**Original Research****EXTRACTION, ISOLATION AND MASS SPECTRAL ANALYSIS OF
CRINUM ZEYLANICUM (BEAUTIFUL CRINUM) BULBS***¹DABEN J.M. and ²DASHAK D.A.¹Department of Science Laboratory Technology, Faculty of Natural Sciences, University of Jos, Plateau State, Nigeria.²Department of Chemistry, Faculty of Natural Sciences, University of Jos, Plateau State, Nigeria.**ABSTRACT**

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The discoveries of pharmacopoeia of scientific medicine which was derived from the herbal knowledge of native people simply as a result of the search of new type of food was lopsided but have been developed through modern experimentations and instrumentations. The extraction, isolation and Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the plant bulbs were carried out using Methanol, Ethyl acetate and Petroleum ether crude extracts. The methods employed were soxhlet extraction, column chromatography (CC), thin layer chromatography (TLC) and GC-MS analysis. Fractions that show single spot on the TLC plate from each solvent were elucidated for GC-MS spectral technique. The GCMS analysis identify the compound accounting for the molecular masses, fragmentation patterns, thermostatically molecular mass ions and the major ions of the fragments. This result showed the presence of 2-methylbutane, methyl benzene, 2,3-propanediol, 1,4-dimethyl benzene, 2-prop-2-enoyl benzoic acid, hexadecanoic acid, methyl-11-methyl dodecanoate, hexanol, 1,3-octadiene, pentane, octanal, hexenal, 2-methylpentane, heptanal, 4,6-dimethyl dodecane, nonane, pentadecane, decane, 2-oxo-phenyl methyl, 2,2-dimethylbutane, phenol, 2,4-bis(1,1-dimethylethyl)-5-methyl, 2,4-octadiene and propanamide. These compounds are indications that the bulb of *C. zeylanicum* has potentials for affordable and locally available alternative plant medications for bone settings and skin troubles.

Keywords: *Crinum zeylanicum*, Mass spectrometry, Gas Chromatography-Mass Spectrometry, Isolation, Medicinal plant and Bone setting.

INTRODUCTION

It is now known as an established fact that the important role of medicinal plant in the health of individuals in rural societies has given rise to their exploitation in modern health care system (Bais et al., 2014). Africa and indeed Nigeria is endowed with a large collection of plants and herbs which are of medicinal importance (Dalziel, 1956; Sofowora, 1993). *Crinum zeylanicum* (L.) is of the family *Amaryllidaceae*, genus-*Crinum* also called milk or wine lily, Ceylon swampy lily and Beautiful crinum. In Nigeria, it is known as gadali (Hausa) and in Plateau State- Doemak

(Dau), Mushere (Lezipir). *Crinumzeylanicum* is a bulbous plant, the leaves are robust and cluster parallel to the ground reaching 75cm long by 6cm wide with strongly scabrous and undulated margin, while the blade are strongly kneeled and wooly dense. The scape range from 60 to 100cm high and bears 4 to 6 yellow flowers with a purple band along the center in a large umbel to 15cm across of slightly damp sites in savanna (Burkill, 1985; Yakandawala and Samarakoon, 2006).

Common to West Africa and widespread throughout most of tropical Africa (Dalziel, 1956; Burkill, 1985) and sub-tropical part of the world Sri-Lanka (Yakandawala and Samarakoon, 2006). *C. zeylanicum* (L.) can be grown from dormant bulbs gathered during the dry season on a temperate and warm temperate region.

C. zeylanicum is distinctly ornamental. In Northern Nigeria, the fresh fruits are pounded with red natron for application to guinea worm blister. The crushed fruits are rubbed on the feet of farmers to prevent injury by hoe. The dried bulbs when pulverized are used as ingredient in some preparations for poisoning fish (Dalziel, 1956; Burkil, 1985).

In middle belt region of Nigeria, the crushed bulbs act as surfactant used on fractured bone for bone setting as used by traditional practitioners. The sliced bulb is heated to produce exudation of the sap, which is then mixed with copper fillings for application to wound (Burkill, 1985).

The ash of the incinerated bulb is mixed with a leaf gall found on *Terminalia avicennioides* (Combretaceae) to produce an ointment said to be effective on swollen joints. According to Afzelius, in Sierra Leone, a cold infusion of the fresh leaves is used to bathe young children suffering from general disability, rickets etc. In Senegal, it is used externally by placing layers of the bulb over areas of skin trouble, on injuries and refractory ulcers. In China, the bulb is used in decoction as a vermifuge. In Tropical Africa, it is used in treating scabies due to the antifungal action. The dried fruit is known to be abortifacient while the root and stem extract are used orally to stimulate uterine contractions in pregnant women. The juice of the leaves is applied to ulcers and bruises, and when mixed with castor oil is applied to eczema on children's leg. *Crinum zeylanicum* have effect on livestock (animals). In Senegal, Mali and Guinea, the plant is commonly known as poison and is recognized as causing diarrhea that is difficult to control. Thus in Senegal, it is not put into oral medicine (Burkil, 1985). The bulb is used as a rubefacient in rheumatism, remedy for earache and for malaria treatment in Dominican Republic. (Tsuda *et al.*, 1984; Strahil *et al.*, 2011). The plant has shown high toxicity against molluscan (*Biomphalaria pfeifferi* and *Lymnae natalensis*) and intermediate hosts of Schistosomiasis (Chifundera *et al.*, 1993; Strahil *et al.*, 2011). These alkaloids Crinidine, Flexinine, 6-hydroxypowelline, Zeylamine, Lycorine, Hamayne, 3-acetylhamayne,

Crinamine, 6-hydroxycrinamine, 6-methoxycrinamine and Galanthamine have been isolate from *C. zeylanicum* (Tsuda *et al.*, 1984; Doepke *et al.*, 1986; 1996; Refaat *et al.*, 2009; Strahil *et al.*, 2011). Many bioactive compounds which exert antiproliferative effects against humane tumor cell, accelerate wound healing processes, depresses the central nerve system, reduces spontaneous motor activity and also contain psychoactive principles that are sedative with possible neuroleptic potentials have also been found present in the bulb of *Crinum zeylanicum*. (Strahil *et al.*, 2011; Tijani *et al.*, 2012).

In our modern society today, research emphasis on the evaluation and characterization of various plants and plant constituents against a number of diseases are based on their traditional claims of the plants given in alternative medicine. The isolation and characterization of plant substances are derivable from phytochemistry. *Crinum zeylanicum* (Figure 1) is a well-known and widely used plant by herbal traditional practitioners in the central and southern parts of Plateau state of Nigeria for bone setting and are also placed over areas of troubled skin. This informs the search to isolation and identifies compounds in *Crinum zeylanicum* bulbs to support the traditional claimed and to proffer a headway to pharmaceutical and drug industries.



Figure 1: *Crinum zeylanicum* plant. Source: (original photo)

MATERIALS AND METHODS

Materials

All reagents used were of analar grade and obtained from Zayo Limited Jos Plateau State Nigeria. Major equipment include Gas Chromatography- Mass Spectrometer GC-MS (QP2010 Plus Shimadzu, Japan).

Sample Collection and Preparation

Crinum zeylanicum bulbs (Figure 2) were obtained from Organic garden in Kopmabar, Doemak District in Qua'an Pan and Ikgwakap-Mushere in Bokkos Local Government Areas (LGAs) of Plateau State-Nigeria. The samples were stored in plastic containers and transported to the laboratory for analysis. They were cleaned free of soil debris and separated into leaves and bulbs. These were then dried in an oven at 60°C. The dried samples were milled and stored in an airtight container for analysis (Harborne, 1985).



Figure 2: *Crinum zeylanicum* bulbs. Source: (original photo)

Extraction of plant material

Fifty grams (50 g) of the sample powder was extracted with Soxhlet extractor using 250 cm³ each of the solvents (Petroleum ether 60-80°C, Ethyl acetate and Methanol). The extracts were concentrated by the use of vacuum rotary evaporator at 35°C, the remaining 20 cm³ of the extracts was left open in a fume cupboard to dry.

Column Chromatography Analysis

The glass column used had the dimension length 60 cm and of diameter 3 cm. The slurry was prepared using 30 g of silica gel (200-400 mesh) in n-hexane. 5 g of the extract was dissolved in n-hexane and then transferred into the column using the pipette. The height of the mobile phases above the packed column was (5-10 cm). The elution was carried out under gravity and the drop rate of the mobile phase was kept constant. The eluent from the column was collected in beakers in fractions of 50 cm³. The Methanol Extract were separated using a gradient eluent mixture of chloroform/methanol ratio 100:0 to 0:100, to obtain 70 fractions. Thin layer chromatographic analysis was used to identify similar fractions which were combined, and labeled FME (Fractions of Methanol Extract).

Similarly, the crude extract of the ethyl acetate was eluted using n-hexane/ethyl acetate/methanol mobile phase in gradient ratios of 100:0:0 to 0:20:80, obtaining 42 fractions. Fractions were combined depending on the similarity in TLC profile and coded as FEE (Fractions of ethyl acetate extract)

The petroleum ether extract fractionation used n-hexane/ethyl acetate/methanol in gradient ratio of increasing polarity, obtaining 55 fractions. Fractions were combined on the basis of TLC profile and coded as FPE (Fractions of Petroleum ether Extract).

Fractions which show single spot on the TLC chromatogram were selected from the extracts of the three solvents systems and analyzed using TLC and GC-MS. Fractions FME03, FEE02 and FPE10 were selected for their good separation.

Thin Layer Chromatographic Analysis

The activated silica gel pre-coated plates (TLC silica gel 60 F254 plates) were spotted with the fractions from different extracts using capillary tubes. Each plate was developed in the three different solvent systems of varied polarity made up of n-hexane/ethyl acetate (9:1, 4:1 and 3:1). The plates were allowed to dry and viewed under UV-light. The separated spots were marked and their R_f values determined (Harborne, 1985). The fractions were pooled together according to their TLC profiles and those that appeared well separated with single spots were identified for further studies. Fractions FME03, FEE02 and FPE10, appeared as single spots and were taken for further analysis.

Identification of Fractions

FME03 (the 3rd fraction obtained from 100% n-hexane ratio) FEE02 (the 2nd fraction obtained using 100% n-hexane) FPE10 (the 10th fraction obtained using 90% n-hexane-10% ethyl acetate solvent system). These fractions were elucidated by Gas Chromatography-Mass Spectrometer GC-MS (QP2010 Plus Shimadzu, Japan).

The GCMS of selected fractions were run and interpreted by comparing with standards, library search of published Electron Impact-Mass Spectral (EI-MS) in the NIST (National Institute of Standards and Technology) database (2012), Shimadzu's Flavours and Fragrance of Natural Synthetic Compounds (FFNSC 1) GCMS library, and other published spectral data. The retention indices were determined based on

a homologous series of n-alkanes internal standard analyzed under the same operating conditions. Calibration based on the Automatic Adjustment of Compound Retention Time (AACRT) function of the GC-MS. Relative concentration of the bulb component were calculated based on GC peak area with computer matching using NIST libraries provided with computer controlling the GC-MS System .

RESULTS AND DISCUSSION

Figure 3 shows the chromatogram of FME03 with the major peaks as peak 2 and 3, identified as methylbenzene and 2,3-propanediol respectively. Peak 4 and 5 were identified as di-methylbenzene and 2-prop-2-enoylbenzoic acid though not among the major peaks.

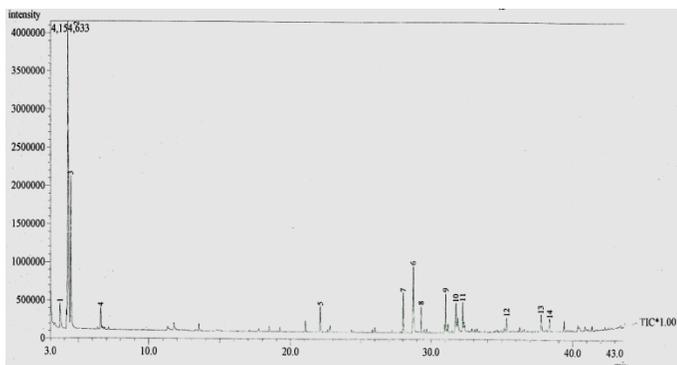


Figure 3: GC-MS Chromatogram of FME03

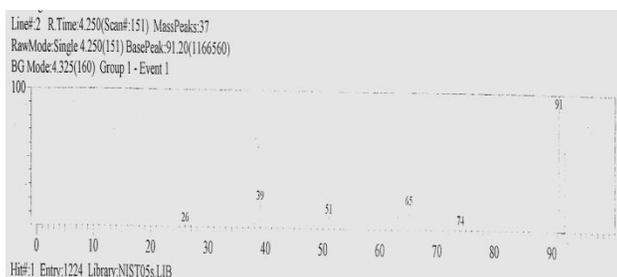


Figure 4: Mass Spectrum of Peak 2 of Figure 3

The fragmentation pattern shown in Figure 4 has its major thermostatically molecular mass ion of the fragments as 91. The mass of fragments and their respective ions loss between the fragmentations are 17(-CH₃, -2H), (9+14)=21(C≡C, +3H), 12(-CH₂, -2H), 13(-CH) as shown in the following fragmentation patterns:

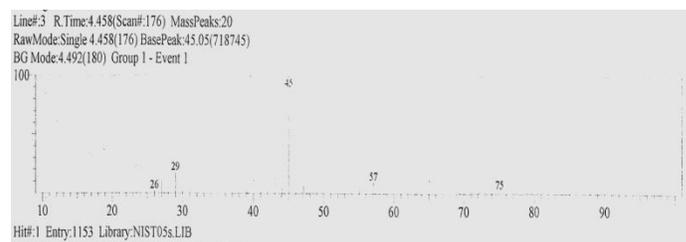
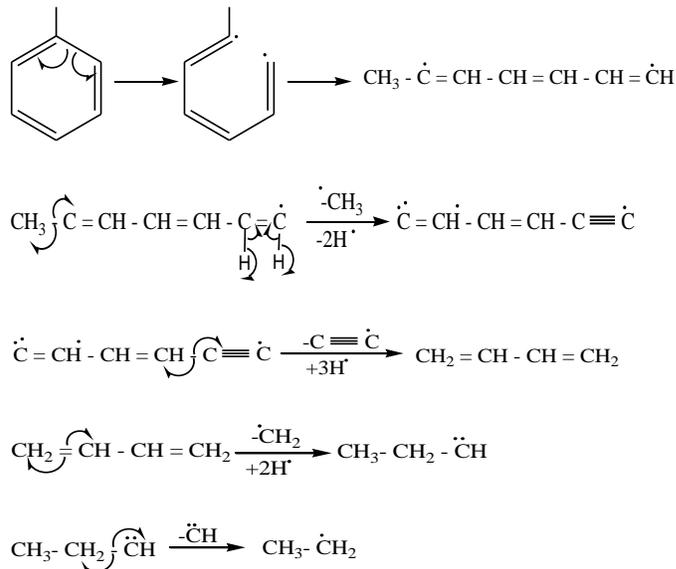
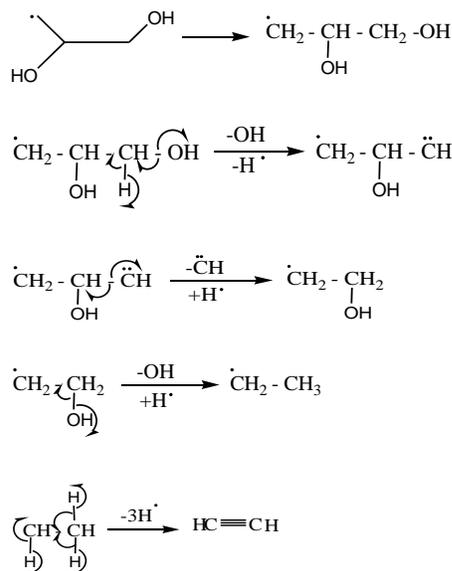


Figure 5: Mass Spectrum of Peak 3 of Figure 3

The fragmentation pattern shown in Figure 5 has its major molecular mass ion of the fragments as 45. The mass of fragments and their respective ions loss between the fragmentations are 18(-OH, -H), 12(CH, +H), 16(-OH, +H), 3(-3H) as shown in the following fragmentation patterns.



The GCMS chromatogram of FEE shown in Figure 6 revealed seven major peaks (4, 7, 9, 12, 15, 18 and 19), but only peaks 4, 12, 15 and 18 could be identified as heptanal, nonane, pentadecane, decane

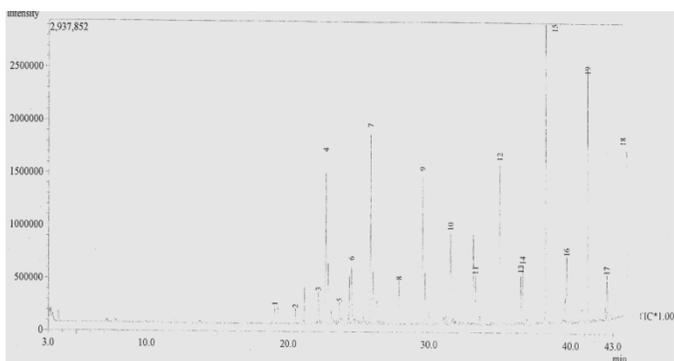


Figure 6: GC-MS Chromatogram of FEE O2

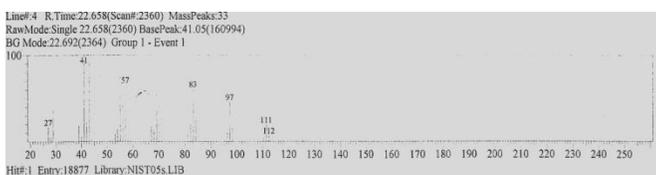


Figure 7: Mass Spectrum of Peaks 4 of Figure 6

The Fragmentation pattern shown in Figure 7 has its major thermostatically molecular mass ion of the fragments as 41 particular for heptanal. The mass of fragments and their respective ions loss between the fragmentations are: 15(CH₂, -H), 14(CH₂), 26(-CH=CH) 16(CH₂, +2H) 14(CH₂) as shown in the structure below.

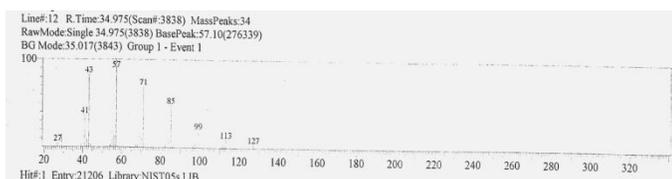


Figure 8: Mass Spectrum of Peak 12 of Figure 7

Figure 8 shows the fragmentation pattern with its major thermostatically molecular mass ion of the fragments as 57 as found in nonane. The mass of fragments and their respective ions loss between the fragmentations are 14(CH₂), 14(CH₂), 14(CH₂), 14(CH₂), 14(CH₂), 2(-H), 14(CH₂) as shown possible in the structure.

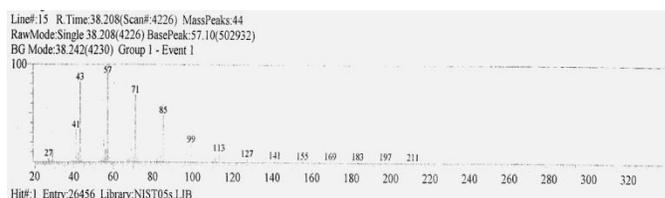


Figure 9: Mass Spectrum of Peak 15 of Figure 6

Figure 9 shows the fragmentation pattern of pentadecane which has its major thermostatically molecular mass ion of the fragments as 57. The mass of fragments and their respective ions loss between the fragmentations are 12[14(CH₂)], 2(-H), 14(CH₂) as shown possible in the structure.

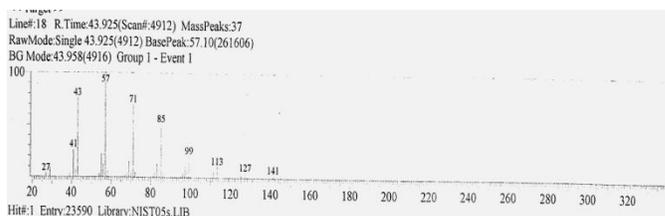


Figure 10: Mass Spectrum of Peak 18 of Figure 6

Figure 10 shows the fragmentation pattern of decane which has its major thermostatically molecular mass ion of the fragments as 57. The mass of fragments and their respective ions loss between the fragmentations are: 9[14(CH₂)] as shown possible in the structure.

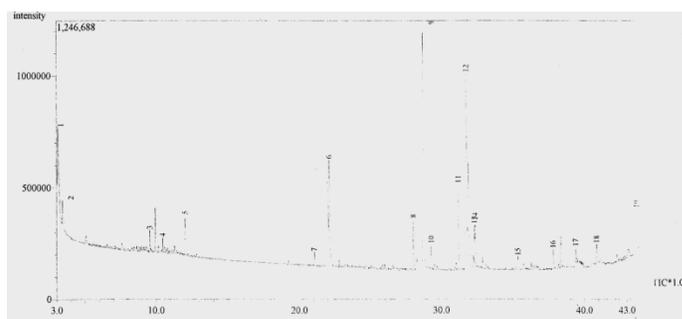


Figure 11: GC-MS Chromatograms of FPE10

Figure 11 shows the chromatogram of FPE10 with major peaks at 6, 9 and 12. Peak 12 was identified as 2,4-octadiene, and peak 7 though not a major was identified as 2,4-di(tert)-butyl-5-methyl phenol.

Some of the compounds identified, such as 2-prop-2-enoyl benzoic acid, methylbenzene and 1,4-dimethyl

benzene, (Table 1), place the plant on high medicinal value. 2-prop-2-enoyl benzoic acid is an effective antifungal, antibacterial and antiseptics agents as earlier reported by Jorg *et al.* (2002). These activities are necessities for fast healing processes of any trouble skin, which agree with the earlier work of Tijani and others (2012), which reported the accelerated wound healing processes of methanolic extract of *C. zeylanicum* bulb. The medicinal potency of methylbenzene and 1,4-dimethyl benzene has been reported, particularly as antidepressants, and being used as disinfectant and as solvents for aiding the penetration of essential oils (Jorg *et al.*, 2002; Cruz *et al.*, 2009).

Table 1: GC-MS Identified compounds from the three solvent fractions extracts

S/No	FME 03	FEE 02	FPE 10
1	2-Methylbutane	Hexanal	2-Methylbutane
2	Methylbenzene	2-methylpentane	Pentane
3	2,3-propanediol	2-prop-2-enoylbenzoic acid	2-oxo-2-phenylmethyl
4	1,4-dimethylbenzene	Heptanal also as peaks	Hexane
5	2-prop-2-enoylbenzoic acid	Hexanal	2-prop-2-enoylbenzoic acid
6	Hexadecanoic acid	4,6-dimethyldodecane	Phehyl,2,4-bis(1-dimethylethyl)-5-methyl
7	Methyl-11-methyldodecanoate	Nonane	Hexadecanoic acid
8	Hexanol	Petadecane	Hexanol
9	1,3-octadiene	Decane	Hexanal
10	1,3-octadiene		2,4-octadiene
11	1,3-octadiene		Propanamide
12	Pentane		
13	Octanal		

The discovery of 2,4-di(tert)-butyl-5-methyl phenol suggest that the plant has the potentials of being used as anti-oxidant, anti-tumor, sores, rheumatism, swelling and inflammatory pain as contributed by Refaat and colleagues (Refaat *et al.*, 2013).

Meanwhile, the presence of hexadecanoic acid (peak 6 of Figure 3, Table 1) suggest that the bulb may serves as possible source of antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavor, hemolytic, 5-alpha reductase inhibitor as earlier reported by Hema *et al.*, (2011) and Omotoso *et al.*, (2014). 2-oxo-2-phenylmethyl (methylbenzoyl formate) increases the interaction between pores wall and active center of a body (Raja *et al.*, 2003)

However, the identification of 2,3-propanediol, hexanol, octanal, hexanal, 2-methylbutane (peak 1, Figure 3), 1,3-octadiene, pentane, 4,6-dimethyldodecane (peak 12, Figure 6), nonane, pentadecane, decane, 2,4-octadiene, 2,2-dimethylbutane and methyl-11-methyldodecanoate (peak 7, Figure 3) in the plant as shown in Table 1 is an indication that the plant can be harnessed in areas of fragrances, flavor and food, and solvents in manufacturing industries (Falbe *et al.*, 2005; Tranzeat *et al.*, 2013).

CONCLUSION

The results of this work have shown that the bulb of *C. zeylanicum* contain many bioactive chemical constituents, and hence has potential uses in treatment of many ailments, particularly in skin diseases.

CONFLICT OF INTEREST

None declared.

REFERENCES

- Bais S, Gill NS, Rana N and Shandil S (2014). A phytopharmacological review on a medicinal plant: *Juniperus communis*. *Int Sch Res Notices* 1-6.
- Burkill HM (1985). The useful plants of west tropical Africa. Families (A-D), 2nd Ed. Royal Botanical Gardens Kew. pp 62-67.
- Chifundera K, Baluku B and Mashimango B (1993). Phytochemical screening and mulluscicidal potency of some Zairean medicinal plants. *Lab Med Plants Herpetol* 28(4): 333-340.
- Cruz S, Chavez PP, Martine ZN and Lopez RC (2009). Toluene has antidepressant-like actions in two animal models used for screening of antidepressant drugs. *Psychopharmacol* 204(2): 279-286.
- Dalziel JM. (1956). Useful plants of west tropical Africa. Crown Agents for Overseas Government, London. pp 242-248.
- Doepke W, Reich E and Sewerin E (1986). Zeylamine a new alkaloid from *Crinum zeylanicum*. *Z Chemie* 26:438.
- Falbe J, Bahrman H, Lipps W and Mayer D (2005). "Alcohols, Aliphatic", *Ullmann's Encyclopedia of Industrial Chemistry*, Weinheim: 2005. Wiley-VCH. Available at: https://en.wikipedia.org/wiki/Hydration_reaction [Accessed 9.9.2016].
- Harborne JB (1985). Phytochemical methods, a guide to modern technique of plant analysis. 2nd Ed. Chapman and Hall, London. pp 1- 192.
- Hema R, Kumaravel S and Lagusundaram A (2011). GC/MS determination of bioactive components of *Murray akeonigii*. *J Am Sci* 7(1): 80-83.

10. Jorg F, Ulrich G and Thomas AS (2002) 'Xylene in Ullmann's Encyclopedia of Industrial Chemistry. Wiley-VCH, Weinheim, pp. 28-433.
11. Omotoso AE, Eseyin OO and Suleiman M (2014). Phytochemical analysis of *Cnidocolusa conitifolius* (Euphorbiaceae) leaf with spectrometric techniques. *Nigerian J Pharm Appl Sci Res* 3(1): 38-49.
12. Raja R, Thomas JM, Jones MD, Johnson BF and Vaughan DE (2003). Constraining organometallic catalysts within mesoporous supports boosts their enantioselectivity. *J Am Chem Soc* 125 (49): 982- 983
13. Refaat J, Abdel-Lateff A, Kamel M S, Ali A A, Ramadan MA, Kino and Nogata Y (2009). Antifouling alkaloids from *Crinum augustum* (Amaryllidaceae). *Pheog Res.* 1:43-52.
14. Strahil B, Stefani R, Maria H, Frances V, Carles C, Georgi M, Iliana I and James B (2011). Anti-proliferative alkaloids from *Crinum zeylanicum*. *Phytother Res* 25:1686-1692.
15. Tijani AY, Salawu OA, Good-luck J, Mbah CC, Madara AA, Ikwuka AC and Chindo B A (2012). Some neuropharmacological effects of *Crinum zeylanicum* in Mice. *Avicenna J Phytomed* 2(3): 162-168.
16. Tijani AY, Salawu, O A, Good-luck J, Mbah CC, Madara AA, Ikwuka AC and Chindo B A (2012). Wound healing activity of *Crinum zeylanicum* L. (Amaryllidaceae) *Phytoparmacol* 3(2): 319-325.
17. Tranzeat L, O'Leary N and Kos W (2013). Malodor counteracting compositions and method for their Patents US 20130164232 A1:2013. Available at: https://www.google.com.ng/?gfe_rd=cr&ei=HfbcV5iPLsnU8gfEwbkw&gws [Accessed 9.9.2016].
18. Tsuda Y, Kashiwaba N and Kumar V (1984). The alkaloidal constituents of Goda Manel (*Crinum Zeylanicum*). *Chem Pharm Bull* 32::3023-3027.
19. Yakandawala DMD and Samarakoon TM (2006). An empirical study on the taxonomy of *Crinum zeylanicum* L. and *Crinum latifolium* L., Amaryllidaceae, Occurring in Sri Lanka. *Ceylon J Sci Biol Sci* 35(1): 53-72.

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