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GC-MS Analysis of Phyto Components from the Stem Bark of *Cola nitida* Schott & Endl

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Abstract: The biochemical constituents of extracts obtained from the stem bark of *Cola nitida* collected from the University of Ibadan, Nigeria is being reported. The ethanol extracts was analysed by Gas chromatography- mass spectroscopy (GC-MS) technique. The main constituents of the extracts were Cycloheptasiloxane tetradeca-methyl (35.287%), Cyclohexasiloxane dodecamethyl (24.941%), Cyclooctasiloxane hexadecamethyl (17.574%), 1H- cycloprop (e) azulen-7-ol-decahydro-1,1,7-trimethyl-4-methylene (7.816%), Cycloconasiloxane octadecamethyl (6.995%), Benzimidazol-5-amine-1-4-ethoxyp (2.265%) and 5-acetyl-2-benzylsulfanyl-6-methyl-nicotinonitrile (1.467%).

Keywords: *Cola Nitida*, GC-MS, Phyto-Medicine

1. Introduction

Evidence exists that plants were used for medicinal purposes some 60,000 years ago. By 3500BC, Ancient Egyptians began to associate less magic with the treatment of diseases and by 2700BC, the Chinese had started to use herbs in a more scientific sense (1). The studies of botany and medicine became very closely linked during the Middle Ages virtually all reading and writing were carried out in the monasteries. Monks laboriously copied and compiled the manuscripts and prepared herbals that described identification and preparation of plants with reported medicinal characteristics. (2). Herbal medicine has become a popular form of healthcare; even though several differences exist between herbal and conventional pharmacological treatments, herbal medicine needs to be tested for efficacy using conventional trial methodology and several specific herbal extracts have been demonstrated to be efficacious for specific conditions. Nevertheless the public is often misled to believe that all natural treatments are inherently safe, herbal medicines do carry risks, so research in this area must be intensified. The main question that has not been often answered satisfactorily deals with the triad absorption/metabolism/efficacy/biochemical constituent of herbs and their extracts which is actually an important unsolved problem in judging their many alleged health effects (3). The search for newer sources of antifungal and antibacterial is a global challenge pre occupying research institutions, Pharmaceutical companies and the academia, since many infectious agents are becoming resistant to synthetic drugs (4)

Cola Schott & Endl. (*Sterculiaceae*) is a genus of about 125 species of tree indigenous to the tropical rain forest.(5). Various medicinal and pharmacological values have been observed in specie of *Cola nitida nuts* extract on elastase/ alpha-1-proteinase inhibitor alone (6) and currently anti-dermatophytic activities as reported by (7). *Cola* has been reported to have very high medicinal values; it has been attributed to the treatment of ringworm, scabies, gonorrhea, dysentery and opthalmia (8). Worthy of note also is the reports of Kim (9) and Jayeola for soft drinks production (10) that alluded to *Cola* been used as a remedy for whooping cough and asthma. *Cola nitida* possess antifungal properties against dermatophytes (*Trichophyton rubrum*, *Trichophyton tonsurans*) and *Candida albicans* as reported by (7). This paper therefore reports the bioactive compounds present in *Cola nitida* ethanol stem bark extracts by GC-MS analysis.

2. Materials and Methods

Cola nitida stem bark were obtained from the University of Ibadan and authenticated at the Department of Botany where a voucher specimen UIH-22487 was prepared and deposited. Dried stem bark were subjected to soxhlet extraction with distilled ethanol as extraction solvent. Extract was filtered, evaporated to dryness in-vacuo, weighed and stored.

3. Analysis of Organic Compounds in the Plant Extracts (Gas Chromatography Mass Spectrometry)

The GC-MS analysis of the stem bark extract of Cola nitida was carried out at the department of Chemical Engineering, University of Ilorin on Agilent 19091S Gas chromatograph (GC) interfaced to a mass spectrometer 433HP-5MS instrument employing the following conditions: silica capillary column fused with 100% phenyl methyl silox, (length; 30m x 250µm; film thickness 0.25µm). For GC-MS detection, an electron ionization system with ionization energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1.5ml/min and an injection volume of 1µl was employed (Split ratio of 50:1) injector temperature-300oC; average velocity of 45.67 cm/sec. The oven temperature was programmed from 100oC (Isothermal for 4 min.) with an increase of 4oC /min to 240oC. Total GC running time was 49 minutes. The relative percentage amount of each component was calculated, by comparing its average peak area to the total areas. The software adopted to handle mass spectra and chromatogram was a turbomass. The detection employed the NIST Ver. 2.0 year 2009 library (Paranthaman et al., 2012). After the performance of the GCMS was the identification of the components detected using their spectra.

4. Identification of Components

Interpretation on mass spectrum of GC-MS was done using the database of National Institute of Standard and Technology (NIST) which contains more than 62,000 patterns. The mass spectra of the unknown components were compared with the spectrum of the known components contained in the NIST library. The name, molecular weight and structure of the components of the test materials were also ascertained using the fragmentation patterns they exhibited and the information available in the library.

5. Results and Discussion

The biochemical constituents obtained from the stem bark of *Cola nitida* from the University of Ibadan, Nigeria are being reported. The ethanol extracts was analysed by Gas chromatography- mass spectroscopy (GC-MS) technique. The main constituents of the extracts were Cycloheptasiloxane tetradeca-methyl (35.287%), Cyclohexasiloxane dodecamethyl (24.941%), Cyclooctasiloxane hexadecamethyl (17.574%), 1H-cycloprop(e)azulen-7-ol-decahydro-1,1,7-trimethyl-4-methylene (7.816%), Cycloconasiloxane octadecamethyl (6.995%), Benzimidazol-5-amine-1-4-ethoxyp (2.265%) and 5-acetyl-2-benzylsulfanyl-6-methyl-nicotinonitrile(1.467%). Advances in high-throughput experimentations have resulted in massive databases of genomic, proteomic and chemical data which in combination with efficient separation methods and powerful spectrometric methods for identification and structure elucidation can be used for identification of active compounds using DNA microarray only (11)

GC-MS chromatogram of *Cola nitida* stem bark extract (table 1, 2 and 3) showed nine(9) peaks indicating the presence of nine compounds. The chemical compounds identified in the extract of the stem bark of *Cola nitida* are presented in Table 1.

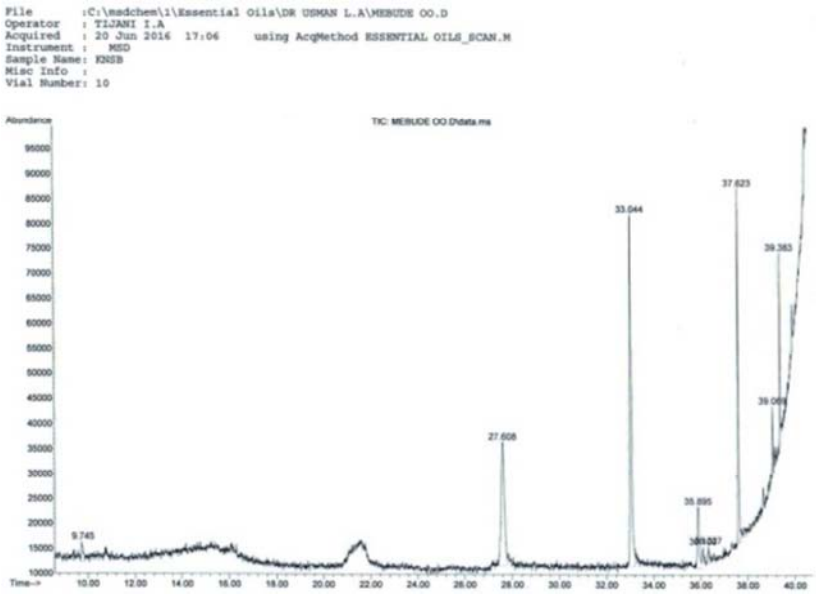
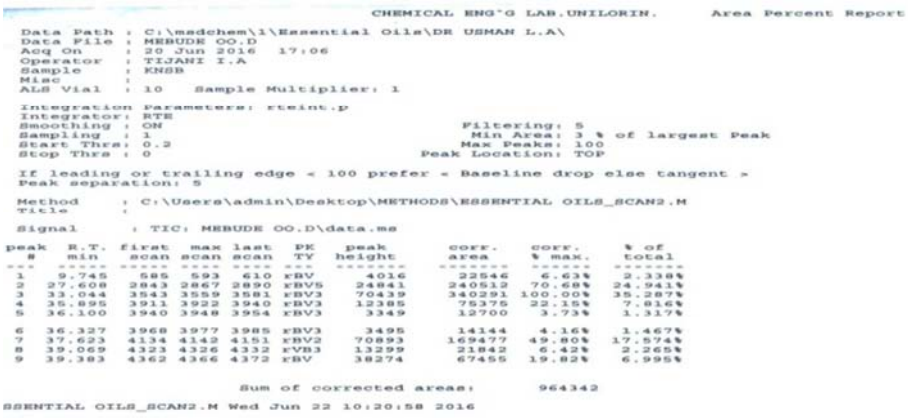


Figure 1. *GC-MS chromatogram of Cola nitida stem bark extract.*



```
                                    CHEMICAL ENG'G LAB.UNILORIN.      Area Percent Report

Data Path : C:\msdchem\1\Essential Oils\DR USMAN L.A\
Data File : MEBUDE OO.D
Acq On    : 20 Jun 2016  17:06
Operator  : TIJANI I.A
Sample    : KNSB
Misc      :
ALS Vial  : 10   Sample Multiplier: 1

Integration Parameters: rteint.p
Integrator: RTE                                  Filtering: 5
Smoothing : ON                                   Min Area: 3 % of largest Peak
Sampling  : 1                                    Max Peaks: 100
Start Thrs: 0.2                                Peak Location: TOP
Stop Thrs : 0

If leading or trailing edge < 100 prefer < Baseline drop else tangent >
Peak separation: 5

Method    : C:\Users\admin\Desktop\METHODS\ESSENTIAL OILS_SCAN2.M
Title     :

Signal    : TIC: MEBUDE OO.D\data.ms

peak   R.T.  first  max  last   PK   peak      corr.    corr.    % of
  #    min   scan  scan  scan   TY  height     area     % max.   total
---  ------ ----- ----- ----  ---  -------   -------   ------   ------
1     9.745   585   593   610  rBV     4016    22546    6.63%    2.338%
2    27.608  2843  2867  2890  rBV5   24841   240512   70.68%   24.941%
3    33.044  3543  3559  3581  rBV3   70439   340291  100.00%   35.287%
4    35.895  3911  3922  3940  rBV3   12385    75375   22.15%    7.816%
5    36.100  3940  3948  3954  rBV3    3349    12700    3.73%    1.317%

6    36.327  3968  3977  3985  rBV3    3495    14144    4.16%    1.467%
7    37.623  4134  4142  4151  rBV2   70893   169477   49.80%   17.574%
8    39.069  4323  4326  4332  rVB3   13299    21842    6.42%    2.265%
9    39.393  4362  4366  4372  rBV    38274    67455   19.82%    6.995%

                              Sum of corrected areas:     964342

SSENTIAL OILS_SCAN2.M Wed Jun 22 10:20:58 2016
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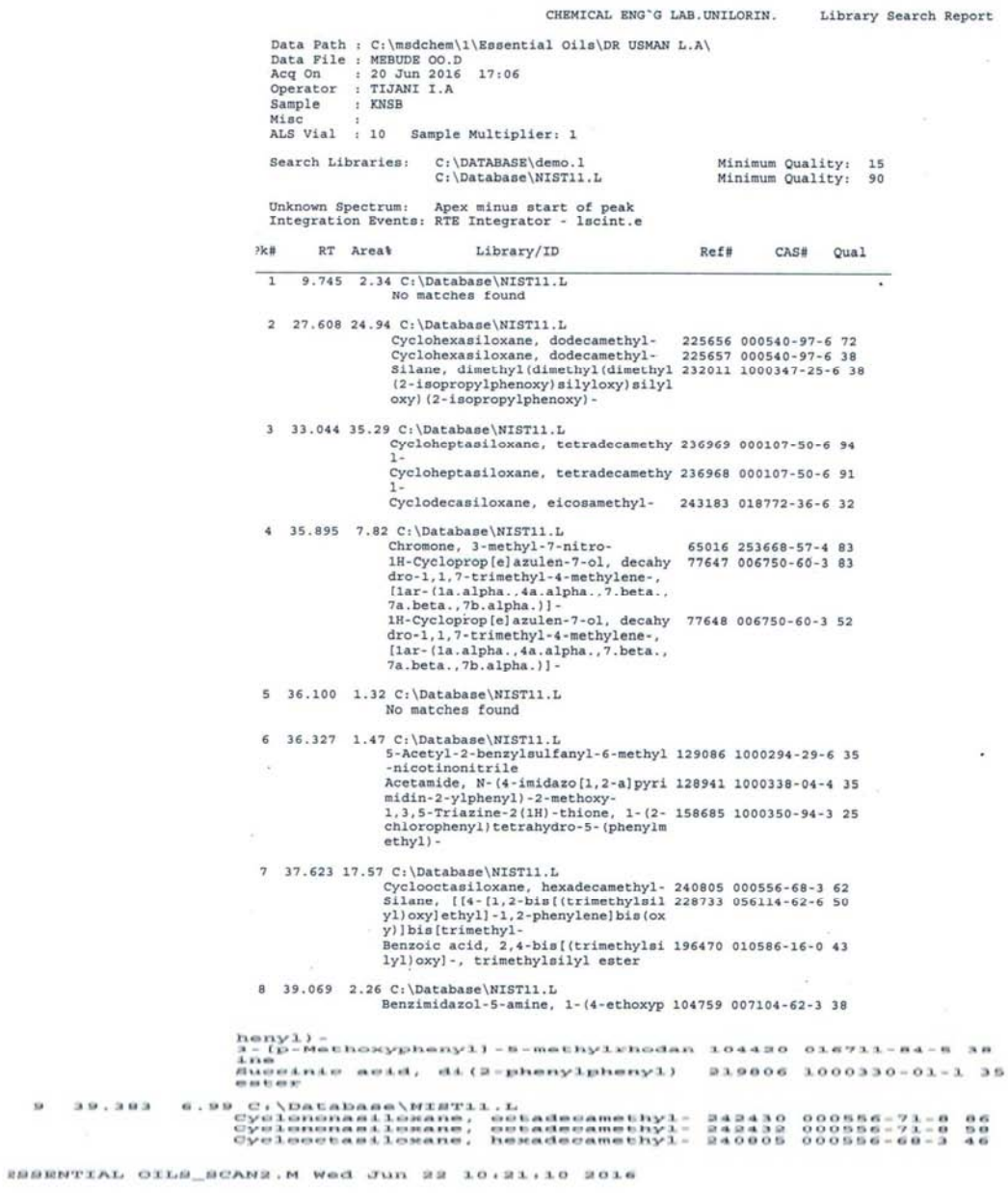
Figure 2. *Showing retention time and peak area of essential oil.*



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                                    CHEMICAL ENG'G LAB.UNILORIN.      Library Search Report

Data Path : C:\msdchem\1\Essential Oils\DR USMAN L.A\
Data File : MEBUDE OO.D
Acq On    : 20 Jun 2016  17:06
Operator  : TIJANI I.A
Sample    : KNSB
Misc      :
ALS Vial  : 10   Sample Multiplier: 1

Search Libraries:   C:\DATABASE\demo.l              Minimum Quality:  15
                    C:\Database\NIST11.L            Minimum Quality:  90

Unknown Spectrum:   Apex minus start of peak
Integration Events: RTE Integrator - lscint.e

Pk#     RT  Area%          Library/ID                    Ref#     CAS#    Qual
---------------------------------------------------------------------------

 1   9.745  2.34 C:\Database\NIST11.L
                 No matches found

 2  27.608 24.94 C:\Database\NIST11.L
                 Cyclohexasiloxane, dodecamethyl-   225656 000540-97-6 72
                 Cyclohexasiloxane, dodecamethyl-   225657 000540-97-6 38
                 Silane, dimethyl(dimethyl(dimethyl 232011 1000347-25-6 38
                 (2-isopropylphenoxy)silyloxy)silyl
                 oxy)(2-isopropylphenoxy)-

 3  33.044 35.29 C:\Database\NIST11.L
                 Cycloheptasiloxane, tetradecamethy 236969 000107-50-6 94
                 l-
                 Cycloheptasiloxane, tetradecamethy 236968 000107-50-6 91
                 l-
                 Cyclodecasiloxane, eicosamethyl-   243183 018772-36-6 32

 4  35.895  7.82 C:\Database\NIST11.L
                 Chromone, 3-methyl-7-nitro-         65016 253668-57-4 83
                 1H-Cycloprop[e]azulen-7-ol, decahy  77647 006750-60-3 83
                 dro-1,1,7-trimethyl-4-methylene-,
                 [1ar-(1a.alpha.,4a.alpha.,7.beta.,
                 7a.beta.,7b.alpha.)]-
                 1H-Cycloprop[e]azulen-7-ol, decahy  77648 006750-60-3 52
                 dro-1,1,7-trimethyl-4-methylene-,
                 [1ar-(1a.alpha.,4a.alpha.,7.beta.,
                 7a.beta.,7b.alpha.)]-

 5  36.100  1.32 C:\Database\NIST11.L
                 No matches found

 6  36.327  1.47 C:\Database\NIST11.L
                 5-Acetyl-2-benzylsulfanyl-6-methyl 129086 1000294-29-6 35
                 -nicotinonitrile
                 Acetamide, N-(4-imidazo[1,2-a]pyri 128941 1000338-04-4 35
                 midin-2-ylphenyl)-2-methoxy-
                 1,3,5-Triazine-2(1H)-thione, 1-(2- 158685 1000350-94-3 25
                 chlorophenyl)tetrahydro-5-(phenylm
                 ethyl)-

 7  37.623 17.57 C:\Database\NIST11.L
                 Cyclooctasiloxane, hexadecamethyl- 240805 000556-68-3 62
                 Silane, [[4-[1,2-bis[(trimethylsil 228733 056114-62-6 50
                 yl)oxy]ethyl]-1,2-phenylene]bis(ox
                 y)]bis[trimethyl-
                 Benzoic acid, 2,4-bis[(trimethylsi 196470 010586-16-0 43
                 lyl)oxy]-, trimethylsilyl ester

 8  39.069  2.26 C:\Database\NIST11.L
                 Benzimidazol-5-amine, 1-(4-ethoxyp 104759 007104-62-3 38

henyl)-
3-(p-Methoxyphenyl)-5-methylrhodan 104430 016711-84-5 38
ine
Succinic acid, di(2-phenylphenyl)  219806 1000330-01-1 35
ester

 9  39.393  6.99 C:\Database\NIST11.L
                 Cyclononasiloxane, octadecamethyl- 242430 000556-71-8 86
                 Cyclononasiloxane, octadecamethyl- 242432 000556-71-8 58
                 Cyclooctasiloxane, hexadecamethyl- 240805 000556-68-3 46

ESSENTIAL OILS_SCAN2.M Wed Jun 22 10:21:10 2016
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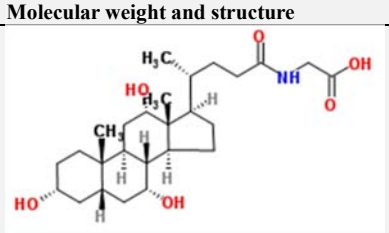
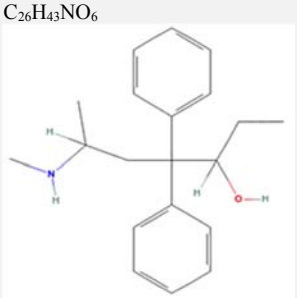
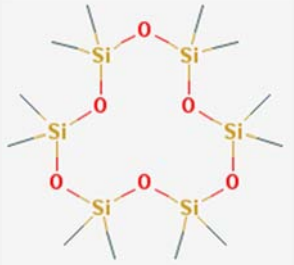
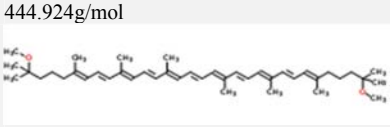
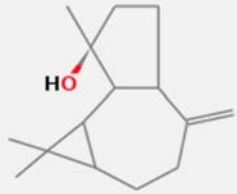
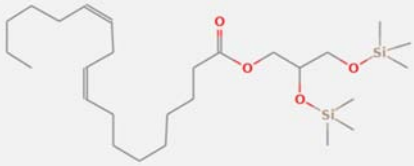
Figure 3. *Showing compounds present in the extract.*

Table 1. *Showing the COMPOUNDS PRESENT IN ETHANOL EXTRACT OF Cola nitida stem bark.*

Name of Compound and Molecular Formula	Molecular weight and structure	Uses
Glycocholic Acid	 $C_{26}H_{43}NO_6$	Acidifier Acidulant
Alpha-N-Normethadol ($C_{20}H_{27}NO$)		An opoid anagelsic and antitussive agent,
Cyclohexasiloxane,dodecamethyl (D6) ($C_{12}H_{36}O_6Si_6$)	 444.924g/mol	Used in personal care products such as hair/skin care products, antiperspirants and deodorants. Antibacterial, Antifungal.
Psi psi,carotene,1,1,2,2-tetrahydro-1,1-dimethoxy($C_{42}H_{64}O_2$)	 600.956	Adulticidal, Repellant, Antibacterial, Antifungal, Anti-inflammatory
Spathulenol	 220.3505	Natural colourant for food and Nutraceuticals, Adulticidal, Anticancer, Antitumor, Anti-inflammatory
1-Monolinoleoylglycerol trimethylsilyl ether ($C_{27}H_{54}O_4Si_2$)	 498.89	Antimicrobial, Antioxidant, Antiinflammatory, Antiarthritic, Antiasthma, Diuretic.

**Source: -Dr. Duke's Phytochemical and Ethnobotanical Databases. Compounds were ran through Dr Dukes database to get the properties of each compound.

The result of the present investigation reveals that the ethanolic extract of Cola nitida stem bark possessed significant anti-inflammatory, anti-oxidant, antitumor, and antimicrobial properties. The various biological activities of the compound present in the ethanolic extract of Cola nitida stem bark suggests its various activities as reported by (7), (9), (10).

The presence of steroid in the extract with strong antimicrobial, anti-oxidant and diuretic properties with the presence of Linoleic acid which confers hepato-protective and antihistaminic effect is worthy of note and it is corroborated by the work of (12) on *Pleiosermium alatum* and (13) on *Senna alata* that extracts with compounds found in *Cola nitida* possesses all the properties stated above. The presence of D_6 (Cyclohexasiloxane, dodecamethyl) which is used in personal care products, anti-perspirant and antifungals alluded to the report of (7) that the plant extract of *Cola nitida* is an effective antifungal agent in the treatment

of fungi infection and a promising alternative/adjunct/ supplement to the azole and allylamine group.

6. Conclusion

In conclusion, this study has shown that the ethanolic extract of *Cola nitida* stem bark possesses various compounds of interest microbiologically and pharmaceutically. Further elucidation to structurally identify the compounds using NMR and isolation of active compounds conferring on the plant the various microbiological attributes is ongoing

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