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RESEARCH ARTICLE

A Study on Endophytic Fungi, Proximate and Chemical Compositions of a Local Variety of Mango Fruit (*Mangifera indica* L.)

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Abstract: Mango (*Mangifera indica* L.) is an important tree which produce edible fruits. Different varieties of mango are available and well-studied. However, little attention has been shown to popular Ogbomoso variety especially for its endophytes and chemical profile. This work aimed at identifying endophytic fungi and carrying out proximate analysis as well as phytochemical analysis of the fruit. Potato Dextrose Agar was used as a medium for the isolation of fungi from the homogenized pulp. Proximate analysis was carried out using standard method of Association of Analytical Chemistry (AOAC). Fourier Transform Infrared Spectroscopy (FTIR) was done to determine characteristic peaks and functional groups of compounds using Thermo Scientific Nicolet iS5 with iD1 transmission. Gas Chromatography- Mass Spectrometry (GC-MS) was used to study the chemical profile of the sample. *Aspergillus niger*. A. *flavus and Rhizopus stolonifer* were the identified endophytes. Moisture and lipid were observed to be highest (76.87%) and lowest (0.38%) respectively in the sample. The amount of carbohydrate was 18.93%. The FTIR result revealed the presence of NH_2, OH, CH_2, C=O (carboxylic) and N-H 1o and 2o amine as important functional groups. A total of 23 compounds were found in the methanolic extract of the sample where Imidazolidin-2-one (18.43%) was the principal compound. N, N-dibenzylhydroxylamine (15.36%), Methyl hexadecanoate (7.33%), Hexadecamethylcyclooctasiloxane (4.51%) were also present in considerable quantities. It is confirmed that the homogenized pulp of Ogbomoso variety of mango fruit is rich in phytochemicals that are useful especially in pharmaceutical industries.

Keywords: *Chemical composition, Endophytes, Mangiferaindica, Proximate analysis, GC-MS*

Introduction

Mango (*Mangifera indica* L.) is a common and an important fruit in both tropical and subtropical regions (Girma *et al*., 2016). It belongs to family Anacardiaceae (Abdulrahman, 2013). The fruit tree is indigenous to the Indian subcontinent, Southeast Asia and Africa (Fowomola *et al*. 2010). It can be propagated by seed and grafting. The tree is erect with alternately arranged evergreen leaves. It produces an edible fruit with stony seed. There are a number of mango varieties differed from one another by not only the fruit shape and textural colour but also aroma which is a prominent characteristic.

Endophytes are the organisms, mostly bacteria and fungi, which live internally in healthy host plants displaying no symptom of disease. These organisms are widely spread and commonly found in all plant species but underexplored (Nisa *et al*., 2015). A symbiotic relationship exists between host and endophytes as the former protects and feed the companion and the latter produces bioactive substances that enhance the growth and competitiveness of the host (Carroll, 1988). These bioactive are also useful in pharmaceutical industries as antibiotics, anticancer, anti-inflammatory, immunosuppressant and many other uses (Priti*et al*., 2009).

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Mango fruit is anatomically sectionalized into epicarp, mesocarp and endocarp. The fleshy mesocarp is commonly used and transformed in food industries to juice, pulp and mango slices for canning. The fruits are rich in nutrients especially vitamin A and ascorbic acid. The physical appearance, texture, flavour and chemical composition are qualities that enhance its acceptability and demand by the consumers (Pleguezuelo *et al.*, 2012; Girma *et al*., 2016). Mango is fondly called super or king fruit because of its usefulness as food, fragrance and ingredient in functional foods (Kittiphoom *et al*., 2012). Mango fruits help in detoxification process, enhancing human complexion and contain an amino acid called tryptophan which is a precursor in serotonin formation, happiness hormone (Ubwa *et al*., 2014).

One popular variety of mango that is commonly taken with high preference in South-West and part of North-Central Nigeria is "Ogbomoso" variety. The demand for the variety is very high because of its innate physical and chemical properties such as shape, appearance, texture, colour, aroma and sweetness. Little information is known about the endophytic fungi and overall chemical composition of the fruit. Besides, data on chemical profile of different varieties mango fruits in Nigeria is scanty (Ubwa *et al*., 2014). This research work aimed at assessing the endophytic fungi, analyzing the proximate elements and unmasking the phytochemicals present in Ogbomoso variety of mango fruit.

Materials and methods

Collection of Mango Fruits

Mature and ripe mango fruits (Ogbomoso varieties) used were harvested at a farm located in Okubi village, Oyo State. The mangoes were collected into a sterilized plastic crate and transported to the Departments of Plant Biology and Chemistry Laboratories, University of Ilorin for subsequent analyses.

Isolation and Identification of fungi

The method of Amadi *et al*. (2014) was adopted. The fruit samples were surface sterilized with 70% ethanol and rinsed in two changes of sterile distilled water. The portions of mesocarp were cut off with sterilized scalpel and homogenized using sterilized laboratory blender. The homogenized sample was aseptically transferred into a sterile beaker, wrapped tightly with aluminium foil and stored under aseptic condition for subsequent use.

Potato Dextrose Agar (PDA) was used as medium for the isolation. The medium was prepared following manufacturer's procedure. Chloramphenicol (30mg/l) was added to inhibit bacterial growth (Adamu *et al*., 2009). Sterile molten medium in petri dishes were inoculated with 1ml of homogenous sample and spread evenly over the surface of the medium. The inoculated plates were incubated at room temperature for 5day observations were made every day. Observed colony development was sub-cultured promptly and bottle slants prepared for storage.

The morphology of the colonies was observed. Slides of pure cultures were prepared for microscopic view. Detailed morphological characteristics of the fungi such as hyphae (septation), reproductive structure (sporangia/conidia) in chain or single and the type of spore were observed and recorded.

Proximate Analysis

Proximate analysis of homogenized pulp was carried out and Moisture, crude protein (micro-Kjeldahl), crude fiber and lipid (Soxhlet) and ash contents were determined using the method as adopted by Orjajogun*et al*. (2014). Total carbohydrate was determined by difference.

Fourier Transform Infrared (FTIR) Spectroscopy

The homogenized pulp of the sample was used for the analysis to identify the types of chemical bonds (functional groups) present in the sample. This was done using FTIR (Thermo scientific Nicolet iS5 with iD1 transmission) spectrophotometer using KBr disc in the range of 4000 cm^{-1} to 400 cm^{-1}. A drop of the pulp was encapsulated with KBr pellet in order to prepare a translucent sample disc which was loaded in FTIR spectrometer.

Gas Chromatography- Mass Spectrometry (GC-MS)

The homogenized pulp of mango fruit was treated with methanol until it was fully dissolved. The GC-MS analysis was carried out using Agilent Technologies 7890A Gas Chromatograph equipped to a mass detector Agilent Technologies 5975C inert MSD with Tripple-Axis Detector, 30m x 0.25mm ID x 1µm of capillary column. The instrument was set to an initial temperature of 110ºC and maintained at this temperature for 2 min. At the end of this period the oven temperature rose up to 280ºC, at the rate of an increase of 5ºC/min, and maintained for 9 min. injection port temperature was ensured as 250ºC and Helium flow as one ml/min. The ionization voltage was 70eV. The samples were injected in spilt mode as 10:1. Mass spectral scan range was set at 45-450(m/z).

Using computer searches on a National Institute Standard and Technology (NIST Ver. 2.1 MS) data library and comparing the spectrum obtained through GC-MS, compounds present in the sample were identified. The names, retention time, molecular formula of the components of the test materials were ascertained.

Results and Discussion

Endophytic fungi

Three endophytic fungi were isolated from the mesocarp of the fruits and identified as *Aspergillus flavus, A. niger* and *Rhizopus stolonifer*. Endophytes are known to produce secondary metabolites with significant activities. The presence of endophytic fungi in mango fruits is a good indicator that bio-active substances may be present in the fruit. Hipol*et al*. (2014) isolated *Aspergillus* spp. as endophytes and confirmed antioxidant potential of these groups of fungi. These antioxidants also play important roles in nutritive values of the fruits. GC--MS analysis reveals production of 2--Phenylethanol from *Aspergillus niger* endophytic in rose which is an essential resource in antiseptics, disinfectants, anti-microbials and preservative in pharmaceuticals (Wani *et al*., 2010). Prasanth *et al*. (2016) confirmed the production of secondary metabolites by endophytic *A. flavus* with antimicrobial properties. Also, *R. stolonifer* produces compounds which have ability to inhibit the growth of bacterial and fungal strains (Sohail *et al*., 2014).

Proximate Analysis

The results of proximate analysis revealed that the homogenized pulp of the mango fruit had moisture content of 76.87% and this was higher than other proximate elements. Both lipid and protein were present in infinitesimal amounts of 0.38% and 0.86% respectively. The amount of carbohydrate was considerably higher (18.93%), second to moisture. Ash and fiber were also present in small amount. The result of proximate analysis was summarized in Table 1.

The percentage of moisture in the sample was higher than other proximate component and this agreed with the result of Girma *et al*. (2016) and Ubwa *et al*. (2014). High water content is usually responsible for quick spoilage of the fruit (Gunawan, 2013) and this by implication, causes short shelf-life (Nwofia *et al*., 2012). Abulude *et al*. (2006) reported that high moisture in fruits encourages microbial spoilage. The protein content of this variety of mango is very low and not a good source of protein following the finding of Aberoumand (2010) who revealed that any food, of plant origin, that provide more than 12% of their calorific value from protein are a good source of protein. Also, the crude fat in the fruit is very low. Excess consumption of fat leads to cardiovascular disorders (Aruah *et al*, 2011).

Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR spectrum of the mango is shown in Fig. 1 and the selected peaks that characterized the mango pulp is presented in Table 2. In the spectrum showed in Fig. 1, the peak at 3626.39 cm^{-1} was assigned to NH_2 in the OH environment at 3192.61 cm^{-1}. The peak at 2926.65 and 1457.52 cm^{-1} were associated with $–CH_2$ stretching and deformation respectively (Ashokkumar and Ramaswamy, 2014). The peaks at 1641.16 and 1457.52 cm^{-1} were due to are asymmetric and symmetric stretching vibrations of C=O in carboxylic groups (Farinella *et al*., 2007; Yang *et al*., 2014). The observed peaks at 1137.73 and 1058.58 cm^{-1} may be due to C-OH of carboxylic acids (Guibavd*et al*., 2003). The peak at 767.72 cm^{-1} was associated to primary and secondary amine.

Gas Chromatography- Mass Spectrometry (GC-MS)

The GC-MS analysis of methanol extract clearly revealed the presence of twenty-three compounds (Fig. 2). Imidazolidin-2-one (18.43%), N,N-dibenzylhydroxylamine(15.36%), Methylhexadecanoate (7.33%) and Hexadecamethylcyclooctasiloxane (4.51%) were the principal compounds while the other nineteen compounds were the minor components from the methanol extract of *Mangiferaindica* (Ogbomosho variety). The active compounds with their retention time (RT), molecular formula, molecular weight and concentration (Peak area %) were presented in Table 3.

Imidazolidin-2-one may be a good source of medicine because a related compound known as Imidazolidine-2-thione had been reported to have anti-HIV activity and antimicrobial properties (Savjani and Gajjar, 2011). and possesses antimicrobial property (Rajeswari *et al*., 2012). Most of these phytochemicals present in the sample are useful in both pharmaceutical and cosmetic industries.

Table 1:- Proximate analysis of *Mangifera indica* (Ogbomosho variety)

Proximate Element	**Amount (%)**
Moisture	76.87±0.06
Ash	1.68±0.06
Lipid	0.38±0.06
Protein	0.86±0.01
Fibre	1.24±0.02
Carbohydrate	18.93±0.06

Table 2: FTIR peaks of the mango pulp and the assigned bonds

Assigned bond	**Wave number (cm^{-1})**
NH_2	3433.35
OH	3192.61
CH_2 stretching and deformation	2926.65 and1457.52
C=O asymmetric and symmetric stretching	1641.16 and 1457.52
CH bending of alkane	1254.88
C-OH of carboxylic acid	1137.73 and 1058.58
O-H carboxylic acid	995.25
N-H 1^o and 2^o amine	767.72

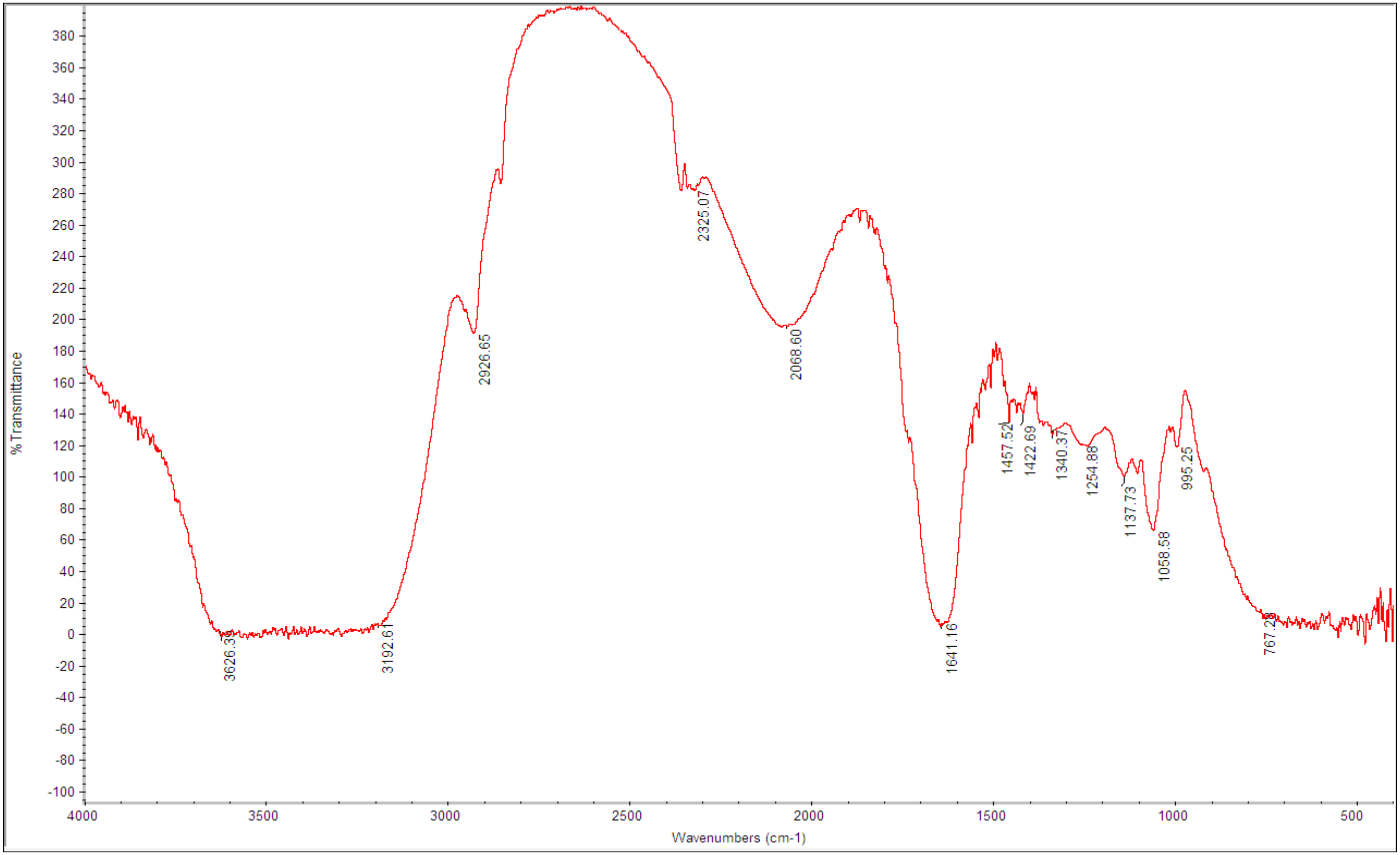

Figure 1: - FTIR spectrum of extract of *Mangifera indica* (Ogbomosho variety)

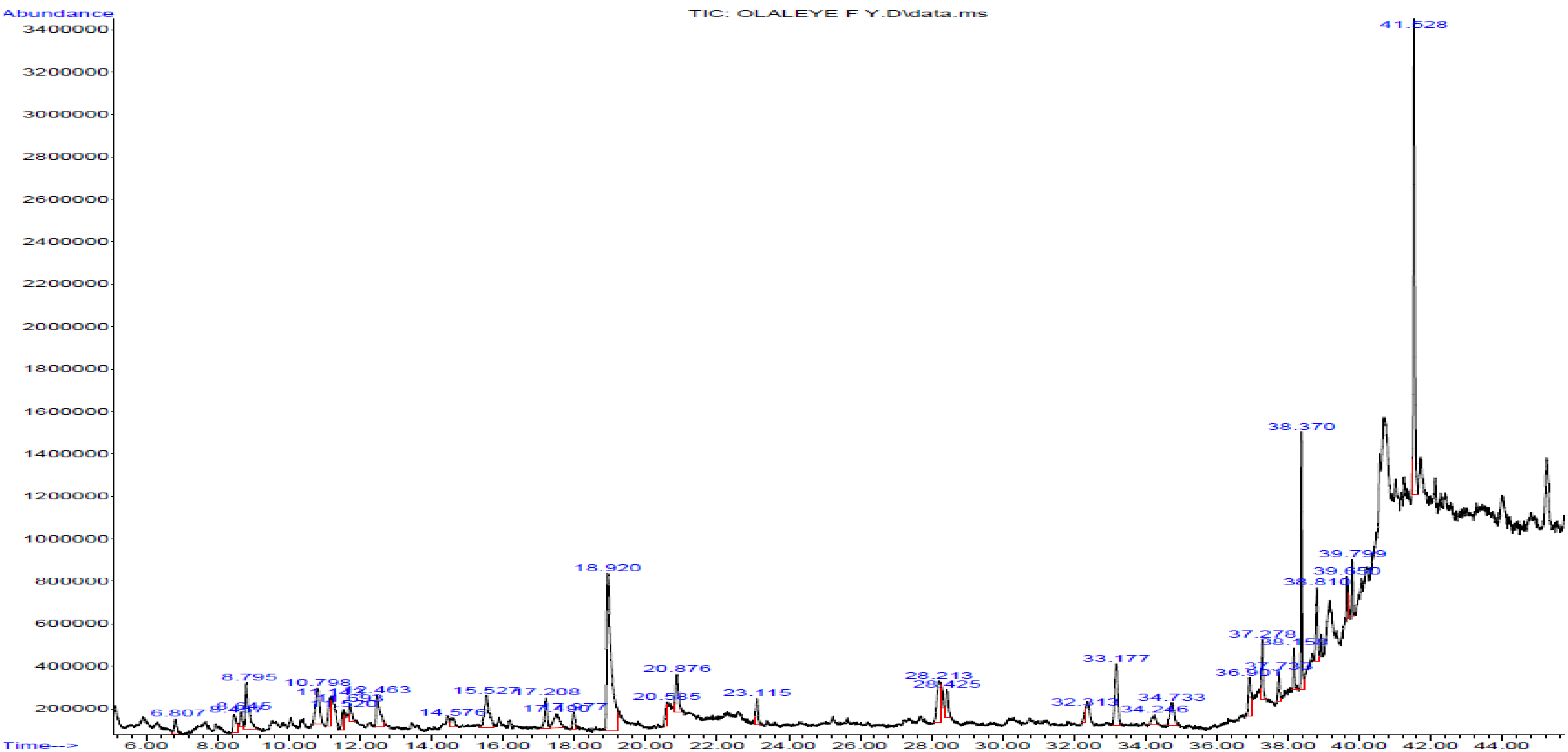

Figure 2: GC-MS analysis of methanol extract of *Mangifera indica* (Ogbomosho variety)

Table 3:- Phyto-compounds identified in the methanol extract of *Mangifera indica*using GC-MS.

S/N	Retention Time	Peak area (%)	Molecular Weight (g/mol)	Name of Compound	Molecular formula
1	10.50	3.67	112.12	Cyclohexane-1,2-Dione	$C_6H_8O_2$
2	11.14	1.80	296.61	Octamethylcyclotetrasiloxane	$C_8H_{24}O_4Si_4$
3	11.52	1.17	94.11	Phenol	C_6H_6O
4	11.69	1.08	220.15	4-(trifluoromethyl)mandelic acid	$C_9H_7F_3O_3$
5	12.46	2.98	112.13	2-Hydroxy-3-methyl-2-cyclopentenone	$C_6H_8O_2$
6	15.53	3.85	179.11	Phenylethanol	$C_8H_{10}O$
7	17.21	2.12	370.77	Decamethylcyclopentasiloxane	$C_{10}H_{30}O_5Si_5$
8	17.98	1.00	306.40	Dipentyl phthalate	$C_{18}H_{26}O_4$
9	18.92	18.43	86.09	Imidazolidin-2-one	$C_3H_6N_{2O}$
10	20.59	1.19	110.11	Catechol	$C_6H_6O_2$
11	20.88	2.55	318.32	diphenyl phthalate	$C_{20}H_{14}O_4$
12	28.21	3.20	519.08	Cycloheptasiloxane	$C_{14}H_{42}O_7Si_7$
13	28.43	1.72	180.56	4-chlorobenzotrifluoride	$C_7H_4ClF_3$
14	32.31	0.62	92.14	Toluene	C_7H_8
15	33.18	4.51	593.23	hexadecamethylcyclooctasiloxane	$C_{16}H_{48}O_8Si_8$
16	36.90	2.49	202.25	11,4-diphenylbutadiene	$C_{16}H_{10}$
17	37.28	2.33	667.39	Octadecamethylcyclononasiloxane	$C_{18}H_{54}O_9Si_9$
18	37.73	1.22	220.26	6,7-dimethoxy-2,2-dimethyl-3-chromene (Procene II)	$C_{13}H_{16}O_3$
19	38.16	1.27	162.21	4-Cinnolinethiol	$C_8H_6N_2S$
20	38.37	7.33	270.45	Methyl hexadecanoate	$C_{17}H_{34}O_2$
21	38.81	3.82	667.39	Octadecamethylcyclononasiloxane	$C_{18}H_{54}O_9Si_9$
22	39.65	0.88	218.29	2-benzylnaphthalene	$C_{17}H_{14}$
23	41.53	15.36	213.28	N,N-dibenzylhydroxylamine	$C_{14}H_{15}NO$

Conclusion

Fruit of the mango of Ogbomoso variety contained endophytic fungi that can produce bioactive substances. The fruit is very nutritional as it possesses health benefit nutrients and important phytochemicals that may be of essential resources to pharmaceutical industries. This work gave background information on the inhabitant endophytic fungi and chemical composition of this variety and provide avenue to explore its significance in all ramifications.

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