTRODUCTION
Plants are renewable sources of secondary metabolites for the treatment of human infectious diseases. This is because of multiple therapeutic properties of the structurally diverse phytochemicals such alkaloids, flavonoids, saponins and terpenoids among others [1]. Numerous medicinal plants have been exploited as effective medicines in the fight against drug resistant bacteria [2]. Plant constituents such as essential oils, terpenoids and sesquiterpenoids have been evaluated as potent antibacterial agents on resistant bacteria using novel molecular targets such as inhibitions of efflux pump [3] and quorum sensing [4]. However, drug combination has evolved as a novel strategy to fight resistant bacteria. It involves the combination of standard antibiotics with plant extracts for effective interactions on drug resistant bacteria [5]. Thus, phytochemicals either as pure compounds, semi-pure or component mixtures can be combined with antibiotics as single therapeutic agent. The interactions via synergistic or additive interventions are recognized in recent years as effective complementary medicine against drug resistant bacteria [6-7].

*Vernonia glaberrima* is a perennial herb distributed in tropical regions of the world especially in Africa and South America [8]. It is found in abandoned fields in Northern Nigeria, and used in traditional medicine against malaria, inflammatory and infectious diseases [9]. Previous phytochemical studies on *Vernonia glaberrima* resulted to isolation of lupeol and coumarins [10]. However, very little information is available on the chemical compositions and their antibacterial efficacies. Hence, *V. glaberrima* leaf and stem hexane extracts were investigated using gas chromatography-mass spectrometry (GC-MS) and evaluated for antibacterial properties alone and in combination with standard antibiotics against selected bacterial strains. This is important to understanding chemical basis of the therapeutic potentials of the plant as source of antibacterial agents especially against resistant bacteria.
MATERIALS AND METHODS
Collection and extraction of plant material
The leaf and stem of *Vernonia glaberrima* were collected (March 2018) in Zaria, Kaduna State, Nigeria. The plant was identified by Umar S. Gallah of the Herbarium, Department of Botany, Ahmadu Bello University, Zaria, Nigeria. A voucher specimen number 215 was deposited there. The plant was air-dried; and leaf and stem samples (100 g each) were subjected to extraction using cold maceration with n-hexane (500 mL) for 12 h on a shaker (Labcon, South Africa). The extracts were filtered and concentrated under reduced pressure on a rotary evaporator (Buchi Rota vapor R-210) at 25°C. The hexane leaf (VGL) and stem (VGS) extracts weighed 9.50 g and 12.70 g respectively.

GC-MS analysis
The GC-MS analysis was carried out on an Agilent Technologies (6890 Series) GC coupled with a (5973 Series) Mass Selective Detector. It was equipped with an Agilent HP-5MS capillary column (0.25 μm film thickness) with dimensions 30 m (length) × 0.25 micron ID. The sample ionization energy of 70eV for GC-MS detection was used. Helium was used as the carrier gas at a pressure of 60 kPa, with the oven temperature programming at 100°C (for 2 min) to 280°C (for 30 min) at a ramping rate of 4°C per min. Diluted sample (2.0 μl) was manually injected while the injection temperature was 280°C with a split ratio of 1:50. The system software was driven by Agilent Chemstation software. The relative percentage of each component was calculated by comparing its average peak to the total areas. The identification of the various compounds was carried out by comparison of their mass spectra with those of authentic samples or those obtained from isolated pure compounds in our laboratory. The NIST/NBS 2005 mass spectral database of the GC-MS system was also used to identify some compounds whose structures were confirmed by published data [11].

Test microorganisms
The bacterial strains: *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, Methicillin resistant *Staphylococcus aureus* (MRSA) and Vancomycin resistant *Enterococci* (VRE) used in this study were obtained as clinical isolates, from the Department of Microbiology, Ahmadu Bello University Teaching Hospital (ABUTH), Shika. The isolates were purified on nutrient agar (OXOID) plates and characterized using standard microbiological and biochemical procedures as previously described [12-13].

Antibacterial susceptibility testing
*In vitro* antimicrobial activity of *V. glaberrima* leaf (VGL) and stem (VGS) hexane extracts was determined by standard agar well diffusion assay as reported [14]. Molten Mueller Hinton agar were seeded with the inoculum (1×10⁸ CFU/ml, 200 μl) and poured into petri dishes and allowed to solidify. The wells were prepared in the seeded agar plates with the help of a cork borer (6 mm). The sample extract each was dissolved in DMSO (5 mg/ml) and extract solution (100 μl) was then introduced into the 6 mm diameter well. The plates were incubated at 37 °C for 24 h. Testing was done in duplicate and Sparfloxacine (30 μg/ml) and Ciprofloxacine (30 μg/ml) discs (Oxoid, UK) were used as standard antimicrobial agent controls and DMSO was used as a negative control. Antibacterial activity was determined by measuring the diameter of the inhibition zone (clear zone) formed around the well in millimeters and classified as follows: Resistant (R): ≤ 10 mm; Intermediate (I): 11-14 mm; Sensitive (S): ≥ 15 mm [15].

Minimum inhibitory concentration (MIC)
Minimum inhibitory concentration was carried out using micro broth dilution in accordance with Clinical Laboratory Standards Institute [16]. Serial dilution of sample extract (0.1 mg/ml to 6.50 mg/ml) was prepared. The tests tubes were inoculated with the suspension of the standardized inocula and incubated at 37 °C for 24 h. MICs were recorded as the lowest concentration of extract showing no visible growth of the broth.

Antibacterial combination studies
*In vitro* antimicrobial combination studies of *V. glaberrima* leaf (VGL) and stem (VGS) hexane extracts each with Sparfloxacine and Ciprofloxacine was determined by standard agar well diffusion assay as reported [17]. Molten Mueller Hinton agar were seeded with the inoculum (1×10⁸ CFU/ml, 200 μl) and poured into petri dishes and
allowed to solidify. The wells were prepared in the seeded agar plates with the help of a cork borer (6 mm). The sample extracts were dissolved in DMSO (5 mg/ml) and the standard drugs each (30 μg/ml). The combined solution of extract with standard drugs (30 μl each) was then introduced into the 6 mm diameter well. The plates were incubated at 37 °C for 24 h. DMSO was used as a negative control. Antibacterial activity was assayed by measuring the diameter of the zone of inhibition formed around the well in millimeters. The experiment was done in triplicate and the average values were calculated for antibacterial activity.

RESULTS AND DISCUSSION
The GC-MS analysis of VGL hexane extract revealed the presence of twenty-one (21) compounds with the abundance of fatty acid/esters such as 9, 12-octadecadienoic acid (Z, Z)-2-hydroxy-1-(hydroxy methyl) ethyl ester (23.7%), hexadecanoic acid (11.6%), hexacosanoic acid, 2-methyl-methyl ester- (8.6%), tetra tetracotane (7.89%), hexadecanoic acid, 1-(hydroxy methyl)-2, 2-ethane diyl ester (5.29%), ethyl tetracosanoate (4.45%) and glycidol stearate (3.90%) which accounted for 68.2% of the total identified compounds. However, the VGS hexane extract contains twenty-three (23) components largely triterpenoids together with aromatic derivatives such as olean-18-ene (15.6%), β-amyrin (14 %), 1, 3, 5-trimethyl benzene (11.21%), 2-methylhexacosane (6.80%), ethanol, 2-(1, 12-octadecadienylxyloxy)- (Z, Z)- (6.28%), lupeol (5.74%), 1, 4-diethyl benzene (3.40%) and 1, 2, 4, 6-tetramethyl benzene (3.24%). These represent 66.3% of the total identified components. The retention times (RT) and relative percent (%) composition of identified components of both VGL and VGS are presented in Table 1. The compounds identified in V. glaberrima (VGL/VGS) are similar to previous GC-MS analysis on V. calvoana leaf ethyl acetate extract [18] and V. cinerea methanol extract [19]. Furthermore, fatty acid esters and aromatic derivatives were identified from the extract of V. arborea as reported [20]. In this study, the GC-MS analysis has revealed numerous bioactive constituents of V. glaberrima leaf and stem hexane extracts, as fat-soluble mixtures of aggregate compounds of different structural motifs with variable content and compositions which may indicate interesting therapeutic properties [21-22].

| Chemical constituents                     | RT (min) | VGL | VGS |
|------------------------------------------|----------|-----|-----|
| 1, 4-dimethyl benzene                    | 4.13     | -   | 1.62|
| 1-ethyl, 2-methyl benzene                | 4.94     | -   | 3.00|
| 1, 3, 5-trimethyl benzene                | 5.05     | -   | 11.21|
| 1-methyl-3-propyl benzene                | 6.16     | -   | 1.45|
| 1, 4-diethyl benzene                     | 6.24     | -   | 3.40|
| 2-ethyl-1, 4-dimethyl benzene            | 6.52     | -   | 1.35|
| 1-ethyl-3, 5-dimethyl benzene            | 6.57     | -   | 2.79|
| 1, 2, 4, 6-tetramethyl benzene           | 7.14     | -   | 3.24|
| 1, 2, 4, 5-tetramethyl benzene           | 7.67     | -   | 1.12|
| Naphthalene                              | 8.30     | -   | 1.06|
| Eicosane                                 | 15.8     | 2.08| -   |
| 2-pentadecane                            | 16.2     | 2.43| -   |
| Hexadecanoic acid                        | 17.2     | 11.6| -   |
| L-(+)-ascorbic acid, 2, 6-dihexadecanoate| 17.6     | 1.26| 2.21|
| Eicosane                                 | 17.9     | 2.34| 1.00|
| ε-11-hexadecanal                         | 19.3     | -   | 1.61|
| 9, 12-octadecadienoic acid              | 19.4     | 2.05| -   |
The results of antibacterial activity on the VGL and VGS hexane extracts are presented in Table 2. Both extracts demonstrated effective antibacterial activity especially on *P. aeruginosa*, MRSA, *E. coli* and *S. aureus*. The VGL was more effective on *P. aeruginosa* (29 mm, MIC 0.125 mg/ml). Similarly, the effects of VGL on MRSA and *S. aureus* (27 mm, MIC 0.125 mg/ml) can be attributed to the therapeutic potentials of fatty acid esters as largely identified constituents from the VGL. This finding is consistent with previous report on the antibacterial activity of fatty acid esters of lipophilic extract of *Pavetta corymbosa* [23]. The susceptibility of MRSA and *P. aeruginosa* to the VGL extract may indicate a broad-spectrum antibacterial potency of the hexane extracts. This is not surprising as *V. ambigua*, *V. blumeoides* and *V. ocephala* have previously been reported with broad-spectrum antibacterial activities [24].

| (E)-9-octadecanoic acid, ethyl ester | 19.5 | 3.23 | - |
| Heptadecanoic acid, ethyl ester | 19.7 | 2.26 | - |
| Tetracosane | 19.8 | 2.97 | - |
| Phytol, acetate | 19.9 | 2.53 | - |
| Hexadecanoic acid, 1-(hydroxy methyl)-2, 2-ethanediyl ester | 20.7 | 5.29 | 1.81 |
| 4, 8, 12, 16-tetramethylheptadecan-4-olide | 21.2 | 1.18 | - |
| 9, 12-octadecadienoic acid (Z, Z)-2-hydroxy-1- (hydroxy methyl) ethyl ester- | 22.1 | 23.7 | - |
| Ethanol, 2-(1, 12-octadecadienyl)oxy- (Z, Z)- | 22.2 | - | 6.28 |
| Tridecanoic acid, 3-hydroxy-ethyl ester | 22.3 | 2.38 | - |
| Glycidosyl stearate | 22.4 | 3.90 | 1.46 |
| Hexacosanoic acid | 22.7 | 1.14 | - |
| Tetracosenal | 26.1 | - | 1.08 |
| Ethyl tetracosanoate | 25.4 | 4.45 | - |
| Hexatriacontane | 26.9 | 2.63 | 1.00 |
| Stigmasta-5, 22-dien-3-ol-acetate (3β-22 Z) | 27.2 | - | 2.15 |
| Hexacosanoic acid | 27.5 | 1.27 | - |
| Lupeol | 28.3 | - | 5.74 |
| Hexacosanoic acid, 2-methyl-, methyl ester- | 28.6 | 8.16 | - |
| β-amyln | 30.0 | - | 14.0 |
| Olean-18-ene | 30.5 | - | 15.6 |
| 2-methylhexacosane | 30.9 | - | 6.80 |
| Tetra tetracontane | 31.0 | 7.89 | - |
| **Total components identified (%)** | **94.7** | **91.0** |

**Table 2**: Antibacterial activity of VGL and VGS hexane extracts alone

| Test organisms | VGL | VGS | Sparfloxacain | Ciprofloxacin | MIC mg/ml |
|----------------|-----|-----|---------------|---------------|-----------|
| **Zone of inhibition (mm)** | **MIC 0** | **MG/ml** |
| MRSA           | 27  | 25  | 22            | 0             | 0.125     | 0.125     |
| *S. aureus*    | 27  | 26  | 27            | 25            | 0.125     | 0.125     |
| *P. aeruginosa*| 29  | 27  | 26            | 24            | 0.125     | 0.125     |
| *E. coli*      | 24  | 26  | 22            | 25            | 0.25      | 0.125     |
The VGS hexane extract also showed similar effects as VGL in terms of potent activity on Gram-negative bacteria P. aeruginosa (26 mm, MIC 0.125 mg/ml) and E. coli (26 mm, MIC 0.125 mg/ml). This indicates the influence of aromatic and sterols components identified (Fig. 1). Previous study of plant extracts containing largely lupeol and β-amyrin demonstrated antibacterial activity with MIC (0.5 mg/ml) on S. aureus [25]. In this study, both VGL and VGS have shown potent activity on Gram-positive and Gram-negative bacteria including resistant MRSA indicating broad-spectrum antibacterial activity of the extracts.

**Table 1**

| S. typhii | VRE | 0 | 0 | 23 | 29 | NT | NT |

MRSA= Methicillin resistant Staphylococcus aureus, VRE= Vancomycin resistant enterococcus VGL=Vernonia glaberrima leaf, VGS=Vernonia glaberrima stem, NT=not tested

The combinations of plant extracts with several classes of antibiotics showed various therapeutic properties on drug resistant bacteria by synergistic or additive interactions [7]. In this study, the antibacterial effects of VGL and VGS in combination each with Sparfloxacin (SFX) and Ciprofloxacin (CFX) are presented in Table 3. The potentiation of SFX when combined with VGL was observed on E. coli and S. typhi. Similar trend of potentiation was observed when SFX combined with VGS on MRSA, P. aeruginosa and S. typhi (Table. 3). This implies that both extracts can improve the therapeutic efficacy of Sparfloxacin on Gram-positive and Gram-negative bacteria. SFX and CFX are broad spectrum antibiotics of the fluoroquinolone family that target bacterial DNA gyrase or the topoisomerase IV enzymes leading to formation of tertiary complexes which
inhibits DNA replication and transcription [26]. It was observed that SFX in combination with VGS remarkably enhanced the antibacterial potency on VRE which otherwise was resistant to the SFX alone (Table 2 and 3). This modulation of SFX activity by VGS suggest the possibility of developing effective phytomedicines against drug resistant bacteria. The combination of CFX with VGL also demonstrated enhanced activity on MRSA and E. coli, which could be attributed to the fatty acid esters largely identified (Fig. 1). However, the interaction of CFX with VGS on MRSA (30 mm) showed significant enhancement of the antibiotic effects (Table 2 and 3). The resistance of MRSA to CFX was modified by additive interaction of the VGS (Table 2 and 3). It was also observed that the potentiation of CFX by the VGS on P. aeruginosa (32 mm) demonstrated the most effective antibacterial efficacy in the whole study. Previous study showed the enhancement of Ciprofloxacin against multi-drug resistant bacteria by Carum copticum extracts of aromatic constituents [27]. Thus, our findings have indicated that phytochemicals from V. glaberrima hexane extracts could significantly potentiate the antibacterial effects of Ciprofloxacin antibiotics especially against resistant pathogens.

**Table 3:** Antibacterial activity of VGL and VGS hexane extracts combined with antibiotics

| Test organisms | VGL+SFX | VGS+SFX | VGL+CFX | VGS+CFX |
|---------------|---------|---------|---------|---------|
| MRSA          | 23      | 29      | 25      | 30      |
| S. aureus     | 24      | 0       | 23      | 0       |
| P. aeruginosa | 27      | 30      | 28      | 32      |
| E. coli       | 30      | 22      | 29      | 20      |
| S. typhii     | 28      | 29      | 32      | 31      |
| VRE           | 27      | 27      | 27      | 27      |

MRSA= Methicillin resistant *Staphylococcus aureus*, VRE= Vancomycin resistant enterococcus, VGL= *Vernonia glaberrima* leaf, VGS= *Vernonia glaberrima* stem, SFX= Sparfloxacin, CFX= Ciprofloxacin

**CONCLUSION**

The GC-MS analysis of *V. glaberrima* leaf (VGL) and stem (VGS) hexane extracts revealed fatty acid esters, triterpenoids and aromatic derivatives. The extracts have demonstrated broad spectrum antibacterial activity alone and in combination with antibiotics. The combinations of Ciprofloxacin (CFX) with VGS showed significant enhancement of antibiotic effects on MRSA. These findings elicit enormous potentials of *V. glaberrima* hexane extracts as therapeutic adjuncts for combating drug resistant bacteria. It will be interesting to evaluate in vivo effects of extracts in combination with antibiotics against drug resistant bacteria.

**ACKNOWLEDGEMENTS**

Authors acknowledge Dr Neil Broomhead of the School of Chemistry and Physics, University of KwaZulu-Natal, Durban, South Africa for running the GC-MS.

**REFERENCES**

1. M. Wink (2015), Modes of action of herbal medicines and plant secondary metabolites. *Medicines*, 2(3), 251-286. [https://doi.org/10.3390/medicines2030251](https://doi.org/10.3390/medicines2030251)

2. P. Nayim, A.T. Mbaveng, B.E.N. Wamba, A.G. Fankam, J.K. Dzotam and V. Kuete (2018), Antibacterial and antibiotic-potentiating activities of thirteen Cameroonian edible plants against Gram-negative resistant phenotypes. *The Sci. World J.*, [https://doi.org/10.1155/2018/4020294](https://doi.org/10.1155/2018/4020294)

3. A.J. Seukep, V. Kuete, L. Nahar, S.D. Sarker, M. Guo (2019), Plant-derived secondary metabolites as the main source of efflux pump inhibitors and methods for identification. *J.*
4. A.B. Aliyu, N.A. Koorbanally, B. Moodley, P. Singh, H. Chenia (2016), Quorum sensing inhibitory potentials and molecular docking studies of sesquiterpene lactones from Vernonia blumeoides. Phytochem., 126, 23-33. DOI: 10.1016/j.phytochem.2016.02.012

5. O.D. Stefanović (2018), Synergistic activity of antibiotics and bioactive plant extracts: A study against Gram-positive and Gram-negative bacteria. In: Kürmusaoğlu, S. (ed.) Bacterial Pathogenesis and Antibacterial Control. Published by IntechOpen, London, UK Chp. 2 pp. 23-48 http://dx.doi.org/10.5772/intechopen.72026

6. M.F. Haroun and R.S. Al-Kayali (2016), Synergistic effect of Thymbra spicata L. extracts with antibiotics against multidrug-resistant Staphylococcus aureus and Klebsiella pneumoniae strains. Iran J. Basic Med. Sci., 19, 1193-1200.

7. D.M. Silva, P.A. Da Costa, A.O.B. Ribon, G.A. Purgato, G. Díaz-Muñoz, M.A.N. Díaz (2019), Plant extracts display synergism with different classes of antibiotics. Ann. Braz. Acad. of Sci., 91(2). e20180117. http://dx.doi.org/10.1590/0001-3765201920180117

8. K. Bremer (1994), Asteraceae: Cladistics and Classification. Portland, Oregon, Timber Press.

9. M.I. Abdullahi, A. Uba, A. Yaro, O. Maxwell, A.J. Yusuf, S. Kabir, A.M. Alhassan, A. Umar, S.S. Bello and I. Nasir (2015). Phytochemical screening, acute toxicity study and evaluation of antidiabetic properties of the methanolic leaf extract of Vernonia glaberrima (Asteraceae). J. Pharm. Chem. Biol. Sci., 3(2), 169-177.

10. A.M. Alhassan, Q.U. Ahmed, J. Latip, S.A.A. Shah, A’a.Y.F. Khan, M.N. Sarian, R.A. Wahab, M. Taher, M.I. Abdullahi, A. Khatib (2018), Phytoconstituents from Vernonia glaberrima Welw. Ex O. Hoffm. leaves and their cytotoxic activities on a panel of human cancer cell lines. South Afr. J. Bot., 116, 16-24. https://doi.org/10.1016/j.sajb.2018.02.391

11. Y. Masada (1976), Analysis of essential oil by gas chromatography and spectrometry. New York: John Wiley & Sons.

12. S.T. Cowan and K.F. Steel (1974), Manual for Identification of Medical Bacteria. 2nd ed, Cambridge University Press, London, pp. 97-115.

13. J.F. McFaddin (1977), Biochemical Tests for Identification of Medical Bacteria. Williams and Wilkins Co., Baltimore, pp. 392-452.

14. C. Perez, M. Paul and P. Bazerque (1990), An antibiotic assay by the agar–well diffusion method. Acta Biol. Med. Exp., 15, 113-115.

15. H.Y. Chenia (2013), Anti-quorum sensing potential of crude Kigelia africana fruit extracts. Sensors, 13, 2802-2817. DOI: 10.3390/s130302802

16. CLSI (2012), Clinical and Laboratory Standards Institute: Performance standards for antimicrobial susceptibility testing; Twenty-second informational supplement. CLSI document M100-S22 Wayne, PA, USA, Clinical and Laboratory Standards Institute

17. I. Ahmad, and F. Aqil (2007), In vitro efficacy of bioactive extracts of 15 Medicinal plants against ESBL-producing multidrug-resistant enteric bacteria. Microbiol. Res., 162, 264-275. DOI: 10.1016/j.micres.2006.06.010

18. I.A. Iwara, G.O. Igile, O.E. Mboso, B.I.A. Mgbje and P.E. Ebong (2017), Evaluation of phytochemical components from ethyl acetate fraction of Vernonia calvoana using gas chromatography-mass spectrometry analysis and its antioxidants activities. Afr. J. Pharm. Pharmacol.,11(42), 534-539.

19. P. Abirami and A. Rajendran (2012), GC-MS analysis of methanol extracts of Vernonia cinerea. Eur. J. Exp. Biol., 2 (1), 9-12.
20. K.S. Janakiraman and S. Chinnagounder (2012), Evaluation of physico-chemical constants and GC-MS analysis of Vernonia arborea. J. Pharm. Res., 5(5), 2900-2905.

21. A.C. Figueiredo, J.G. Barroso, L.G. Pedro, and J.J.C. Scheffer (2008). Factors affecting secondary metabolite production in plants: volatile components and essential oils. Flav. Frag. J., 23, 213-226. https://doi.org/10.1002/ffj.1875

22. L.J. McGaw, A.K. Jäger and J. van Staden (2002), Antibacterial effects of fatty acids and related compounds from plants. South Afr. J. Bot., 68, 417-423. https://doi.org/10.1016/S0254-6299(15)30367-7

23. A.B. Aliyu, M.A. Ibrahim, H. Ibrahim, M.B. Dambatta, A.O. Oyewale (2017), GC-MS analysis of Pavetta corymbosa lipophilic extract and its antimicrobial activity. Ife J. Sci., 19(2), 363-368. DOI: 10.4314/ijs.v19i2.16

24. A.B. Aliyu, A.M. Musa, M.S. Abdullahi, H. Ibrahim, A.O. Oyewale (2011). Phytochemical screening and antibacterial activities of Vernonia ambigua, Vernonia blumeoides and Vernonia oocophala (Asteraceae). Acta Pol. Pharm., 68, 67-73.

25. S. Abu-Lafi, M. Rayan, M. Masalha, B. Abu-Farich, H. Al-Jaas, M. Abu-Lafi, A. Rayan (2019). Phytochemical composition and biological activities of wild Scolymus maculatus L. Medicines. 6 (53), 1-11. DOI: 10.3390/medicines6020053

26. L.J.V. Piddock and M. Zhu (1991), Mechanism of action of Sparfloxacin against and mechanism of resistance in Gram-negative and Gram-positive bacteria. Antimicrob. Agents Chemother. 2423-2427. DOI: 10.1128/aac.35.11.2423

27. M. Maheshwari, A.S. Althubiani, H.H. Abulreesh, F.A. Qais, M.S. Khan, I. Ahmad (2019), Bioactive extracts of Carum copticum L. enhances efficacy of ciprofloxacin against MDR enteric bacteria. Saudi J. Biol. Sci., 26, 1848-1855. https://doi.org/10.1016/j.sjbs.2017.12.008