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Phenolic Profiling of *Hyphaene thebaica* by LC-ESI-Mass: Iron Nanoparticles Significance and Cytotoxic Activity

Sahar A. M. Hussien[a]*, Amani M. D. EL-Messalamy[b], Mai S. M. Seleim[b]

[a] *Phytochemistry and Plant Systematics Department,National Research Centre,Egypt,*

[b] *Chemistry Department, Faculty of Science, Zagazig University, Egypt.*

Abstract

Phenolic profiling of edible parts *Hyphaene thebaica* (Doum palm) are identified using LC-ESI-MS, the Overall polyphenolic constituents demonstrated by means of LC-ESI-Mass profiling . Twenty three isolated compounds were identified as ; Caffeic acid, protocatechuic acid, rhamnetin, catechin, quercitrin, vanillic acid , kaempferol 3-*O*-acetyleglucoside , cinnamic acid, apigenin-7-*O*-glucose , intricatin-3-*O*-htyrosol, luteolin, quercetin, naringenin , kaempferol ,vanillic acid-4-β-D-glycoside coumaric acid, ferulic acid, luteolin-6-arbinose-8-glucose, *p*-coumaroyl malic acid eriocitrin, apigenin and hesperetin

Iron nanoparticles (FeNps) of *H. thebaica* fruit EtOAc extract was freshly prepared and characterized with Dynamic Light Scattering (DLS) with particles size 150.7 nm. The anti-proliferation activity of the crude extract of EtOAc and the synthesized Fe Nps was evaluated using MTT assay on human colon (Caco-2) and liver (HepG2) cancer cell lines.

The results declared that half maximal inhibitory concentration (IC_{50}) of EtOAc extract on colon (IC_{50} 35.4 µg/ml) and on liver cancer cell lines (IC_{50} 72.02 µg/ml) while nanoparticles portion of EtOAc was found more pronounced on colon cancer cell lines (IC_{50} 19.44 µg/ml) and on liver (IC_{50} 15.5 µg/ml). So, the Fe Nps of EtOAc of *H.thebaica* fruit extract with particles size 150.7 nm is more effective as antitumor than the crude EtOAc extract.

Keywords: *Hyphaene thebaica* L. Fruit (Doum palm), HPLC/MS , polyphenolic constituents ,Iron Nanoparticles Fe Nps , Antitumor activity.

INTRODUCTION

Hyphaene thebaica L. Fruit (Doum palm) is a member of the palm family called Arecaceae, which is indigenous to the Nile Valley and grows very well in Nigeria's southern portion **[1]** . The plant possesses brown edible oval fruits with outer fibrous flesh which is rich in poly phenolic compounds and potent antioxidants **[2]** . The fruit kernel is edible when it's unripe, but difficult when it's ripe, it's used for local craft, building and can be used in medicinal applications. In traditional locations in Egypt, fruits are frequently consumed as drinks **[3]**. Apart from food uses, the crude extract of the fruit was reported to be active against acute myeloid leukemia **[4].** Cancer is an abnormal form of tissue development in which the cells show uncontrolled division leading to a rise in the amount of cells that divide **[5].** Among non-communicable diseases, cancer is second leading cause of death worldwide after cardiovascular diseases **[6].**

Considering the above facts this study focuses on the development of an economic, simple and eco-friendly synthesis of iron nanoparticles using active phenolic compounds and flavonoids *H. thebaica* fruit EtOAc extract and its anti-proliferation activity on human colon and liver cancer cell lines.

Nano scale technology development has been commonly recognized as a revolutionary model change for cancer identification and remediation. Indeed, the increase in study attempts investigating the design and preparation of nanoparticles (NP) devices has resulted in the development of many material forms with promising therapeutic and diagnostic impacts in a single nano drug for the therapy of different kinds of cancer **[7].**

*Corresponding author e-mail: drsahar90@yahoo.com.; (Sahar Awad Allah Hussein).

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The Production of Nps from plant extracts that are eco-friendly materials, has drawn more attention of researchers because of its abundant applications in different fields due to their deep-rooted properties **[8].** Different types of metallic Nps were synthesized using different metal atoms such as silver, gold, zinc, copper, titanium and iron. The plant extracts operate as metal ion reduction and capping agents that have more benefits than other biological procedures **[9].**

The extracts of these plants contain molecules with alcoholic OH- functional groups. These molecules can be used to reduce and form stable complicated compounds **[10].**

Materials and Methods

The *H. thebaica* fruits were collected from the arid Aswan desert region of Southern Egypt in November (2018). The taxonomic and authenticated by Prof.Dr. Salwa Kawshty , A voucher herbarium specimen was deposited in herbarium of the National Research Center (NRC), Egypt ,with voucher specimen (A 362) . After scraping from the seed using stainless steel knife, the pericarp and the edible part were crushed. The broken part was placed in a refrigerator at 5ºC until processing and evaluation. The powdered fruit coat (100 g) was exhaustively extracted with (500 ml) of ethyl acetate (95% purity) for 24 h at 78ºC which yielded (4.2 gm) of dried plant crude extracts kept in refrigerator for analysis.

LC-ESI-Mass Analysis

High performance liquid chromatography LC-ESI-Mass analysis was performed in Drug Discovery and Development, Research Centre (Ain shams University, Giza, Egypt) by inverse stage C_{18} column (ACQUITY UPLCBEH C_{18} 1.7 µm particle size 2.1 omb 5 mm Column) in order to identify flavonoids and phenolics of *H.thebaica* fruit extract. The sample (100 µg/mL) solution was prepared with grade solvent of MeOH, filtered by using a membrane disc filter (0.2 µm) then subjected to LC-ESI-MS analysis. The amounts of samples (10µl) were injected into the fitted HPLC tool. The portable sample stage was prepared by filtering with the 0.2 µm filter membrane disk and degassed before injection with the sonication. Mobile phase elution was made with the flow rate of 0.2 mL/min using gradient mobile phase comprising two eluents: eluent A is H_2O acidified with 0.1% formic acid and eluent B is MeOH acidified with 0.1% formic acid. The parameters for analysis were carried out using negative ion mode as follows: source temperature 150°C,cone voltage 30 eV, capillary voltage 3 kV, desolation temperature 440°C, cone gas flow 50 L/h and desolvation gas flow 900L/h. Mass spectra were detected using the ESI negative ion mode between m/z 100–1000. Using the Maslynx 4.1 software, peaks and spectra were processed and tentatively recognized by comparing their retention time (RT) with mass range and international data.

All chemicals were purchased from Merck KGaA, Darmstadt, Germany.

Synthesis of Iron Nanoparticles

The iron nanoparticles were obtained by the co-precipitation method which means to carry down by a precipitate of substances normally soluble under the conditions employed **[11],** in Nawah Scientific Institute (Al mokattam , Cairo, Egypt) . In distilled water, molar ratio of ferric ion to ferrous ion in the solution of two Fe_3SO_4 .7 H_2O and of $FeCl_3$ .6 H_2O were dissolved. The solution was heated to 80ºC and stirred forcefully (1000 rpm). Then, NH_4OH (50 mL) was dropped under stirring, and the solution was stirred for 40 minutes. Heating and stirring were turned off and the solution rested until reaching room temperature. The magnetic nanoparticles (Fe_3SO_4) were washed with distilled water. After that, they were filtrated, and dried in oven (60ºC) for 2 hours. The obtained iron nanoparticle (25 mg) mixed with (2 g) of ethyl acetate extract dissolved in (100 ml) of distilled water and 200 µl DMSO and the resulting liquid was stored for further characterization.

Characterization of Iron Nanoparticles

Synthesized iron nanoparticles were characterized with the help of a particle size analyser. The hydrodynamic size distributions of nanoparticles were analysed by a Dynamic Light Scattering (DLS) which is a technique in physics that can be used to determine the size distribution profile of small particles in suspension or polymers in solution. **[12].** Using a Nanotrack wave device (microtrack, Boston, USA).

Cell Culture and Treatment

The cytotoxicity assay was performed at the (Veterinary Serum and Vaccine Research Institute , Cairo, Egypt) under approved investigations. The human colon cancer cell line Caco-2 was grown in Roswell Park Memorial Institute medium (RPMI)-1640 containing 10% fatal bovine serum (FBS) while Hepatic cancer cell line HePG2 was grown in Dulbecco's modified Eagle's medium (DMEM) containing10%(FBS) and 1% penicillin-streptomycin antibiotics. The cells were grown in a CO_2 incubator containing 5% CO_2 and 95% air at 37ºC. The cells were sub-cultured for 4 days of regular interval. Cells were seeded at a density of 7× 103 cells/ml. And

passaging by a brief incubation with trypsin / EDTA. Cells growing at the exponential phase were used to perform all the experiments.

In *vitro* Assay for Cytotoxicity Activity by (MTT assay)

The MTT assay is a colorimetric assay for evaluating cell metabolic activity. NAD (P) H-dependent cellular oxidoreductase enzymes may, under defined conditions, reflect the number of viable cells present. These enzymes are capable of reducing the tetrazolium dye MTT 3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to its insoluble formazan, which has a purple color. Other closely related tetrazolium dyes including XTT, MTS and the WSTs are used as a conjunction for the intermediate electron acceptor, 1-methoxy phenazine methosulfate (PMS). With WST-1, which is cell-impermeable, reduction occurs outside the cell through plasma membrane electron transport **[13]**. Since reduction of MTT can only occur in metabolically active cells the level of activity is an indicator for the viability of the cells(flat bottom)and incubated for 24h. After 24h, cells were treated with various concentrations from *H.thebaica* fruit (0, 6.5, 12.5, 25, 50 and100 µg/mL) for both; the synthesized Fe Nps and the crude ethyl acetate extracts. After that, MTT reagent (20 mg/mL) were added along with fresh culture medium into each well and incubated for 4 h in 37ºC in dark. The formazan products which formed by MTT were solubilized by DMSO and mixed well . Additional incubation for1h in 37ºC in dark. Plate in ELISA Reader was measured according to EIA/ELISA **[14].** The optical density (OD) in 57nm was measured . By using Graph Pad Prism software (GraphPad Software, San Diego,CA,USA) the median inhibition concentration (IC_{50}) was calculated.

Statistic analysis

Statistical analysis were performed by a digital computer, using Excel and SPSS version 17.0 programs according to the technique adopted by **[15].** Differences at $P \leq 0.001$ were considered statistically significant.

Results and Discussion

Identification of Phenolic Acids and Flavonoids Of EtOAc Extract, Of *H.thebaica* LC-ESI-Mass :

Ethyl acetate extract was examined with HPLC/MS to prove the presence of 23 major peaks within 31 min. The electrospray ionization mass spectrometry in negative ion mode for all the compounds was detected and mass fragmentation patterns compared with literature data. Twenty three isolated compounds were identified **(Table1),(Fig.1) ;** Caffeic acid, protocatechuic acid, rhamnetin, catechin, quercitrin, vanillic acid , kaempferol-3-O-acetyleglucoside , cinnamic acid, apigenin-7-*O*-glucose , intricatin 3-*O*-htyrosol, luteolin, quercetin , naringenin ,kaempferol ,vanillic acid-4-β-d-glycoside coumaric acid, ferulic acid, luteolin-6-arbinose-8-glucose, *p*-coumaroyl malic acid eriocitrin, apigenin and hesperetin.

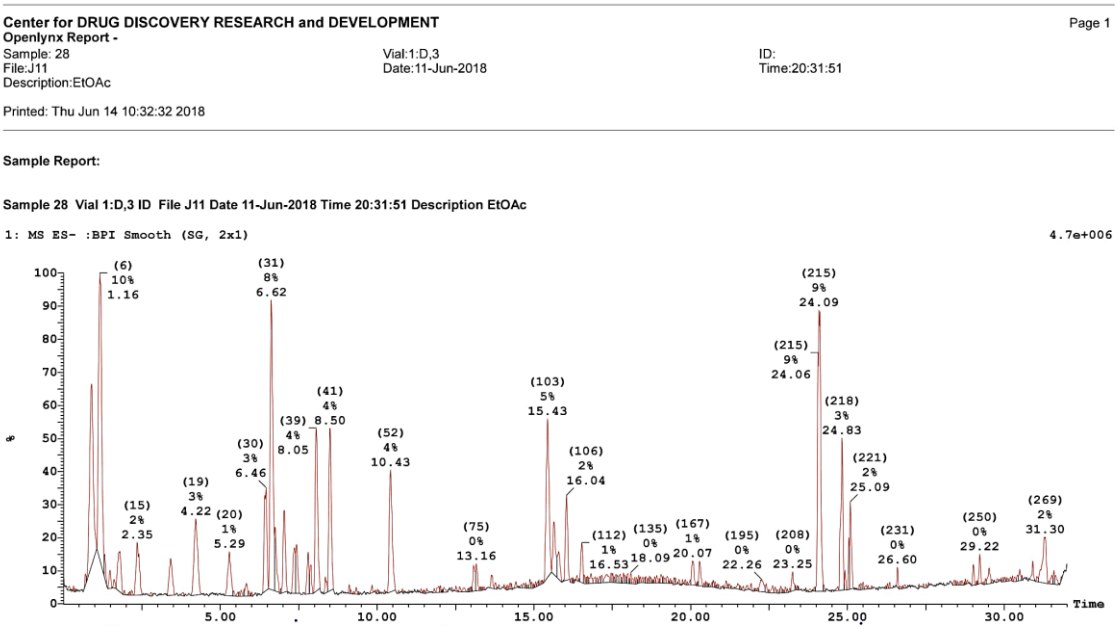

Fig 1. HPLC-MS Chromatographic Analysis Of Ethyl Acetate Extract

Table 1 : Phenolic acids and flavonoid compounds identified by HPLC/MS of EtOAc extract of *H. thebaica* Lfruits

Peak No.	Rt (min)	Area (%)	$[M-H]^-$ of found mass (m/z)	Identification	M.wt (g/mol)
1	0.89	6	179	Caffeic acid	180.159
2	1.16	10	153	Protocatechuic acid	154.12
3	2.35	2	315	Rhamnetin	316.26
4	3.41	1	289	Catechin	290.27
5	4.22	3	447.1	Quercitrin	448.38
6	5.29	1	167	Vanillic Acid	168.14
7	6.46	3	489.2	Kaempferol-3-*O*-Acetyleglucoside	490.41
8	6.62	8	147	Cinnamic Acid	148.16
9	6.75	3	431.2	Apigenin-7-Glucose	432.21
10	8.05	4	311.1	Intricatin	312.32
11	7.48	4	137	3-Hydroxy Tyrosol	138.16
12	8.00	0	285	Luteolin	286.23
13	8.50	4	301	Quercetin	302.28
14	10.43	4	271	Naringenin	272.25
15	13.16	0	285	kaempferol	286.23
16	15.43	5	329.3	Vanillic acid 4-β-D-glycoside	330.28
17	16.04	2	163.2	Coumaric acid	164.16
18	20.07	1	193	Ferulic acid	194.18
19	23.25	0	669	Luteolin-6-arbinose -8-glucose	670
20	24.09	9	279.2	*p*-coumaroyl malic acid	280.23
21	24.83	2	595.5	Eriocitrin	596.5
22	25.09	2	269.2	Apigenin	270.24
23	31.30	2	301.2	Hesperetin	302.28

Reaction of bioactive compounds of *H.thebaica* fruit ethyl acetate extract with synthesized iron nanoparticles, appeared to be a potent source of phenolic acids and flavonoids.

Preparation of iron nanoparticles of EtOAc extract, the color changed to black, by HPLC/MS examination proved the presence of 17 major peaks namely : Caffeic acid , Cinnamic acid, Vanillic acid 4-β-D-glycoside,Coumaroyl malic acid quercetin, quercitrin, kaempferol-3-*O*-acetyleglucoside and apigenin-7-glucose, **(Table 2)**.

So, the change of color to black revealed to the reaction with compounds of EtOAc , extract to form zero valent iron NPs complex. The phenolic and flavonoid OH- groups are important for metal-binding activity which were involved in the reduction of Fe ions into Fe NPs (**[16]**. As example; caffeic acid (as phenolic acids) and quercetin (as flavonoids) were synthesized as coated magnetic nanoparticles. The particle size distribution of synthesized FeNPs shown in **(Fig 2)**

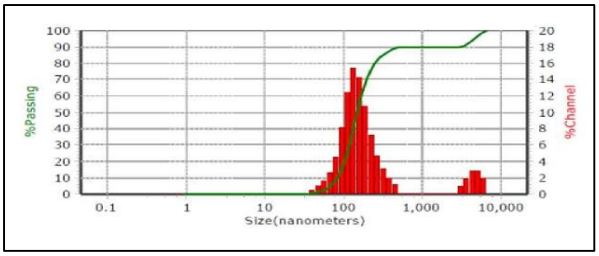

Fig.2 particle size distribution of synthesized FeNPs

Table 2 : The phenolic and flavonoids bioactive compounds of EtOAc extract to form zero valent FeNPs complex.

Peak No.	Rt (min)	Area (%)	$[M-H]^-$ (m/z)	Identification	M.wt (g/mol)
1	0.92	5	179.1	Caffeic acid	180.159
2	0.93	19	377.1	Oleuropein aglycone	378.37
3	1.20	16	451	Catechin-7-glycoside	452.1
4	1.91	3	153	Protocatechuic acid	154.12
5	2.04	2	315.1	Rhamnetin	316.26
6	4.19	1	137	3-hydroxy tyrosol	138.16
7	5.09	1	353.1	Chlorogenic acid	354.31
8	6.33	2	461.1	Luteolin 7-O-β glucuronoide	462.363
9	6.52	8	145.1	Coumarin	146.14
10	8.35	2	593	Kaempferol-3- β –(6''-*O*-*p*-coumaroy) - glucopyranoside	594
11	9.54	2	607.2	Kaempferol-3-7-dirhamnoside	608.5
12	11.75	1	285.2	Luteolin	286.23
13	12.96	0	269.1	Apigenin	270.24
14	19.65	1	271	Naringenin	272.25
15	22.32	4	433.3	Quercetin-3-D-xyloside (Reinutrin)	434.3
16	24.31	1	594.4	Cyanidin 3-*O*-rutinoside	595.53
17	31.29	1	431.3	Apigenin-7-glucose (vitexin)	432.32

Examination of Iron Nanoparticles Extract

Synthesis of iron nanoparticles was evaluated using spectrophotometer and Plasmon resonance displaying the size of the homogenous particle as it is not aggregated and is not resolved. Fe Nps were collected in the form of a solution for less than two weeks and were not settled down. The results of the diagram's significant change stated that FeNP result populations are narrowly distributed, implying the development of homogeneous particle size **(Fig. 4).** It is clearly noted that when the amount of fruit extracts is higher more metallic iron is produced. This behavior can be attributed to the amount of polyphenols (reducing agent) contained in the extracts.

Particle size measurements show that firstly; the average particle diameter is (150.7 nm) when passing the pure EtOAc extract is 50% to the Fe NPs (**Table 3,4).** .Secondly; peaks summary table show that the diameter of 89.6% of nanoparticles volume is 142.2 (nm) which were used as the best size for NPs in cell lines experiments **(Table 5) ,** whereas only 10.4% of Fe Nps contain micronsized nanoparticle agglo merates which were neglected in our study on cancer cell lines. PDI is basically a representation of the distribution of size populations within a given sample. The numerical value of PDI ranges from 0.0 (for a perfectly uniform sample with respect to the particle size) to 1.0 (for a highly poly disperse sample with multiple particle size populations). Values of 0.2 and below are most commonly deemed acceptable in practice for polymer-based nanoparticle materials **[17].** The range of polydispersity index (ISO PDI) of the synthesized Fe NPs is 0.027 and the average particle diameter is (150.7 nm) .

Table 3. Summary of particle size measurement of Fe Nps of EtOAc *H.thebaica* fruit extract

Data	Value
MI(nm):	616.0
MN(nm):	85.90
MA(nm):	144.3
Cs:	41.58
SD:	100.3
PDI:	0.02653
Mz:	182.4
Qi:	0.732
Ski:	0.719
Kg:	17.07

Table 4. Perecentiles of particle size measurement of Fe Nps of EtOAc *H. thebaica* fruit extract

%Tile	Size(nm)
10.00	85.10
20.00	104.9
30.00	120.2
40.00	134.8
50.00	150.7
60.00	170.4
70.00	199.1
80.00	253.8
90.00	3020
95.00	4570

Table 5. Peak summary of particle size measurement of Fe Nps of EtOAc *H.thebaica* fruit extract.

Dia(nm)	Vol%	Width
4530	10.4	2052
142.2	89.6	127.6

Comparative Cytotoxicity Activity

The results of the assay revealed that iron Nps extract inhibit growth of all two cell lines used in a dose depending passion as depicted .The anti-proliferation effect of Fe Nps of EtOAc fruit extract and the crude extract of *H. thebaica* on human colon and liver cancer cell lines were determined using MTT assay. The cytotoxicity of the Nps was possibly due to their interference with normal cellular functions by inducing cellular chemistry changes and interrupting cellular proteins synthesis **[18]**.

This extract has cytotoxic effect according to the guidelines from American National Cancer Institute, which considered that IC_{50} for potential plant should be <30 g/ml **[18]**. Reduction in cell number was noticed following incubation with increasing concentration of nanoparticles of the fruit of *H.thebaica* compared to control with IC_{50} Value of pure EtOAc extract and iron nanoparticles.

In caco-2 cell lines; Fe Nps of ethyl acetate fruit extract of *H.thebaica* were found to inhibit Cancer cell line growth after treatment in dose dependent manner ,so IC_{50} Value of crude EtOAc extract and Fe NPs were (35.4 and 19.44 µg/ml) . The Fe NPs extract is more effective than the pure EtOAc extract on Caco-2 cell line. **(Table 6,7).**

The cell Proliferation of Caco-2 cell line using MTT for both the pure EtOAc extract and the synthesized iron nanoparticles of EtOAc extract of *H.thebaica* fruit. **(Fig 3).**

Table 6. Anticancer effect of EtOAc extract synthesize from *H.thebaica* on Caco-2 cells

S.No.	Conc. (µg/mL)	Absorbance (O.D)	Cell viability (%)	IC (50%)
1	0	0.494	100	
2	6.5	0.463	93.72469636	
3	12.5	0.455	92.10526316	**35.4**
4	25	0.248	50.20242915	
5	50	0.219	44.33198381	
6	100	0.082	16.59919028	

Table 7. Anticancer effect of **iron nanoparticles** synthesized from *H.thebaica* extract on Caco-2 cells

S.No.	Conc. µg/mL)	Absorbance (O.D)	Cell viability (%)	IC (50%)
1	0	0.494	100	
2	6.5	0.469	94.93927126	
3	12.5	0.361	73.07692308	**19.44**
4	25	0.157	31.78137652	
5	50	0.077	15.58704453	
6	100	0.058	11.74089069	

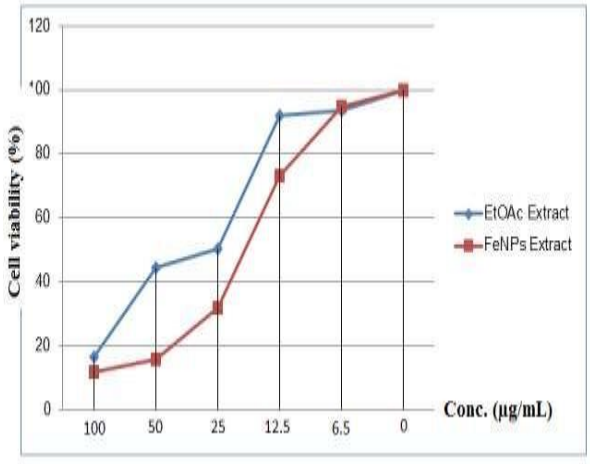

Fig.3 Cell Proliferation of Caco-2 cell line using MTT for both the pure and the synthesized iron nanoparticles EtOAc extract of *H.thebaica*

In HePG2 cell lines; Fe Nps of ethyl acetate fruit extract of *H.thebaica* were found to inhibit Cancer cell line growth after treatment in dose dependent manner so, the concentration necessary to produce 50% death rate of crude EtOAc extract and Fe NPs were (72.02 and 15.5 µg/mL) respectively . **(Table 8**

, 9) Cell Proliferation of HePG2 cell line using MTT for both the pure EtOAc extract and the synthesized iron nanoparticles of EtOAc extract of *H.thebaica* fruit. **(Fig. 4).**

Table 8. Anticancer effect of EtOAc extract synthesized on HEpG2 cells.

S.No.	Conc. (µg/mL)	Absorbance (O.D)	Cell viability (%)	IC (50%)
1	0	0.356	100	72.02
2	6.5	0.318	89.3258427	
3	12.5	0.291	81.74157303	
4	25	0.248	69.66292135	
5	50	0.201	56.46067416	
6	100	0.158	44.38202247	

Table 9. Anticancer effect of **iron nanoparticles** synthesized extract on HEpG2 cell

S.No.	Conc. (µg/mL)	Absorbance (O.D)	Cell viability (%)	IC (50%)
1	0	0.356	100	15.5
2	6.5	0.323	90.73033708	
3	12.5	0.205	57.58426966	
4	25	0.075	21.06741573	
5	50	0.068	19.1011236	
6	100	0.054	15.16853933	

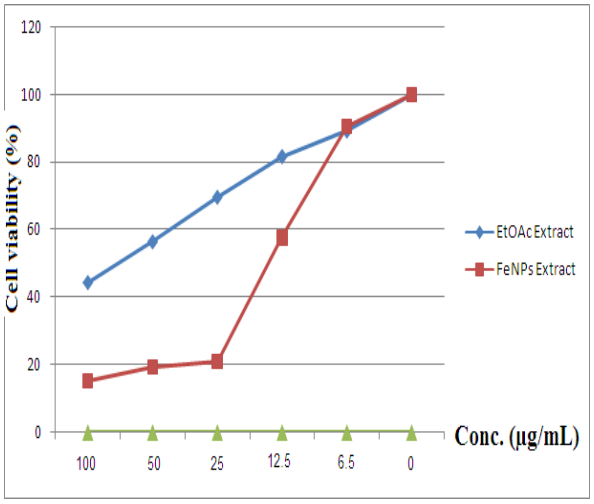

Fig.4 Cell Proliferation of HePG2 cell line using MTT for both the pure and synthesized iron nanoparticles EtOAc extract of *H.thebaica*

In this study, it was observed that the synthesized Fe NPs induces a concentration dependent inhibition of Caco-2 cells and HepG2 cells. Cytotoxic effects of iron nanoparticles were likely owing to the reality that FeNPs may interfere with the correct functioning of cellular proteins and cause subsequent modifications in cellular chemistry resulting in elevated hydrophobicity within bovine hemoglobin which generates alpha helix modifications to beta sheets. Other reports suggest that Fe Nps are likely to interact with thiol-rich enzymes. Therefore, it is possible that once penetrated into cells, Fe Nsp can attack functional cell proteins resulting in partial unfolding and protein aggregation as is the case with bovine hemoglobin **[19].**

CONCLUSION

This study provides an overall approach for analysis of *H.thebaica* L. fruit, providing insight into chemical composition of ethyl acetate fruit extract. HPLC-MS showed an accurate profiling of major flavonoids and phenolic compounds. EtOAc extract of the fruit was selected based on its antioxidant activity as reducing agent for the production of iron nanoparticles suspensions. The size of synthesized Fe Nps was 150.7 nm. It was demonstrated that the production of iron nanoparticles using green synthesis is an inexpensive, efficient and environmentally friendly than the classical chemistry.

Therefore, synthesized Fe Nps of *H.thebaica* EtOAc extract can be of both clinical and environmental applications. FeNPs extract of *H.thebaica* L. fruit was found to be active against the proliferation of human colon (Caco2) and liver (HepG2) cancer cell lines in concentration dependent manner .The results showed that Fe Nps fruits extract is more effective as antitumor than the crude one. The effect of Fe Nps fruit extract was found more pronounced in liver followed by colon cancer cell lines as indicated by corresponding IC_{50} values.

REFERNCES

[1] Aremu A., Fadele O. (2011). Study of some properties of doum palm fruit(*H. thebaica* Mart.) in relation to moisture content, Afr. J. Agric. Res 6: 3597-3602. http://www.Acade micjournals.org/ AJAR

[2] Hsu B., Coupar I. M., Ng K. (2006) . Antioxidant activity of hot water extract from the fruit of the Doum palm, *Hyphaene thebaica*, Food Chem 98:317-328. https:// doi.org/10.1016/j.foodchem.2005.05.077

[3] Eldahshan O. A., Ayoub N. A. , Singab B., Al-Azizi M. M. (2009) .Potential antioxidant phenolic metabolites from doum palm leaves. Afr.J Pharm. Pharmacol 3:158-164. http://www.academicjournals.org/ajpp

[4] Elalla M. A., Faten (2009) . Antioxidant and anticancer activities of doum fruit extract (*Hyphaene thebaica*). African Journal of Pure and Applied Chemistry , 3 (10), 197-

201,(2009)http://www.academicjo urnals.org/AJPAC
[5] Kanchana A. and Balakrishna M. (2011). Anticancer effect of saponins isolated from Solanum trilobatum leaf extract and induction of apoptosis in human larynx cancer cell lines. Int J Pharm.Pharm Sci3(4):356-364 https://innovarea cademics .in/ journal/ijpps/Vol3Suppl4/2567.pdf
[6] World Health Organization . (2015) .Global Health Estimates, Switzerland
[7] Sanna V., Pala N., Sechi M. (2014) .Targeted therapy using nanotechnology : focus on cancer. Int J. Nanomed 9:467-83. https://dx.doi.org/10.2147%2FIJN.S36654
[8] Kuppusamy P., Yusoff M. M. , Maniam G. P. , Govindan N. (2016) . Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications An updated report . Saudi Pharm J 24:473-484, doi:10.1016/j.jsps.2014.11.013
[9] Valli J. S., Vaseeharan B. (2012). Biosynthesis of silver nanoparticles by Cissus quadrangularis extracts. Mater Lett 82:171-173. https://doi.org/10.1016/j.matlet.2012.05.040
[10] Nadagouda M.N., Castle A., Murdock R., Hussain S., Varma R. (2010). In vitro biocompatibility of nano scale zero valent iron particles (NZVI) synthesized using tea polyphenols. Green Chem .12:114–122
[11] Patnaik P. (2004). Dean's Analytical Chemistry Handbook, 2nd ed. McGraw-Hill, New York Berridge M.V., Herst P.M., Tan A.S. (2005) . Tetrazolium dyes as tools in cell biology: new insights into their cellular reduction. Biotechnology Annual Review11:127-152. https://doi.org/10.1016/ S1387-2656(05)11004-7
[12] Berne B. J., Pecora R.(2000). Dynamic Light Scattering. Courier Dover Publications . Mineola 0-486-41155. https: //do.org/10.1016/0307-4412(77)90025-5
[13] Van Weemen B.K., Schuurs A.H.W.M. (1971) .Immunoassay using antigen enzyme conjugates. FEBS Letts 15:232–6 .https:// doi.org/10.1016/0014-579371)80319 -8
[14] Norusis M. J. (2007). SPPS 17.0 advanced statistical procedures. Upper Saddle River, N J. Prentic Hall Patnaik P. (2004). Dean's Analytical Chemistry Handbook, 2nd ed. McGraw-Hill, New York
[15] Norusis M. J. (2007). SPPS 17.0 advanced statistical procedures. Upper Saddle River, N J. Prentic Hall Patnaik P. (2004). Dean's Analytical Chemistry Handbook, 2nd ed. McGraw-Hill, New York
[16] Clarke S. (2013). Development of Hierarch-ical Magnetic Nanocomposite Materials for Biomedical Applications. Ph.D. Thesis, Dublin City University, Northside, Dublin
[17] Rajeshkumar S. , Malarkodi C. , Vanaja M. , Annadurai G. (2016). Anticancer and enhanced antimicrobial activity of biosynth esizd silver nanoparticles against clinical pathogens. J. Mol. Struct1116:165-173https://www.Science direct . com/journal/journal-of-molecularstructure/vol/ 1116/ suppl/C
[18] Alenka L., Barbara K., Roland L., Linda N. (2000) .Activity of edible mushrooms against the growth of human t4 leukemic cancer cell, hela cervical cancer cells and Plasmodium falciparum. J.Herbs,Spices and Medicinal Plants 6:49-58
[19] Shawkey A.M.,Rabeh A.M.,Abdulall A.K., Abdellatif.A.O.(2013).Green nanotechnol-ogy : Anticancer activity of silver nanopa rticles using Citrullus colocyn this aqueous extracts. Adv. Life Sci Technol.13: 60-70