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BIOACTIVE COMPOUNDS OF *MORICANDIA NITENS* AND ITS ANTICANCER EFFECT

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Abstract:
Glucosinolates are a class of organic anions that can be hydrolyzed either enzymatically with myrosinase or non-enzymatically to form primarily isothiocyanates and/ or nitriles. Investigation of the hydrolyzed glucosinolate products using GC-MS with natural autolysis and exogenous myrosinase enzymatic hydrolysis methods for the aerial parts and roots of Moricandia nitens, showed that the glucosinolate compounds; Ethyl isothiocyanate, Isobutyl isothiocyanate, 5- (methylthio)-4- pentene nitrile, 3-butenyl isothiocyanate, Allyl isothiocyanate, Benzyl isothiocyanate, 3- (Methyl thio) propyl isothiocyanate, 4- (methyl sulphonyl) butane nitrile and 3- (methyl sulphonyl) propyl isothiocyanate percentages were detected in both the aerial parts and roots, with relatively higher percentage in roots than that of the aerial parts, while 4-(methylthio) butanenitrile, 5- (methylthio) pentane nitrile and 4-Methylthio-3-butenyl isothiocyanate were higher percentages in the aerial parts than that of roots. The amount of total glucosinolates content in the aerial parts and roots were 1.7 and 2.1mg/gm, respectively. GC-MS analysis of the aerial parts and roots of M. nitens showed the presence of 50 phytochemical constituents for each part. When comparing the mass spectra of some bioactive constituents, with our detected compounds, the detected bioactive compounds were identified. It was noticed that, the amount of total terpens content of the aerial parts and roots of M. nitens were 2.72 and 5.04 mg/ gm, respectively. Meanwhile the percentage of the total alkaloids were 0.02% for the aerial parts and roots of the plant. Cytotoxic activity for the aerial parts and roots of M. nitens (in vitro) against (HCT) showed remarkable cytotoxic activity at IC_{50}= 99.8µg/ml and 63 µg/ml for aerial parts and roots, respectively, beside remarkable cytotoxic activity against (HEPG2) at IC_{50}= 153 µg/ml and 200 µg/ml for aerial parts and roots, respectively.

Key words: *Moricandia nitens, Glucosinolates, bioactive compounds, Gas chromatography-mass spectrometer and Cytotoxic activity.*

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INTRODUCTION:

Moricandia nitens (Viv.) E. A. Durand & Barratte belong`s to family Brassicaceae, which is an economically important family for its many food and oil seed crops as well as containing many important ornamental plants and noxious weeds. Crucifers are characterized by the presence of a group of secondary compounds called glucosinolates [1]. Glucosinolates are a diverse class of S- and N-containing secondary metabolites that are found mainly in members of the Brassicaceae [2]. Glucosinolates play a variety of roles for plant defense responses and cancer prevention. They are relatively nonreactive, hydrophilic, nonvolatile compounds that are stored within plant vacuoles [3,4]. The hydrolysis of glucosinolates is catalyzed by endogenous myrosinases (*b*-thioglucoside glucohydrolases) [5]. Myrosinases are encoded by small gene family and are found in idioblasts [6] in most tissues of glucosinolate-producing plants [7,8]. Six glucosinolates were identified in leaves, roots and ripe seeds of *Erysimum corinthium* Boiss, in addition to other volatile constituents, e.g., terpenes and fatty acids esters were also identified. Seeds and leaves showed higher antimicrobial activity than roots. Seeds showed a marked cytotoxicity *in vitro* against colorectal, hepatic and Hela cell lines [9]. Terpenoids are the most widespread, chemically interesting groups of secondary metabolites with over 30,000 known compounds including steroids [10,11]. Many terpenes have biological activities and are used for human treatment diseases. Among the pharmaceuticals, the anticancer drug Taxol® and the antimalarial drug Artimesinin are two of the most renowned terpene-based drugs [10]. *Moricandia nitens* included the flavonoids, coumarins, tannins, sterol and/ or tepens, resin, glycosides and/ or carbohydrates and traces of alkaloids, also the same author isolated five flavonoid compounds and demonstrated that the methanol extract was the best activity against some bacteria and fungi [12]. No previous studies available on the investigation of glucosinolates and terpens of *Moricandia nitens*, which encourage us to the study the glucosinolates and other bioactive compounds of *Moricandia nitens*.

MATERIALS AND METHODS:

Plant material:

Moricandia nitens (Viv.) E. A. Durand & Barratte was collected at full flowering stage from Ageba area, Mersa-Mattruh at April 2011, then the aerial parts and roots of the plants were separated from each other. The aerial parts and roots were air dried, then ground to fine powder and kept to be used for different analysis.

Preparation of the hydrolysis products: According to Al Gendy and Lockwood [13]**.**

Natural autolysis

Five gm of each of the aerial parts and roots were separately mixed with distilled water (100 ml) and left for natural autolysis overnight (17h) at room temperature, where Dichloromethan (20ml) was added and the mixtures were shaken for 30 min, then they were centrifuged for 5 min at 3500 rpm. The separated organic layer was separated, dried over anhydrous sodium sulphate and concentrated under nitrogen to about 1 ml. The concentrated hydrolysate was kept in dark vial in a freezer .

Exogenous myrosinase enzymatic hydrolysis

Two samples of 5gm of each of the aerial parts and roots separately were mixed with distilled water (100 ml), myrosinase enzyme (1&2 units, Sigma) and 2-5 mg of L- ascorbic acid were added and allowed to hydrolyze for 1h& 2h. followed by extraction as described before.

Identification of the hydrolyzed products using GC-MS analysis

GC-MS analysis was carried out on a Hewlett-Packard 6890 gas chromatograph fitted with a fused silica HP-5MS capillary column (30 m × 0.25 mm; film thickness 0.25 µm). The oven temperature was programmed from 60ºC at 3ºC/min. Helium was used as carrier gas at a flow rate of 2 ml/min. The gas chromatograph was coupled to a Hewlett-Packard 6890 mass selective detector. The MS operating parameters were: ionization voltage, 70 eV; ion source temperature 200ºC.

Estimation of the total glucosinolates of *Moricandia nitens* parts: According to Hu et al., and Thies [14,15].

Extract preparation (hydro-alcoholic extraction) of other bioactive compounds

About 120gm of each of the aerial parts and roots of *Moricandia nitens* were separately extracted with 750ml of 70% ethanol and the mixtures were shaken for 72h at room temperature. The extract was filtered with Whatman No. 1 filter paper and the filtrate was evaporated to dryness in crucibles using a temperature-regulated water bath pre-set at 50°C. The obtained residues were weighed and the drying extract was preserved at 4°C in an airtight container until use at GC-MS apparatus as above [16].

Identification of bioactive compounds

The identification of components was based on GC-MS apparatus library. The relative abundance level of each component was calculated as percentages by comparing its average peak area to the total area. The results obtained were tabulated and the bioactive compounds identified by GC-MS analysis were carried out.

Estimation of the total terpenes of *Moricandia nitens* parts: According to Indumathi et al., [17].

Estimation of the total alkaloids of *Moricandia nitens* parts: According to British Pharmacopoeia [18].

Determination of cytotoxic activity

Potential cytotoxicity of the aqueous extract (70% ethanol) was tested using the method of Skehen et al., [19], where the tumour cell lines were HCT (colon carcinoma cell line) and HEPG2 (liver carcinoma cell line).

RESULTS AND DISCUSSIONS:

Investigation of the glucosinolate hydrolysis products

The aerial parts and roots of *M. nitens* when subjected to natural autolysis and two samples of each part were subjected to enzymatic hydrolysis using one and two units of external myrosinase enzyme, then they were subjected to GC-MS analysis. GC-MS analysis of the aerial parts and roots revealed the presence of twelve glucosinolate compounds:

Ethyl isothiocyanate: Mass spectrum revealed M/Z (relative Abundance %); 87 (M^+, 34%), 72 (40%), 69.1 (50%), 65.2 (15%), 57 (20%) and 26 (100%).

Isobutyl isothiocyanate: Mass spectrum revealed M/Z (relative Abundance %);133(M^++ 3, 18%), 91.1 (100%), 77.1 (76%), 65.2 (34%), 54 (26%) and 25 (46%).

4-(methylthio) butane nitrile: Mass spectrum revealed M/Z (relative Abundance %);133(M^++ 1, 18%), 119.1 (100%), 91.1 (94%), 65.1 (48%), 50 (36%) and 24 (71%).

5- -(methylthio)-4- pentane nitrile (Dehydroerucin nitrile): Mass spectrum revealed M/Z (relative Abundance %);127(M^+,10%), 85.1 (18%), 72 (30%), 57.1 (36%), 25.4(100%) and 23 (84%).

5- (methylthio) pentane nitrile (Erucin nitrile): : Mass spectrum revealed M/Z (relative Abundance %);136 (M^+, 10%), 121.1(22%), 91.1 (100%), 77.1 (80%), 65.4(40%) and 24 (82%).

3- butenyl isothiocyanate (Napin): Mass spectrum revealed M/Z (relative Abundance %);113 (M^+, 8%), 85.1(18%), 71.1 (30%), 57.1 (38%), 27.2(100%) and 27 (74%).

4-Methylthio-3-butenyl isothiocyanate (Erucin) : Mass spectrum revealed M/Z (relative Abundance %);87 (M^++ 52, 10%), 95.1(16%), 79.1 (28%), 55.1 (18%), 27.2(100%) and 26 (56%).

Allyl isothiocyanate: Mass spectrum revealed M/Z (relative Abundance %);99 (M^+, 8%), 85.1(14%), 71.1 (24%), 57.1 (34%), 27 (100%) and 26 (54%).

Benzyl isothiocyanate: Mass spectrum revealed M/Z (relative Abundance %);150 (M^+ +1, 10%), 107.1(8%), 85.1 (18%), 71.1 (38%), 55.2 (36%), 27 (100%) and 25 (62%).

3- (Methyl thio) propyl isothiocyanate (Iberverin): Mass spectrum revealed M/Z (relative Abundance %); 147 (M^+, 6%), 73.1(100%), 59 (8%) and 25 (8%).

4- (methyl sulphonyl) butane nitrile: Mass spectrum revealed M/Z (relative Abundance %); 165 (M^+ + 4 , 3%), 113.3 (4%), 85.2 (16%), 71.1 (30%), 57.1(44%) and 28 (100%).

3- (methyl sulphonyl) propyl isothiocyanate: Mass spectrum revealed M/Z (relative Abundance %); 197 (M^+ + 12 , 3%), 162.3 (3%), 99.1 (5%), 85.1 (17%), 71.1 (34%), 57.1(30%) and 28 (100%).

Those results were analogous to that obtained by other authers [13, 20-22]. The difference of the hydrolyzed glucosinolates products obtained from natural autolysis and exogenous myrosinase enzymatic hydrolysis of the aerial parts and roots of *M. nitens* were showed at Table (1) with its retention time.

Table 1: Glucosinolates hydrolysis of *M. nitens* aerial parts and roots using GC-MS.

Compounds	Aerial parts				Roots			
	RT (min)	Natural autolysis (%)	Exogenous myrosinase (%)		RT (min)	Natural autolysis (%)	Exogenous myrosinase (%)	
			1h	2h			1h	2h
Ethyl isothiocyanate	9.05	3.4	5.2	3.3	9.69	4.1	6.0	3.7
Isobutyl isothiocyanate	9.72	0.27	0.21	-	10.4	1.34	1.02	0.03
4-(methylthio) butane nitrile	10.41	0.32	0.15	0.06	10.57	0.28	0.02	-
5- (methylthio)-4- pentene nitrile	11.11	0.43	0.21	0.10	11.09	0.78	0.45	0.18
5- (methylthio) pentane nitrile	11.54	3.03	1.08	0.81	11.28	0.33	0.21	-
3- butenyl isothiocyanate	12.75	0.32	0.06	-	14.06	0.49	0.10	-
4-Methylthio-3-butenyl isothiocyanate	14.08	0.76	0.12	0.02	14.73	0.35	0.09	-
Allyl isothiocyanate	20.24	0.29	0.05	-	18.82	1.76	1.02	0.03
Benzyl isothiocyanate	20.79	0.40	0.45	0.62	20.77	0.69	0.72	1.05
3- (Methyl thio) propyl isothiocyanate	24.48	0.36	0.41	0.51	24.46	0.66	0.84	0.95
4- (methyl sulphonyl) butane nitrile	27.46	0.34	0.24	0.22	27.13	1.35	0.76	0.25
3- (methyl sulphonyl) propyl isothiocyanate	27.76	0.23	0.01	0.05	28.10	0.39	0.02	0.06

The obtained results showed that, the compounds Ethyl isothiocyanate, Isobutyl isothiocyanate, 5-(methylthio)-4- pentene nitrile, 3- butenyl isothiocyanate, Allyl isothiocyanate, Benzyl isothiocyanate, 3- (Methyl thio) propyl isothiocyanate, 4- (methyl sulphonyl) butane nitrile and 3- (methyl sulphonyl) propyl isothiocyanate percentages were higher in roots than that of the aerial parts, while 4-(methylthio) butanenitrile, 5-(methylthio) pentane nitrile and 4-Methylthio-3-butenyl isothiocyanate were higher in the aerial parts than that of roots. All handled reviews showed that the glucosinolate compounds of *M. nitens* were unidentified before.

Total glucosinolates content of *M. nitens* parts:

The total glucosinolates content for *M. nitens* were estimated quantitatively using spectrophotometeric methods, where the amount of total glucosinolates content of the aerial parts and roots were 1.7 and 2.1mg/gm, respectively.

Identification of bioactive compounds by GC-MS

The GC-MS analysis of The aerial parts and roots of *M. nitens* indicated the presence of 50 phytochemical constituents in each part. Comparison of the mass spectra of bioactive constituents, where their bioactive compounds were identified. The active principles with their respective retention time and concentration (%) were presented at Table 2.

Table 2: GC-MS spectral analysis of the extracts for *M. nitens* aerial parts and roots.

No	Compounds	R T (Mins)	Aerial part (%)	Roots (%)	Compound Nature
1	Sabinene	8.53	0.96	1.06	Monoterpene
2	áMyrcene	9.05	--	0.34	Monoterpene
3	Lauric acid	9.69	0.76	--	Fatty acid
4	çTerpinene	10.04	1.34	4.86	Monoterpene
5	O- Cymene	10.41	--	0.32	Monoterpene
6	Levomenthol	10.46	1.28	1.22	Monoterpene alcohol
7	L Phellandrene	10.57	0.70	0.27	Monoterpene
8	Decane	11.09	6.78	10.43	Hydrocarbon
9	silane trichloro docosyl pentane 1 bromo	11.28	1.33	1.20	Alkaloid
10	à-Pinene	11.52	1.80	1.94	Monoterpene
11	5Cyclopropylcarbonyloxypentadecane	12.06	1.27	--	Hydrocarbon
12	àTerpinolene	12.48	0.66	0.28	Monoterpene
13	Santolina triene	12.49	--	0.86	Monoterpene
14	2,4,6,8Tetramethyl1undecene	12.73	0.72	3.22	Hydrocarbon
15	Octane (CAS)	12.75	0.31	3.32	Hydrocarbon
16	Cis Sabinene hydrate	13.22	1.63	0.38	Monoterpene
17	Trans sabinene hydrate	13.23	--	1.11	Monoterpene
18	3Octyn2ol	14.06	1.49	--	Acohol
19	1-Terpineol	14.08	--	1.37	Monoterpene alcohol
20	Terpinen-4-ol	16.10	9.76	24.54	Monoterpene alcohol
21	Myrcenol	16.70	2.33	--	Monoterpene alcohol
22	àTerpineol	16.72	--	2.53	Monoterpene alcohol
23	Linalool	18.26	1.28	--	Monoterpene alcohol
24	Linalyl acetate	18.29	--	0.47	Diterpene
25	Pentadecane	18.82	2.06	--	Hydrocarbon
26	Hexadecane (CAS)	19.15	--	1.07	Hydrocarbon
27	tetramethyl Silane	19.17	1.09	0.51	Organosilicon
28	2Bromononane	19.31	0.33	--	Monoterpene
29	Ether, 6methylheptyl vinyl	19.34	--	0.24	alkene group and an alkyl group
30	Oxalic acid, allyl nonyl ester	19.56	1.55	1.22	Ester
31	Hentriacontane	20.21	0.85	4.11	Hydrocarbon
32	Dodecane (CAS)	20.43	3.81	2.38	Hydrocarbon
33	Tridecane- 4-methyl	20.77	0.69	1.40	Hydrocarbon
34	Trans Caryophyllene	23.96	1.74	1.77	Sesquiterpene
35	Silane, tetramethyl(CAS)	24.48	--	0.36	Organosilicon
36	Tetradecane	25.71	1.98	2.81	Hydrocarbon
37	Docosane	26.21	2.45	3.20	Alkane
38	9Octadecen12ynoic acid, methyl ester	26.23	--	0.58	Ester
39	Silane, trichlorodocosyl	26.61	--	1.21	Organosilicon
40	Eicosane	27.45	2.54	0.34	Alkane
41	Sulfurous acid, decyl 2propyl ester	33.33	1.56	0.34	Ester
42	Sulfurous acid, hexyl tridecyl ester	34.84	1.45	1.27	Ester
43	Velleral	37.83	2.69	--	| Sesquiterpen
44	Stearic acid hydrazide	38.26	--	0.25	Fatty acid

45	Oleic Acid	38.77	--	0.19	Fatty acid
46	Heptane, 2,6dimethyl	42.24	0.67	0.29	Hydrocarbon
47	Oxiraneoctanoic acid, 3octyl, Methyl ester, trans(CAS)	43.02	1.33	1.39	Ester
48	3Hexadecyloxycarbonyl5(2hydroxye thyl)4methylimidazolium ion	43.64	1.30	1.12	Imidazole
49	1Propene1,2,3tricarboxylic acid, tributyl ester	43.83	1.95	--	Ester
50	2-Piperidinone, N-(4-bromo-n-butyl)	44.16	0.33	--	Alkaloid
51	2Myristynoyl pantetheine	45.27	1.30	--	Protein
52	Tributyl acetylcitrate	45.74	8.12	--	citric acid ester
53	1 Butyl Pentyl trifluoro methane sulfonate Tetra Acetyl D'Xylonic nitrile	47.46	1.36	1.78	Glucosinolate
54	4Bromophenyl) bis(2,4dibromophenyl)amine	49.56	0.68	--	Indole
55	7MethylZtetradecen1ol acetate 9-Octadecenoic acid (Z)(CAS)	50.33	1.30	--	Fatty acids
56	Erucic acid	51.22	--	2.22	Fatty acids
57	Dinoctyl phthalate	51.53	1.41	1.65	Phenolic
58	Squalene	56.53	5.37	6.22	Triterpene
59	Andrographolide	57.06	--	0.06	Diterpene
60	Triacontane	58.09	4.80	--	Hydrocarbon
61	9Octadecenoic acid	60.66	1.27	1.32	Fatty acids
62	Cholesterol -3-O-[2acetoxy ethyl]	61.27	0.58	1.21	Sterol
63	Lucenin	61.71	1.45	--	Flavonoid
64	Palmitic acid	62.97	0.89	--	Fatty acid
65	Stigmasterol	63.65	--	0.61	Sterol
66	áSitosterol	64.96	1.15	1.82	Sterol
67	Gynolutone	66.83	4.21	2.66	Hormone

The identified compounds with high percentages of the aerial parts and roots like Terpinen-4-ol (Monoterpene alcohol) (9.76% and 24.54% for aerial parts and roots, respectively), Tributyl acetylcitrate (citric acid ester) (8.12% at aerial parts), Decane (Hydrocarbon) (6.78% and 10.43% for aerial parts and roots, respectively), Squalene (Triterpene) (5.37% and 6.22% for aerial parts and roots, respectively) showed a wide range of potent bioactivity.

Squalene acts as a protective agent and has been shown to decrease chemotherapy-induced side-effects. Moreover, squalene alone exhibits chemopreventive activity. Although it is a weak inhibitor of tumor cell proliferation, it contributes either directly or indirectly to the treatment of cancer due to its potentiation effect. In addition, squalene enhances the immune response to various associated antigens, and it is therefore being investigated for vaccine delivery applications [23].

It was interesting that, our GC-MS fingerprints revealed the presence of sesquiterpenes which have been identified as the active constituents present in several medicinal plants with a wide range of biological properties including anti-infective, anti-oxidant, anti-inflammatory, anticancer and anti-cholinesterase activities [24].

The total terpenes content of *Moricandia nitens* parts:

Estimation of the total terpens content of the aerial parts and roots for the first time spectrophotometrically, revealed that the total terpens content of the aerial parts and roots of *M. nitens* were 2.72 and 5.04 mg/ gm, respectively.

The total alkaloids content of *Moricandia nitens* parts:

The percentage of the total alkaloids was 0.02% for each of the aerial parts and roots of *M. nitens*.

Cytotoxic activity of *M. nitens* aerial parts and roots

Cytotoxic activity (in vitro) of the aerial parts and roots against (HCT) showed remarkable activity at IC_{50}= 99.8µg/ml for aerial parts (Fig. 1), (HCT) at IC_{50}= 63 µg/ml for roots (Fig. 3), (HEPG2) at IC_{50}= 153 µg/ml for aerial parts (Fig. 2) and (HEPG2) at IC_{50}= 200 µg/ml for roots (Fig. 4). It may be attributed to the presence of different classes of bioactive compounds which detected by GC-MS analysis beside the presence of different compounds of glucosinolates. It may be also attributed to the presence of compounds with antioxidant properties such as terpenoid compounds which were detected in our extract. These finding also coincide with those reported by Barla et al., [25].

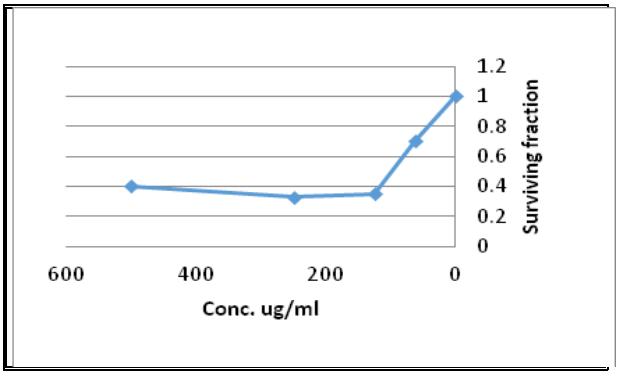

Fig. 1: Cytotoxic potency for the aerial parts against of *M. nitens* (HCT) & IC_{50}= 99.8µg/ml

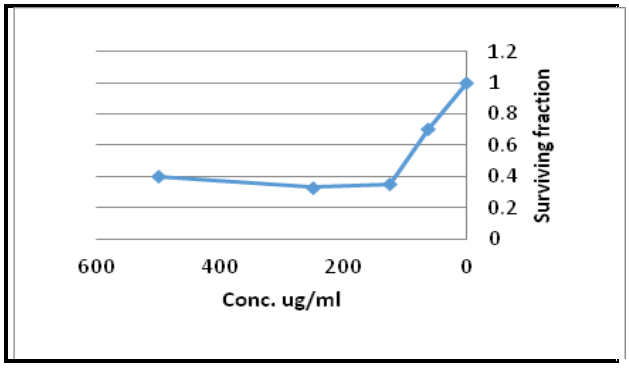

Fig. 2: Cytotoxic potency for the aerial parts of *M. nitens* against (HEPG2) & IC_{50}= 153 µg/ml

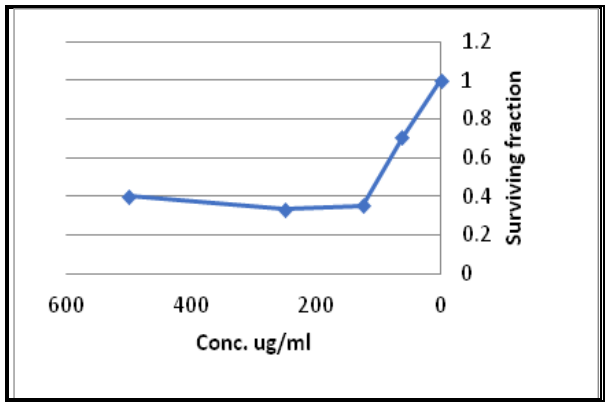

Fig. 3: Cytotoxic potency for the roots of *M. nitens* against (HCT) & IC_{50}= 63µg/ml

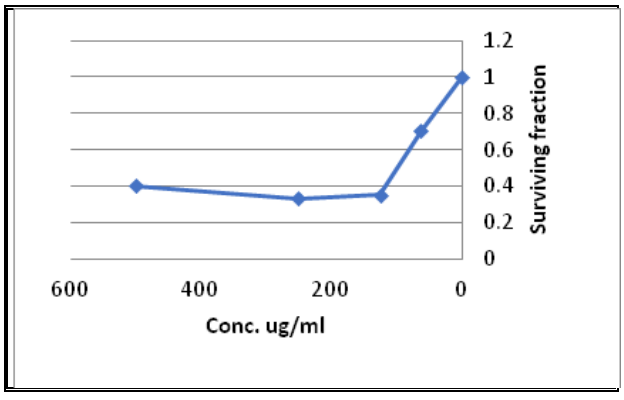

Fig. 4: Cytotoxic potency for the roots of *M. nitens* against (HEPG2) & IC_{50}= 153 µg/ml.

CONCLUSION:

The present study identified an array of glucosinolates and the bioactive compounds present in the aerial parts and roots of *Moricandia nitens* and reported their compounds nature. In addition to the determination of total glucosinolates, total terpenes and total alkaloids. Cytotoxic activity of the aerial parts and roots of *M. nitens* (in vitro) against (HCT and HEPG2) showed remarkable cytotoxic activity.

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