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GC-MS analysis and identification of constituents present in the root extract of *Mitragyna inermis*

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Abstract
The active compounds of the acetone extract of *Mitragyna inermis* were isolated using solvent- solvent extraction and column chromatography. Seven phytochemical constituents have been identified by GC-MS analysis by comparing the fragmentation of the unknown compounds with that of library database. The compounds identified are Ethanone 1-(2,3,4trimethylphenyl)-, 4H-pyran-4-one-3-acetyl-, 2-6-dimethyl, 3-acetyl pentan-2,4-dione, and phenol, 2,5-dimethyl- acetate.

Keywords: *Mitragyna inermis*, GC MS analysis, fragmentation pattern

1. Introduction
Medicines derived from plant have been used by man in traditional medicine throughout the ages because they are cheap, available and their holistic treatment and scientists in various part of the world concentrate on the study of these remedies in order to use them as an alternative to expensive and imported drugs (Sofowora, 1993) [8]. Plant have ability to synthesis medicine wide variety of chemical compounds that are used to perform important biological functions and to depend against attack from predators such as insects, fungi and herbivorous mammals (Tapsell *et al.*, 2006) [10]. At least 12, 000 of such compounds have been isolated which less than 100% of the total compounds (Tapsell *et al.*, 2006) [10]. Ethnomedicinal studies is also receiving more attention because of the side effect of some of the drugs in used and resistant by microorganism (Catlin *et al.*, 1982) [4]
Mitragyna inermis is a shrub or tree with a dense, wide crown. Grown in the sub-Sahara Africa (Konko *et al.*, 2008 [6], and Burkill *et al.*, 1985) [2]. It is called giyayya in Hausa, *Mitragyna inermis* is a bushy tree and grows up to 16m high, it bole is up to 60cm in diameter with branches usually from low (Burkil 1985) [2]. *Mitragyna inermis* is grown on dump perennially flooded site, swampy savannah or inland site of coastal mangrove (Adoum, 2012) [1]. The plant is common across the region from Mauritania to west Cameron and in to the Congo basin and Sudan (Burkil, 1985) [2].
Mitragyna inermis is widely known and use in traditional medicine in West African to treat several disease (Konko *et al.*, 2008) [6]. The leaves and bark are febrifuge (Von 1990) [11]. The plant is diuretic. it is used in the treatment of various conditions including constipation, stomach disorder, dysentery, rheumatism, malaria, gonorrhoea, syphilis, leprosy, bilharzias, jaundice, mental disorder and epilepsy (Von 1990) [11].
A lot of analytical techniques are available for the separation, identification and characterization of phytochemicals. GC-MS has proven to be a valuable methods for the analysis of non polar, volatile essential oils, lipids and alkaloids (Mythili *et al.*, 2013) [7]. This study aim at isolation of phytoconstituents present in the root extract of *Mitragyna inermis*

2 Materials and Methods
2.1 Plants Identification
The Plants were identified by taxonomist at the herbarium of Ahmadu Bello University Zaria, Nigeria and corresponds to the voucher numbers of 259.

2.2 Sample Collection and Treatment
Fresh root of *Mitragyna inermis* was collected from Hadejia-Nguru wet land Area of Jigawa State. It was washed in water and re-washed in distilled water, air dried and ground to fine powder.

2.3 Method of Extraction
Finely grounded root of *Mitragyna inermis* (100g) was soaked in n-hexane (1000ml) with

occasional stirring for 48hrs, the soaked material was filtrated and the extract was concentrated using rotary evaporator and air dried, weighed and kept for further uses. The residue after extraction with hexane was soaked in ethyl acetate for 48hrs with occasional stirring for 48hrs, the mixture was then filtered and the filtrate was concentrated using rotary evaporator and dried in moist free environment, weighed and kept for further uses. The residue was soaked in acetone for 48hrs followed by filtration, concentration, drying and weighing.

2.4 Isolation and purification

The acetone extracts of the root of *Mitragyna inermis* was washed in hexane, ethyl acetate, acetone and methanol to obtained hexane extract of the acetone extract, ethyl acetate extract of the acetone extract, and acetone extract of acetone

The ethyl acetate extract of the acetone above was load on column guided by TLC and eluted with methanol: chloroform (1:1), the procedure give 9 fractions (1-9) and TLC was performed on all the 9 fractions. Fraction 8 show single spot and it was derivatized using acetic un hydride and Zinc chloride before subjected to GC MS analysis. Derivatization will render highly polar material to be sufficiently volatile so that can be eluted at reasonable temperature without thermal decomposition (Knapp 1979) [4].

2.5 Component Identification

The constituent of the extract was identify by matching the peak computer libraries and confirmed by comparing mass spectra of the peaks and those from literature.

3. Results

Table 1: Molecular weight and important ions present in the mass spectra of acylated compounds in *Mitragyna inermis* root extract.

Name of the proposed identity	R.T	Fragments and their relative abundance
1-(2,3,4 trimethyl phenyl) Ethanone	13.286	147(100%), 119(51.9%) 93(22.2%) 53(5.5%) 162(24.1%)
Phenol 2,5 – dimethyl acetate	12.242	164(24%), 122(100%) 103(46.9%), 78(14.3%), 94(8.1%) 66(6.1%)
3- acetate pentane-2,4-dione	7.628	14z2 (24%), 85(100%), 127(32%), 100(6%)
4H-pyran 4-one- 3-acetyl-2,6-dimethyl	16.493	67(100%) 124(12%) 148(14%) 109(42.1) 85(10%)

Discussion

1-(2, 3, 4, trimethyl phenyl) ethanone

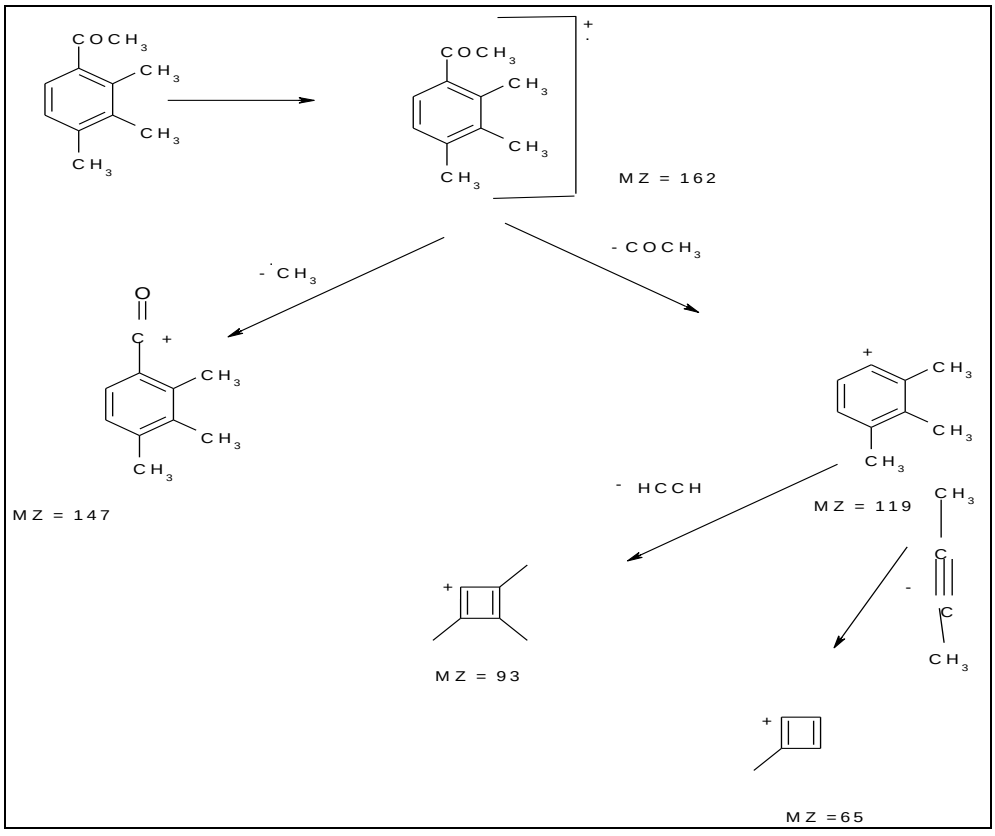

Fig 1: Fragmentation pattern of Ethanone 1-(2, 3, 4 trimethylphenyl)-,

1-(2, 3, 4-trimethyl phenyl) ethanone, this fragment by loss of an electron to form a molecular ion with mass of MZ=162 which further fragment by loss of CH_3 (15) and $COCH_3$ to give ion with mass of M/Z=147(base peak) and M/Z=119 respectively and the ions with mass of 119 fragment further by loss of ethyne and butyne to give ions with mass MZ=93 and M/Z=65.

4H-pyran-4-one-3-acetyl-2-6- dimethyl

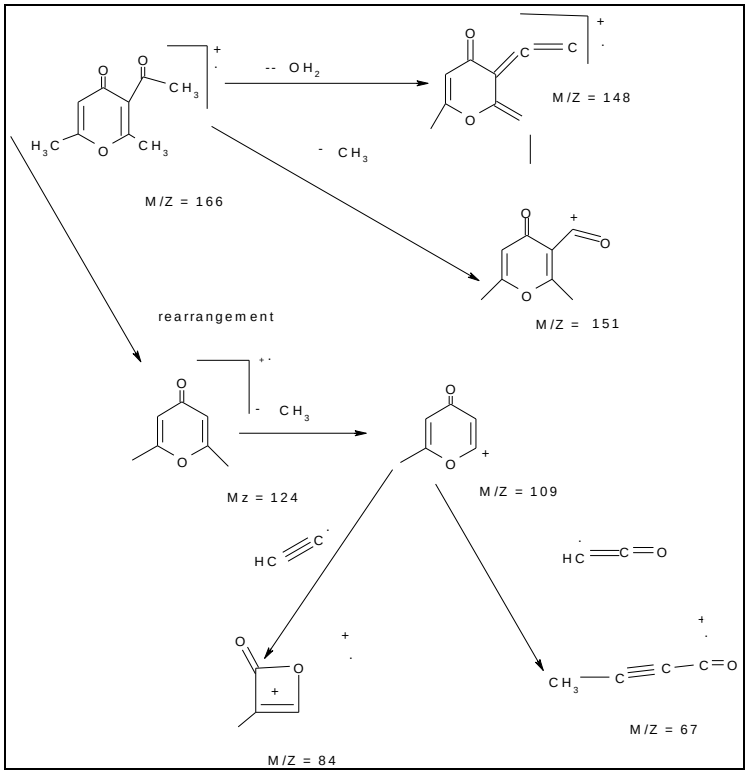

Fig 2: Fragmentation pattern of 4H-pyran-4-one-3-acetyl-2-6- dimethyl,

4H-pyran-4-one-3-acetyl-2-6- dimethyl, loss of H_2O from molecular ion (MZ=166) give ion with mass of M/Z =148, loss of methyl radical from the molecular ion give ion with massM/Z= 151 and Mclafferty rearrangement of molecular ion give ion with mass of 124 which fragment further by loss of methyl radical to give ions with M/Z = 109, ion mass of 109 fragment to give ions with masses of 67 and 85 respectively.

3-acetate pentane -2, 4- dione

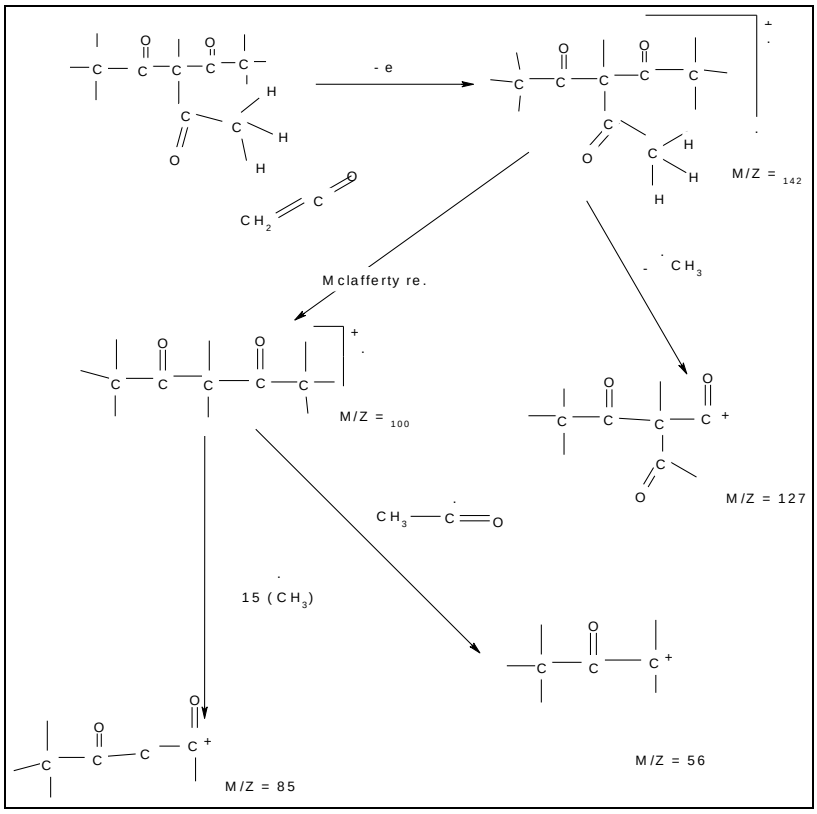

Fig 3: Fragmentation pattern of 3-acetyl pentan-2,4-dione,

Mass spectrum of 3-acetate pentane -2,4- dione show molecular ion with mass M/Z =142, the loss of methyl radical from the molecular ion give rise to a fragment with mass ofM/Z= 127. The Mclafferty rearrangement of the molecular

ion give ion with mass of M/Z =100 which fragment further by loss of methyl radical to give ion with mass of 85.

Phenol 2, 5- dimethyl acetate

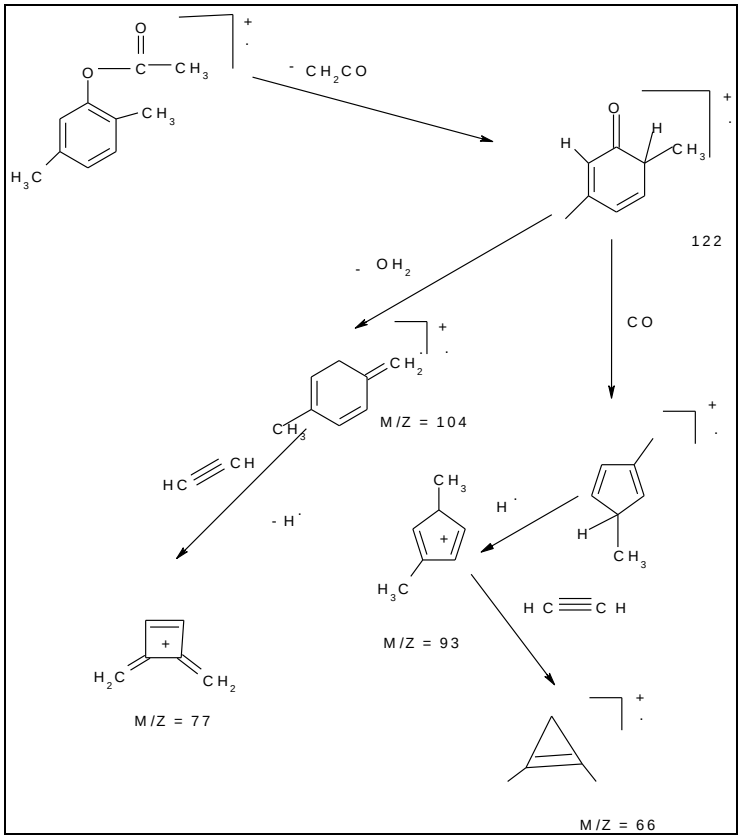

Fig 4: Fragmentation pattern of phenol, 2,5-dimethyl- acetate

The Mclafferty rearrangement of phenol 2, 5 dimethyl give phenolic ion as molecular ion with mass of M/Z = 122. The molecular ion fragment further through two pathways.

1. The loss of water (18) from the molecular ion give ion with mass of M/Z=104, which further fragment further by loss ethyne (26) to give ion with mass of 77.

The Molecular ion also fragment by loss of CO(28) to give ion with mass of 94 which fragment further by loss of ehtyne (26) to give ion of mass of 66.

Conclusion

The result show that extract of the root of *Mitragyna inermis* contain Ethanone 1-(2,3,4trimethylphenyl)-, 4H-pyran-4-one-3-acetyl-2-6- dimethyl, 3-acetyl pentan-2,4-dione, and phenol, 2,5-dimethyl- acetate which may be responsible for the antimicrobial activity of the plant.

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