

Full Length Research Paper

Effect of solvent type on the yields and mineral compositions of the leaf extracts of *Moringa oleifera* L.Adeoye, M. Dasola¹, Lawal, A.Tunbosun^{2*}, Azeez, L. Adeyemi¹ and Olayiwola, O. Abidemi³¹Industrial and Environmental Chemistry Unit, Chemical Sciences Department, Fountain University, Osogbo Osun State, Nigeria.²Industrial and Environmental Chemistry Unit, Chemical Sciences Department, Al-Hikmah University, Ilorin Kwara State, Nigeria.³Chemical Sciences Department. Oduduwa University, Ipetumodu, Ile-Ife, Osun State, Nigeria.

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The present study was conducted for the leaves extract of *Moringa oleifera* L (*Moringaceae*) in different solvents: Fermented maize marsh (Omidun /FMM), hot water (HW), cold water (CW), palm-wine (PW), ethanol, dichloromethane (DCM) and n-hexane to determine the effects of solvent properties on the extraction yields and the compositions of dietary minerals (K, Ca, Mg, Zn, Cu, and Fe) for *M. oleifera*. The observed yields follow the trend: PW > CW > FMM > ethanol > HW > DCM > n-hexane. Fermented maize marsh extracts recorded the highest mean compositions for Ca and Mg (560.50 ± 34.5 and 485.75 ± 67.32); palm-wine extracts recorded the highest compositions of Fe and Cu (7.42 ± 1.22 and 0.50 ± 0.01), while cold water and hot water extracts recorded the highest compositions of K (2045.14 ± 265.02) and Zn (2.16 ± 0.11) respectively. The lowest concentrations of these trace metals which range from 0.06 ± 0.01 to 6.47 ± 0.35 was recorded in n-hexane extracts. Statistical analysis of the mineral constituents using two sample t-tests studies at $p < 0.05$ also revealed great significant differences between the means of the mineral elements in the extracts except in few cases. The high yields of these extracts in PW, CW and FMM indicate that these solvents have great affinity and similar solubility properties for the different compositions of *M. oleifera*.

Key words: *Moringa oleifera*, fermented maize marsh, solvatochromic, extraction, toxicological.

INTRODUCTION

Green plants provide most of the world's molecular oxygen and are the basis of a good number of the earth's ecologies, especially on land. Plants enrich our lives as flowers and ornaments, and generally contain high amounts of essential nutrients, vitamins, minerals and fatty acids. These are important in animal feed and human nutrient for osmotic adjustment and to activate

enzymes, hormones and other organic molecules that enhance the growth, function and maintenance of life processes by living organisms (Aslam et al., 2005). Minerals cannot be synthesized by animals and must be provided from plants or mineral-rich water (Mosha et al., 1995; Al-Kharusi et al., 2009).

Moringa oleifera Lam [syn. *M. pterygosperma* Gaertn.]

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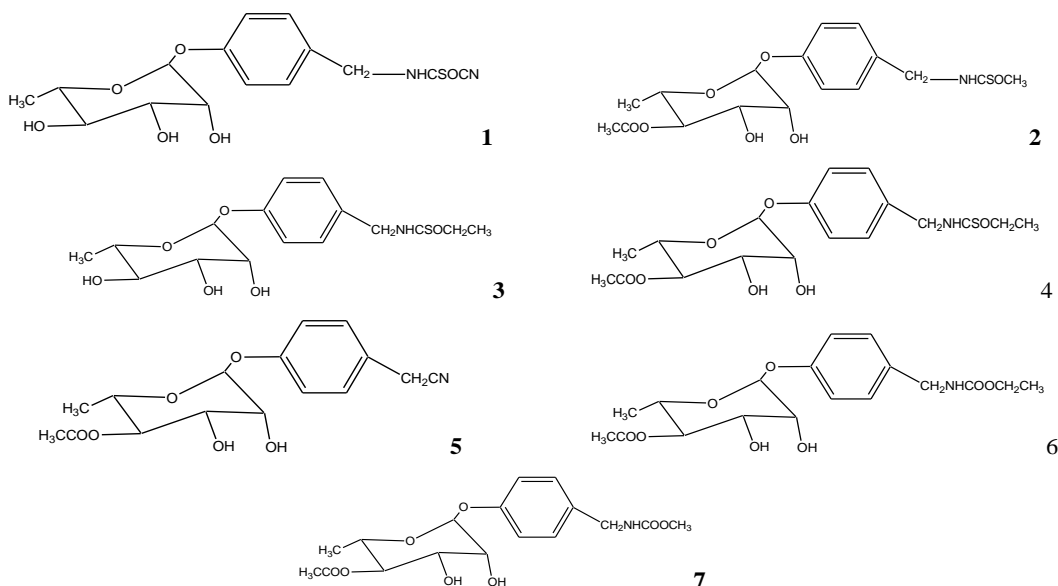


Figure 1. Structures of some selected phytochemicals from moringa (1) niazidin (2) niazicin (3) niazimicin (4) niaziminin (5), niazirin (6) niazirin (7) niazicin (Reubena, 1995).

plants, widely distributed and naturalized species of a monogeneric family Moringaceae is commonly called ben oil tree but locally known as *Zogeli* among the Hausa speaking people of Nigeria (Toba et al., 2010). It has been reported to be a rich source of protein, vitamins, β -carotene, aminoacids, various phenolics and essential minerals like calcium (Ca) and potassium (K) (Toba et al., 2010; Makkar and Becker 1996). It has also been reported to be a good source of natural antioxidants such as ascorbic acid, flavonoids, phenolics and carotenoids which enhance the shelf-life of fat containing foods. Its oil and micronutrients have also been acclaimed to contain antitumour, antiepileptic, antidiuretic, anti-inflammatory and venomous bite characters (Hsu, 2006), while its aqueous leaf extracts has been reported to regulate thyroid hormone and can be used to treat hyperthyroidism since it exhibits an antioxidant effect (Kumar and Pari, 2003). The biodiesel derived from *M. oleifera* oil by alkali-catalyzed trans-esterification with methanol is reported to be an acceptable substitute for petrol and diesel. Its cetane number was found to be the highest reported for a biodiesel fuel with much better oxidative stability (Mahajan et al., 2007). The seeds also serve as one of the cheap and best coagulants for water treatment (Kalogo et al., 2000).

The minerals and nutritional values of *M. oleifera* and their bioavailability have been a subject of great studies and there have been reported considerable variations among these constituents in both the leaves and seeds of this plant. These depend on factors like genetic background, location, environment and cultivation methods (Aslam et al., 2005; Brisibe et al., 2009; Toba et al., 2010).

Cold water, hot water, palm wine or fermented maize mash (omidun) has been used traditionally as solvent for herbal extraction in Nigeria. The leaves, barks or roots of some herbal plants are either cooked or soaked in these solvents to treat certain diseases. However, there are limited reports on the influence of these solvents on the mineral and bioactive compositions of these herbal extract. Most of the bioactive components contained in plant matrices are medium-sized molecules which due to the presence of aromatic delocalized π -electrons in it, the

molecules are highly polarizable. Their high polarizability makes the molecules liable to a variety of specific interactions with various solvents, e.g., protonation, hydrogen bonding, and specific solvation (Andri et al., 2009). Thus, for toxicological reasons, drug and medicine manufacturers are increasingly required to minimize the number of solvents employed in pharmaceutical processes (Kolar et al., 2002). The presence of a solvent in the extract may also affect the kinetics of crystallization and the morphology of the crystals, which is an important factor in determining the product quality (Andri et al., 2009).

To date, twenty three active compounds had been isolated and elucidated from *M. Oleifera* leaves and these had been claimed to depend on their species, sources and ages (Rubeena, 1995; Toba et al., 2010). These compounds are novel glucosides with hypotensive properties; some of these are niaziminin, niazinin, niazicin, niazimicin, niazirin, niazirin, niazicin and niazimin in Figure 1 (Rubeena, 1995). It is therefore pertinent in this work to confirm the presence/ retention of these compounds in the extracts of *Moringa* leaves



Figure 2. Flow diagram for the preparation of ogi /omidun (FAO, 1999).

ecotype in Osogbo, a South Western Nigeria town through their functional groups and to study the effect of solvent on its mineral compositions.

MATERIALS AND METHODS

Solvents

Fermented maize mash (*omidun*), prepared by soaking and fermentation of maize grains in cold water for 28 to 72 h, wet milled, sieved and left to sediment for 48 h (Figure 2) were collected from traditional processors of ogi in Osogbo, Osun State, Nigeria. This solvent was from ogi prepared from white maize grains (FAO, 1999; Abdulrahman and Kolawole, 2006). The *omidun* samples were collected aseptically in sterile screw cap bottles. Fresh palm-wine was bought in undiluted form from the local palm-wine taper while other reagents/chemicals (dichloromethane, ethanol, n- hexane) used were analytical grade products of British Drug House (BDH). The reagents were redistilled twice and their boiling points were used as index of purities. The standards used were prepared using distilled water.

Collections and identification of plant materials

Fresh leaves of *Moringa* were collected from *M. oleifera* plants located in Fountain University compound, Osogbo in Osun State southern/western Nigeria between January and February, 2013. The plant materials had earlier been taxonomically identified by Dr. Awodoyin, R.O. of Botany Herbarium, University of Ibadan. The leaves were air- dried until a constant weight was obtained (one week). They were pulverized to powdery form using a well-cleaned 240 v 4 L blender machine. The powdered samples were then stored in airtight containers, properly kept and labeled for further analysis. Soil samples of the land on which *M. oleifera* is grown were also taken within 10 cm depth. And, its pH determination was done at ratio of 1:1 in distilled water (Anjorin et al., 2010).

Determination of moisture and ash contents

2 g fresh samples of *M. oleifera* leaves (in duplicate) were weighed. The moisture contents in the samples were determined using the method of the Association of Official Analytical Chemists (AOAC, 1990). This was done by weighing the leaves in the crucible and drying in oven at 105°C until a constant weight was obtained. Determination of the ash content was done by ashing at 550°C for about 3 hrs (Ogbe and John, 2011). The ash resulted from the combustion was then allowed to cool to lowest temperature and weighed.

Preparation of *M. oleifera* extracts

The extracts of *M. oleifera* in different solvents were prepared by soaking 8 g of dried powdered samples each in 100 ml of distilled coldwater, distilled hot water, *omidun*, palmwine, dichloromethane, n- hexane, ethanol respectively for 24 h in an airtight reagent bottles. These were then filtered. The filtrates were concentrated in rotary vacuum evaporator to yield an oily golden yellowish mass. The concentrates of all extracts mentioned above were finally air dried to constant weight and the total yield of each sample were determined. 2 g of each of the concentrates were treated with 20 ml of concentrated Trioxonitrate (V) acid (HNO_3) heated to reflux on an electric hot plate at 70 to 90°C until digestion was completed (Anjorin et al., 2010). Side by side, 20 ml of HNO_3 was also added to empty beakers which served as blank. The contents of the beaker were allowed to cool, filtered through whatman No. 2 filter paper into volumetric flasks and made up to volume of 100 ml with distilled water before the Atomic Absorption Spectroscopy analysis was done. These were carried out in triplicates.

Determination of the compositions of dietary minerals in the *M. oleifera* extracts

Chemical analysis

Macro elements (K, Ca, Mg) and trace elements (Zn, Cu, Fe) concentrations in the *M. oleifera* extracts in different solvents were estimated on Atomic Absorption Spectrophotometer (AAS) (Buck Scientific Modelling 2010/2011 V.G.T made in England) at the Agronomy Department of the University of Ibadan. Infra-red analysis of the functional groups in the *M. oleifera* extract and UV-Visible spectroscopy analysis of the concentrates in the wavelength range of 190 to 800 nm in different solvents were also determined.

Statistical analyses

The analyses in this study were carried out in triplicates. For each, a mean value and standard deviation were calculated. Statistical analysis of the results obtained involved the use of Aspin-Welch unequal variance two sample t-tests.

RESULTS AND DISCUSSION

Characterization of the extracts of *M. oleifera* leaves

The extracts of *M. Oleifera* leaves were obtained in amorphous form as golden to dark brown colour in different solvents. The moisture contents, the extracts yields, the mineral composition and the results of the spectra analysis (UV and IR) showing the effect of

Table 1. Yields of *M. oleifera* extracts in different solvents.

Solvent	Yield (g)
Hot water	1.13 ± 0.02
Cold water	2.0 ± 0.01
n-hexane	0.2 ± 0.02
D.C.M	1.0 ± 0.11
Ethanol	1.2 ± 0.03
F.M.M	1.9 ± 0.01
Palm-wine	6.77 ± 0.12

F.M.M., Fermented maize mash; DCM, dichloromethane.

Table 2. Mineral composition of *M. oleifera* leaves extracts in different solvents.

Extracts	Ca (mg/L)	Mg (mg/L)	K (mg/L)	Fe(mg/L)	Cu (mg/L)	Zn (mg/L)
Cold water	484.33±75.77 ^a	292.82±16.09 ^a	2045.14±265.02 ^a	1.38±0.04 ^a	0.08±0.01 ^a	0.64±0.12 ^a
Hot water	512.18±17.18 ^b	197.25±26.37 ^b	1775.99±98.66 ^b	2.78±0.17 ^a	0.06±0.01 ^b	2.16±0.11 ^b
F.M.M	560.50±34.5 ^b	485.75±67.32 ^c	1875.15±183.53 ^b	3.47±0.07 ^b	0.11±0.02 ^a	1.14±0.05 ^c
D.C.M	20.40±1.43 ^c	12.71±3.02 ^d	33.93±3.77 ^c	6.63±0.33 ^c	0.09±0.02 ^a	0.29±0.02 ^d
n-hexane	4.22±0.28 ^d	1.56±0.09 ^e	6.47±0.35 ^d	0.70±0.01 ^d	0.06±0.01 ^b	0.14±0.03 ^e
Ethanol	5.19±0.04 ^d	54.75±4.12 ^f	625.21±28.27 ^e	5.89±1.38 ^c	0.13±0.05 ^a	0.33±0.02 ^d
Palm-wine	152.50±24.47 ^e	212.50±35.85 ^b	216.50±12.56 ^f	7.42±1.22 ^c	0.50±0.01 ^b	0.44±0.03 ^f

D.C.M, 1, 2-dichloromethane, F.M.M, fermented maize mash (Omidun). Data are expressed as Mean ±SD. Means with different superscripts in a column are significantly different at $p < 0.05$.

Table 3. Summary of the Infra-red (IR) analysis showing the different functional groups in the extracts of *M. oleifera* leaves.

Functional groups	(u _{max}) cm ⁻¹						
	Cold water	Hot water	F.M.M	Palm- wine	Ethanol	D.C.M	n-hexane
U O-Hstr	3396	3425	3430	3404s	3457.82	3453	3445.71
U OH/ N-Hstr	-	-	-	3373	-	-	-
U C-H str in CH ₂	2976.95	2980.2	-	2978.34	-	-	2927.75
U C=C str (aromatic ring)	1648.66	1643.09	1637.86	1643.94	1639.21	1638.36	1638.54
U C=O str (carbamate)	-	-	-	-	-	-	1726.42
U -C=O amide	-	-	-	-	-	-	1589.63
U C-O thioamide	1400	1398	-	1399.50	-	-	1374.23
U C-OH stretch/def	1047	1048.30	1090	1048.86s	1048.57	-	-
u Subst bzc	662.26	668.33	609.32	662.26	601.00	-	-

F.M.M, Fermented maize mash; D.C.M, dichloromethane

different functional groups and the effect of extractive solvents in the spectra shifts of *M. oleifera* leaves extracts are as presented in Tables 1 to 4 and in Figures 3 to 15. Moisture contents: 10.22% ± 0.11. Ash values: 7.93% ± 0.32

pH Values and soil samples

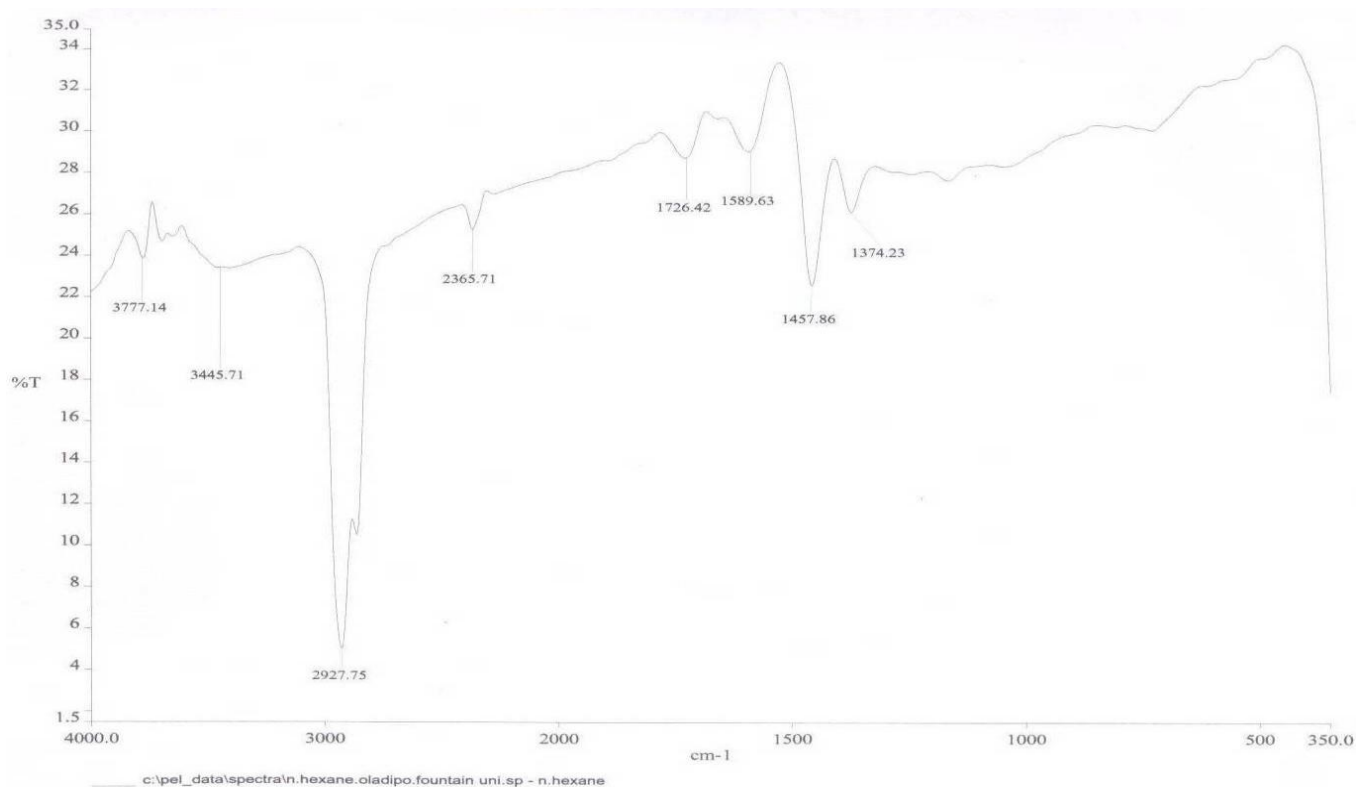
The soil on which the plants grow is loamy soil while its

pH was slightly acidic (pH 6.55). This was in line with the findings of Anjorin et al. (2010). The moisture contents (10.22% ± 0.11) and total ash values (7.93% ± 0.32) for *M. oleifera* leaves obtained in this study were slightly higher than those reported by Moyo et al. (2011) and Ogbe and John (2011) for *M. oleifera* leaves. The variations in the reported values of these parameters may be due to differences in the ages of the trees or their different stages of maturity and possibly due to difference in agro-climatic conditions (Ogbe and John, 2011).

Table 4. UV-Visible Absorption Bands of different solvents extracts of *M. oleifera*.

Extracts	Band I		Band II		Band III		Band IV	
	(λ_{max})nm	Absorbance	(λ_{max})nm	Absorbance	(λ_{max})nm	Absorbance	(λ_{max})nm	Absorbance
Cold water	300	2.304	-	-	-	-	-	-
Hot water	295	1.565	-	-	-	-	-	-
F.M.M	300	2.081	-	-	-	-	-	-
Palm wine	300	2.018	-	-	-	-	-	-
Ethanol	295	1.553	410	0.452	-	-	665	0.116
D.C.M	295	1.331	410	0.724	-	-	665	0.250
n-Hexane	-	-	415	0.234	435	0.126	470	0.143

F.M.M., Fermented maize mash; D.C.M., dichloromethane.

**Figure 3.** IR Spectrum of n-hexane extract of *M. oleifera*.

Extracts yields

Plant matrices including leaf, stem and root contain various solute molecules with more than one functional group. Therefore it is difficult to predict the solubility and interactions of the solute in a particular solvent (Andri et al., 2009). Polarity was defined by Snyder as the relative ability of a molecule to engage in strong interactions of all kinds and is the sum of all possible interactions (Andri et al., 2009). Thus, the concept of polarity can be used as alternative way of considering solubility.

The total yields of *M. oleifera* in the solvents of choice

(dichloromethane, n-hexane, Fermented Maize Mash, Ethanol and water) as shown in Table 2 indicated that palm-wine and non polar (n-hexane) extracts had the highest and lowest yields respectively. All the extracts obtained from the solvents used were observed as dark brown or golden brown semi-solids. The high yields of these extracts in palm-wine and fermented maize mash (Omidun) indicated that these solvents has high affinity and similar solubility properties for the different compositions of *M. oleifera* because they contain hydroxyl group which is hydrophilic showing that the molecules in these extracts dissolves better in these

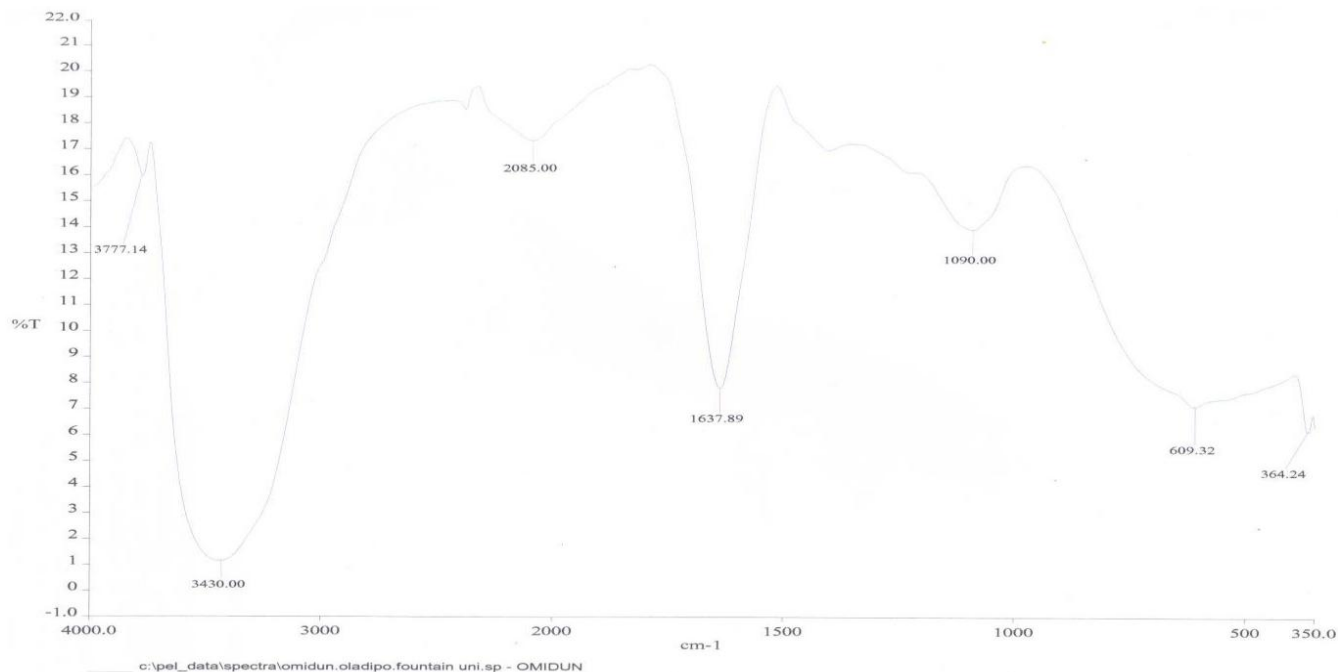


Figure 4. IR Spectrum of Fermented maize mash extracts of *M. oleifera*.



Figure 5. IR Spectrum of Hot water extracts of *M. oleifera*.

solvents. The extract yields of the plants decreased with decreasing solvent polarity. Low polar solvents such as dichloromethane and n-hexane were not able to significantly extract the *M. oleifera*. However, there is also

requirement from environment considerations and toxicological implication to reduce the quantity of organic solvents used in particular chlorinated solvents (including dichloromethane) in the extraction of consumable items

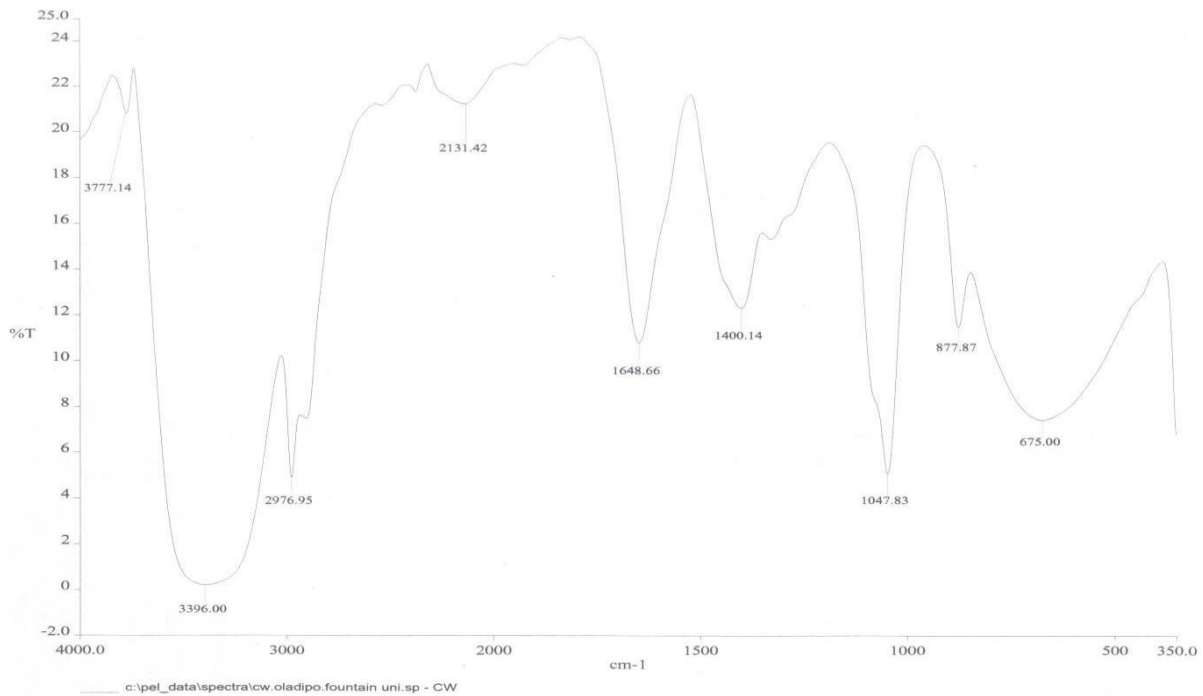


Figure 6. IR Spectrum of cold water extracts of *M. oleifera*.

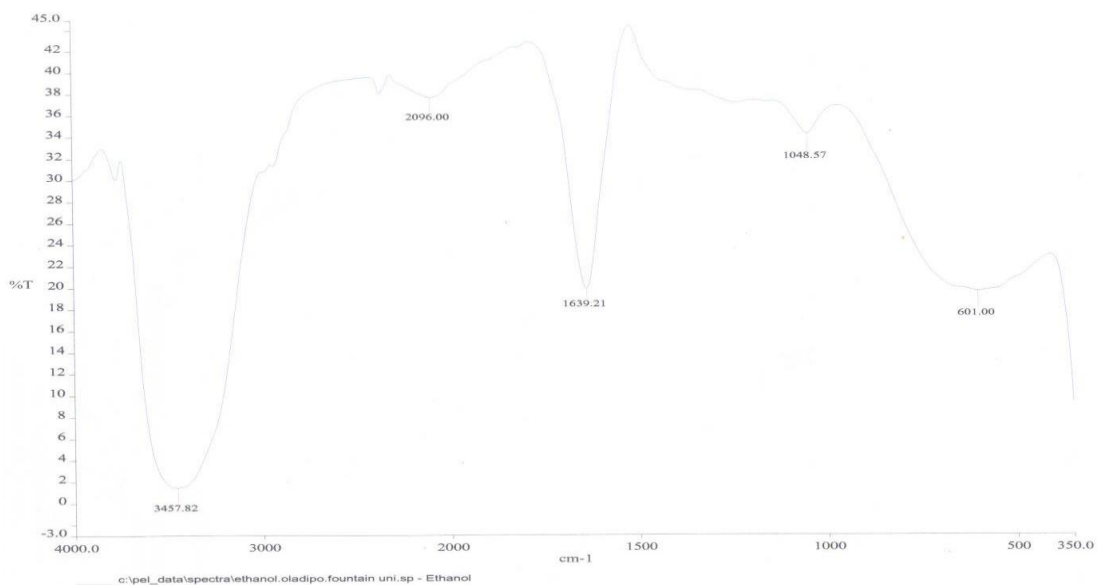


Figure 7. IR Spectrum of ethanol extracts of *M. oleifera*.

(Barwick, 1997).

Distribution of minerals in *M. oleifera* extracts

The level of the important dietary mineral constituents (k, Ca, Mg, Zn, Cu and Fe) that are determined in this study

in different solvent extracts of *M. oleifera* are as summarized in Table 2. In all the extracts and for the macronutrients analysed, concentrations of K was the highest while that of Mg was the lowest. For the micronutrients analysed, Fe concentrations was the highest while Cu was the least. This report supported the previous findings of Moyo et al. (2011) that *Moringa*

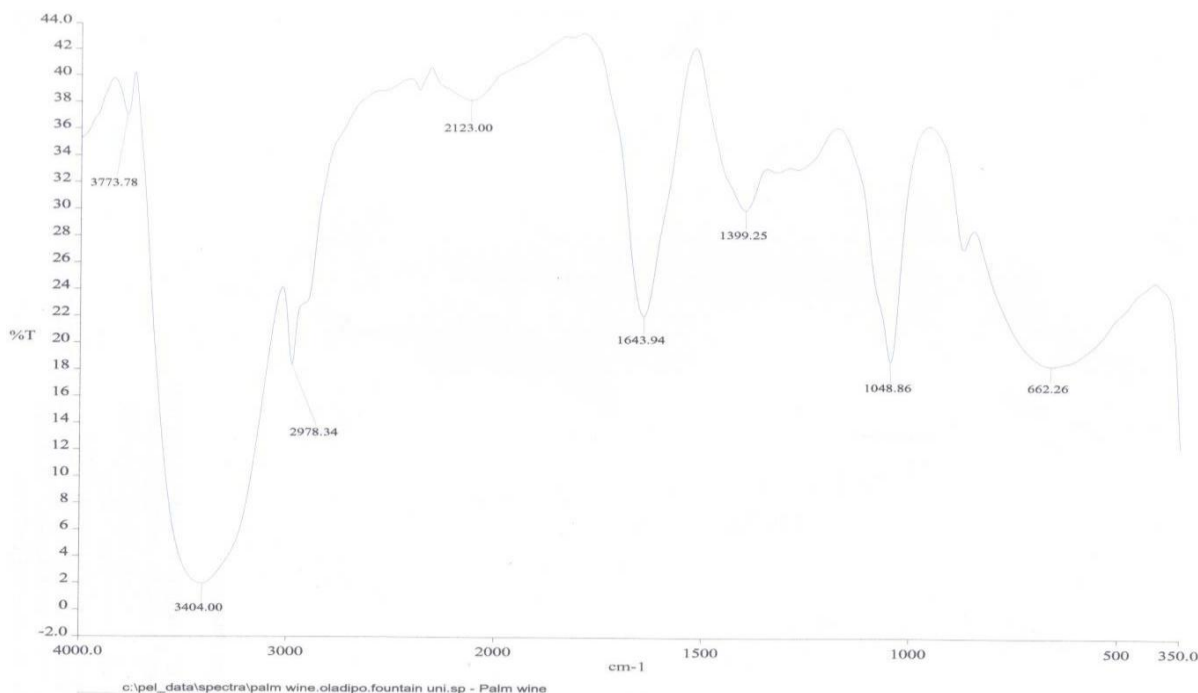


Figure 8. IR Spectrum of palm wine extracts of *M. oleifera*.

seeds and leaves could be a potential source of Fe supplement for humans and livestock.

Fermented maize marsh extracts recorded the highest mean compositions for Ca and Mg (560.50 ± 34.5 and 485.75 ± 67.32); palm-wine extracts recorded the highest compositions of Fe and Cu (7.42 ± 1.22 and 0.50 ± 0.01) while cold water and hot water extracts recorded the highest compositions of K (2045.14 ± 265.02) and Zn (2.16 ± 0.11) respectively. The lowest concentrations of these trace metals which range from 0.06 ± 0.01 to 6.47 ± 0.35 was recorded in n-hexane extracts. Statistical analysis of the mineral constituents using two sample t-tests studies at $p < 0.05$ also revealed great significant differences between the means of the mineral element in the extracts except for Ca contents in hot water and FMM extracts, as well as, in n-hexane and ethanol extracts pair; K concentrations in hot water and FMM extracts; Fe concentrations in cold water and hot water, in addition to, dichloromethane and ethanol extracts pair; Cu in cold water, FMM and dichloromethane; and Zn concentrations in ethanol and dichloromethane. This indicates that solvents polarity and types has great influence on the mineral and bioactive compositions of *M. oleifera* extracts.

The high values of Ca and K in Omidun extracts may be as a result of the high nutritional and therapeutic values of *ogi*. Earlier reports have also shown the presence of microorganisms and their attendant minerals, vitamins and soluble nitrogenous compounds present in *ogi* which cannot also be ruled out in *omidun*. This might be an added advantage for the highest yields of these

elements in the *omidun* extracts. This indicated that the extraction process is a function of the solvents compositions.

The similarity in some of the mineral contents of the extracts obtained with cold water, hot water and *omidun* also attests to the high affinity and similar solubility properties of these polar solvents for *M. oleifera*.

Ultra violet/visible spectroscopy (UV/Visible) analysis

From the ultra violet/ visible spectral (Figures 9 to 15) and the summary of the ultra violet/ visible spectra analysis, that is, the wavelength maximum absorption (λ_{max}) and their corresponding absorbance for the leaves extract of *M. oleifera* in different solvents media at room temperature in Table 4, two prominent bands were observed for the extracts in the organic solvents used: Polar protic (ethanol), polar aprotic (dichloromethane), and non polar (n-hexane) while three bands were observed in the aqueous solvents (cold water, boiled water, FMM and palm-wine). The highest absorption bands of these extracts appear at 295 nm/300 nm while the lowest were obtained at 210 to 285 nm in all the solvents except in non polar n-hexane. This non appearance of band 1 in the spectra of the extracts in organic solvent could be due the non transparency of this band to these solvent.

Band 1(295 nm) in cold water is shifted to longer wavelength, in both palm-wine and FMM and the intensities as observed also increased in this solvent.

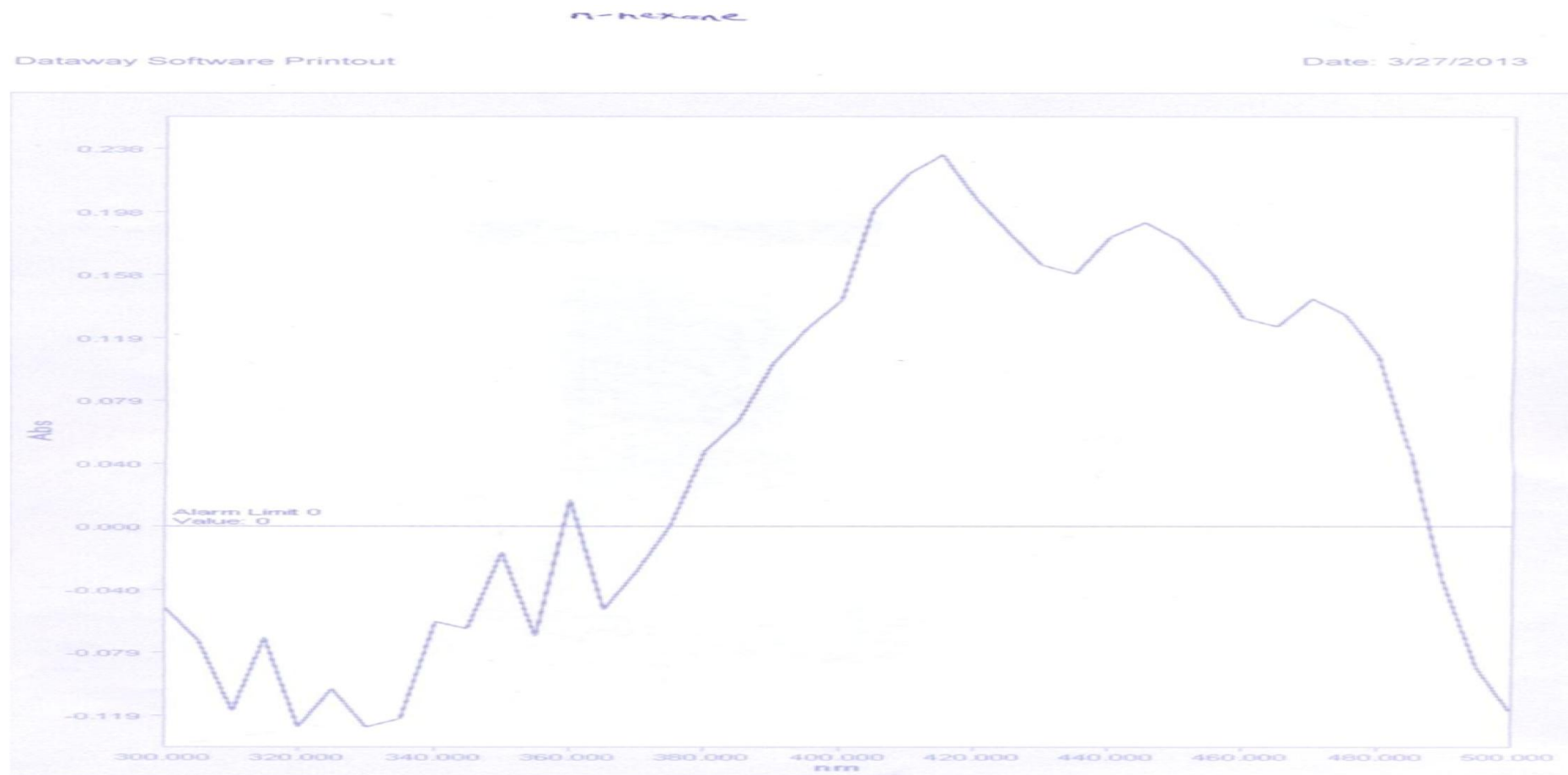


Figure 9. UV-Visible spectrum of n-hexane extracts of *M. oleifera*.

This shows that solvents have great influence (through interaction e.g., protonation, hydrogen bonding, and specific solvation effect) on the molecules of the compounds (Kolar et al., 2002). The absorbance at 295 nm was retained for the extracts in dichloromethane (DCM) and ethanol but with lower intensity (hypochromic shifts), Band

2 (410 nm) was also retained in ethanol but with increase in intensity of absorption. Increase in solvents polarity has little or no effects on the absorption maximum (λ_{max}). The band which occurred at 295 nm in DCM, boiled water and ethanol were shifted to longer wavelength, that is, 300 nm (Bathochromic shift) in Omidun and

cold water.

Infrared spectroscopy (IR)

Two or more compounds are one if their spectra agree in position and relative intensities

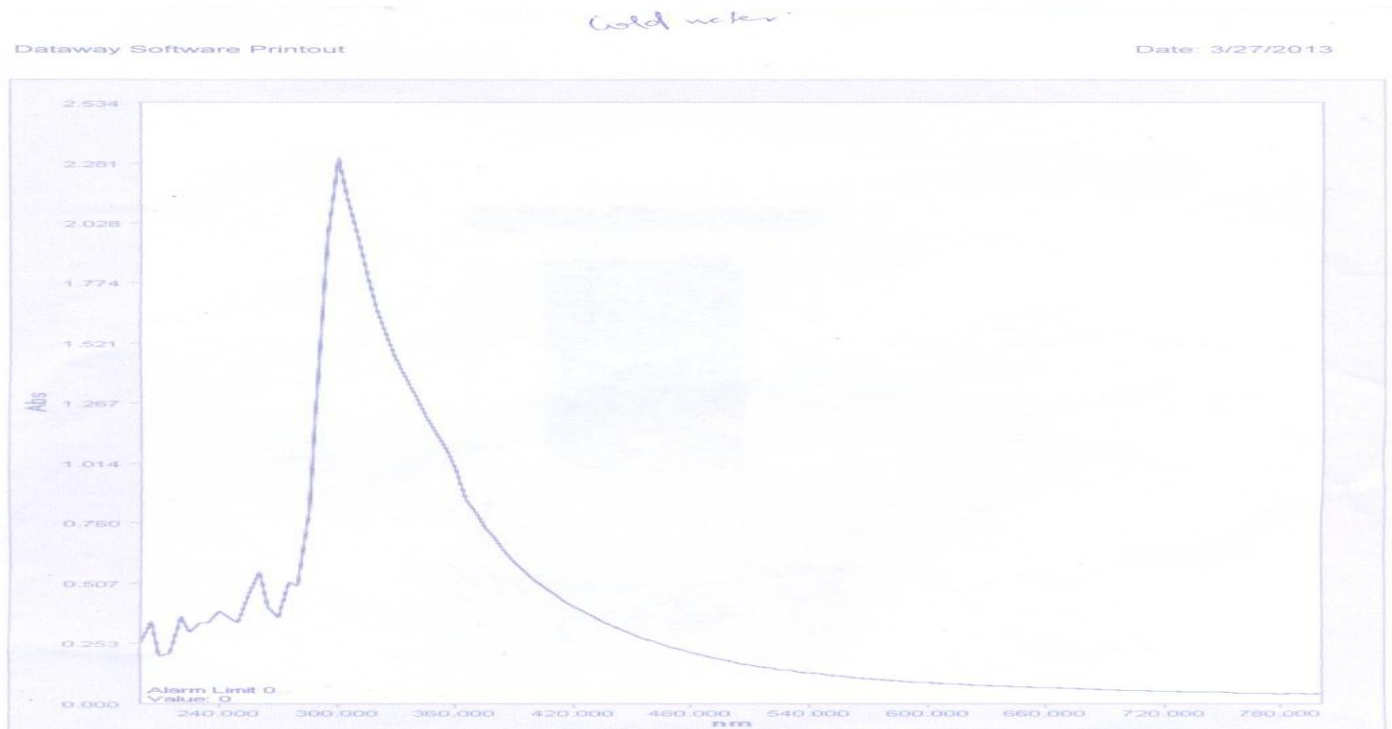


Figure 10. UV-Visible spectrum of cold water extracts of *M. oleifera*.

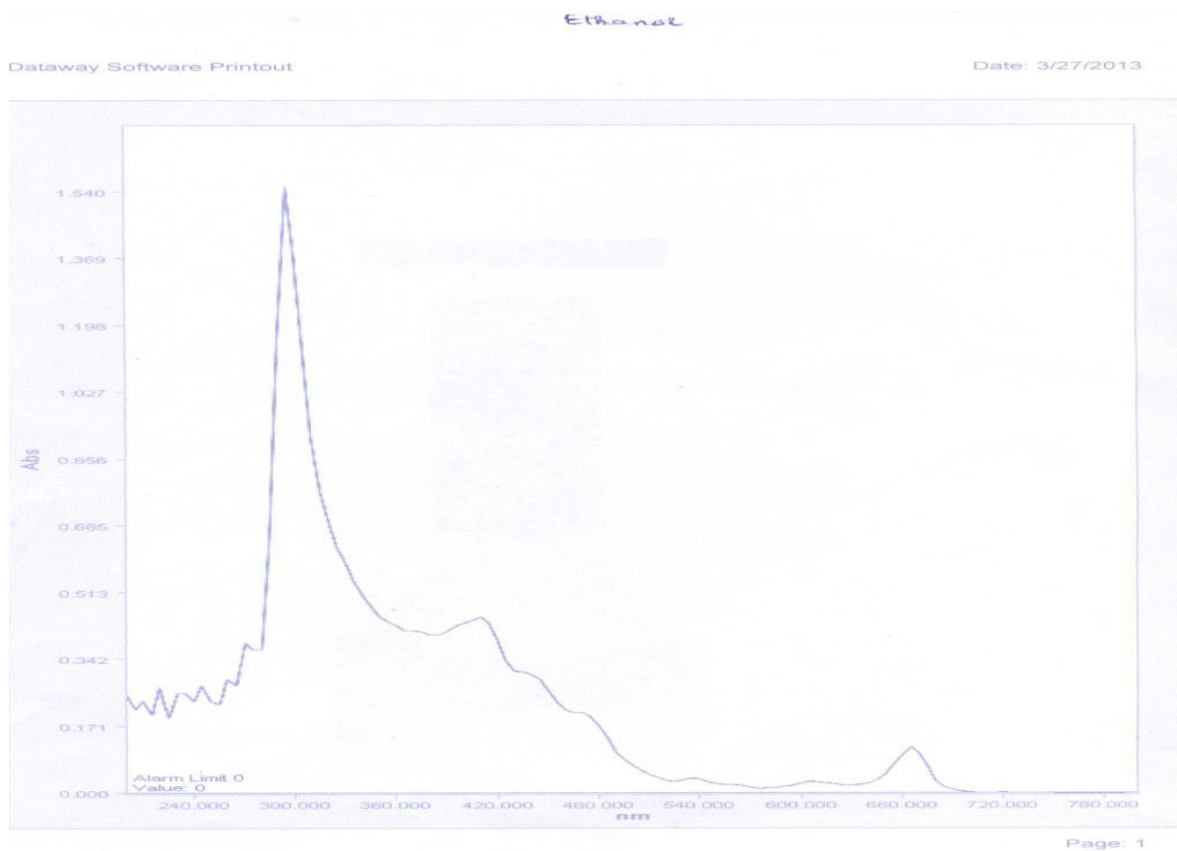


Figure 11. UV-Visible spectrum of ethanol extracts of *M. oleifera*.

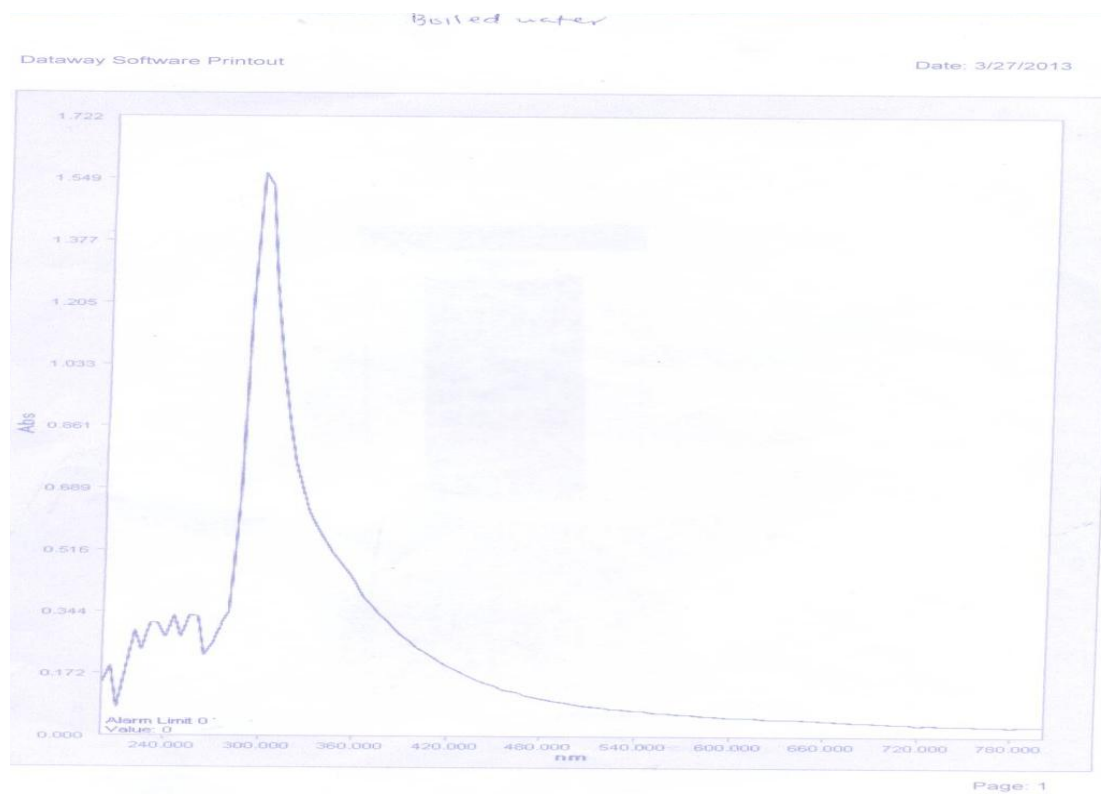


Figure 12. UV-Visible spectrum of Hot water extracts of *M. oleifera*.

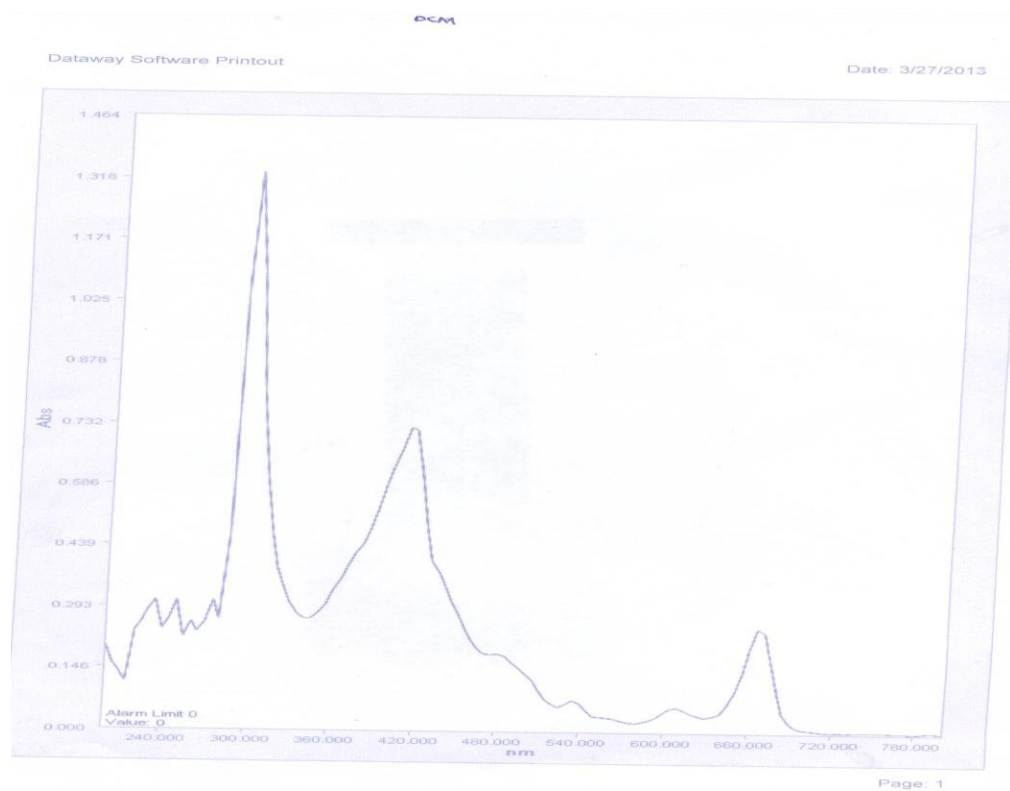


Figure 13. UV-Visible spectrum of Dichloromethane extracts of *M. oleifera*.

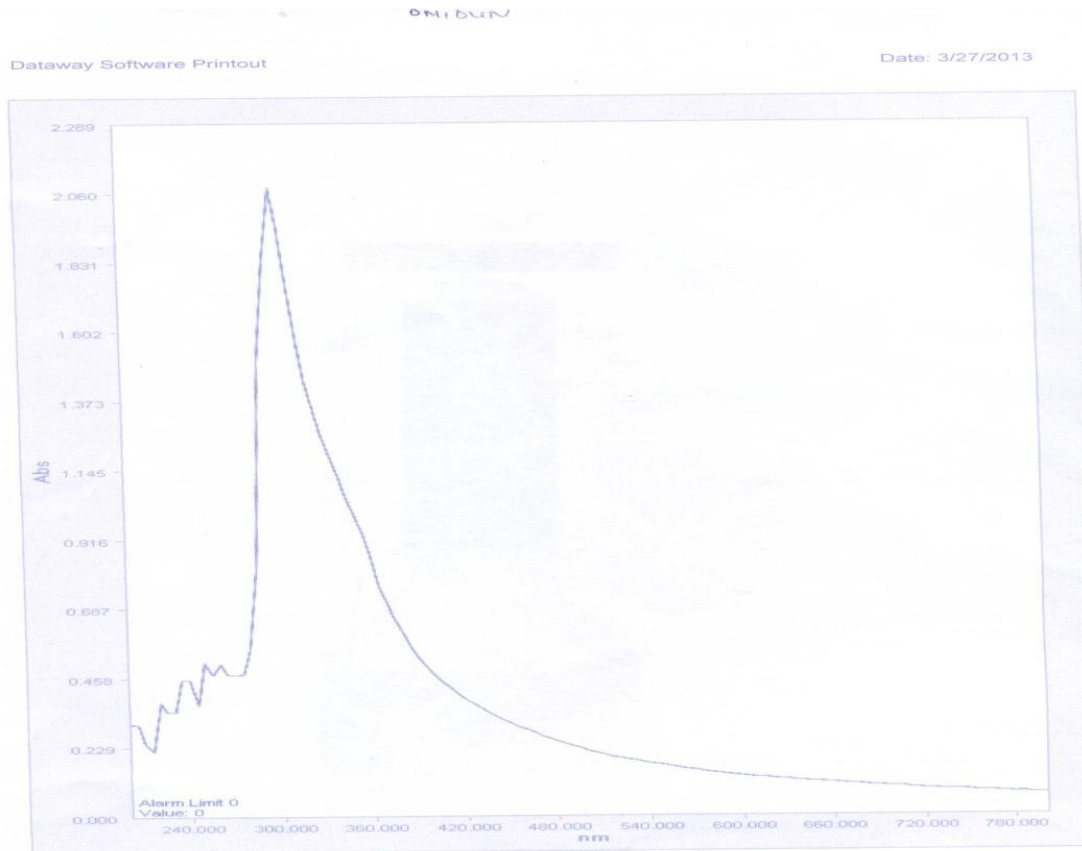


Figure 14. UV-visible spectrum of fermented maize marsh extracts of *M. oleifera*.

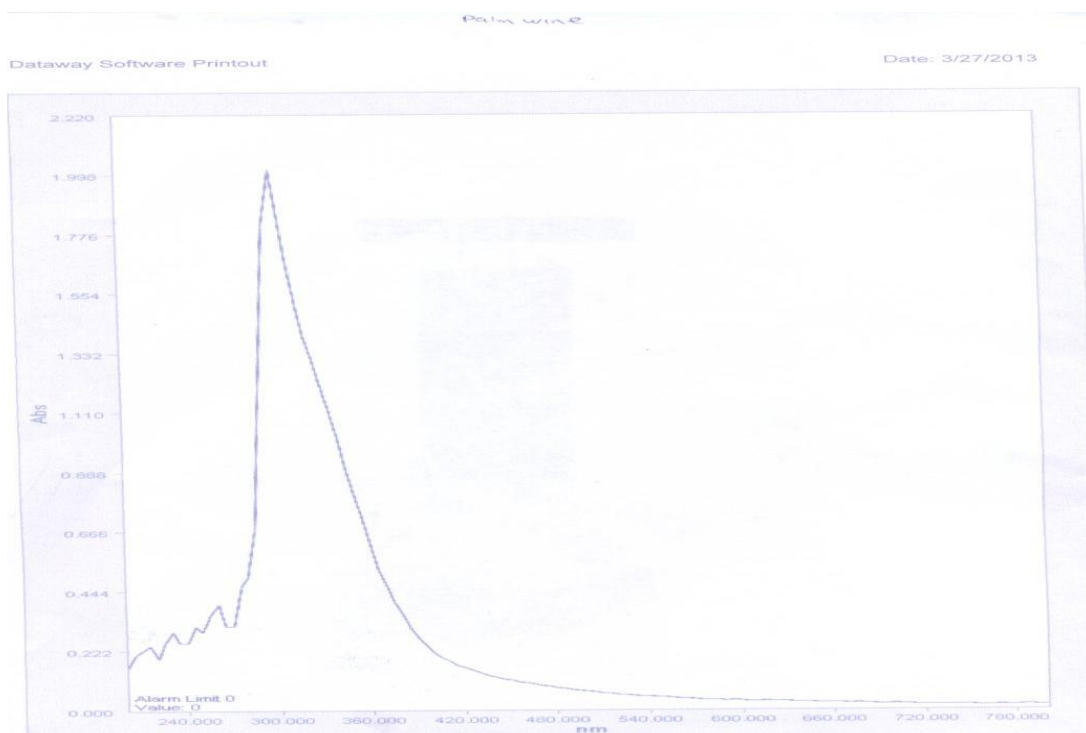


Figure 15. UV-Visible spectrum of palm-wine extracts of *M. oleifera*.

of the bands. Thus, the functional groups of the active compounds (Figure 1) in the studied *M. oleifera* extracts and the effect of different solvents on them were confirmed by (IR) analysis. Figures 3 to 8 and Table 3 show the characteristic absorption bands exhibited by the IR spectrum of the studied leaf extracts in different solvents. These observed functional groups are comparable with those obtained from the proposed structures of the isolated compounds in Figure 1 with slight variations due to changes in solvent (Rubeena, 1995).

Conclusion

This study shows that solvents polarities and types have great influence on the various chemical compositions of *M. oleifera* plant extracts due to its various interactions with them. Palm wine gave the highest yields of the extracts and highest compositions of micronutrients (Fe and Cu). Fermented maize mash gave highest composition of the macronutrients (Ca and Mg) and micronutrients (Zn) components. Statistical analysis of the mineral constituents using two sample t-tests studies at $p < 0.05$ generally reveals great significant differences in the attendants studied mineral compositions in the extracts except in few cases. The Moringa from this source was found to be a good source of important minerals and this further confirmed that this plant is a viable food supplement feeds additives for dietary minerals in animal and human beings.

Conflict of Interest

The authors have not declared any conflict of interest.

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