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High‑Performance Liquid Chromatography‑Fingerprint Analyses, *In vitro* Cytotoxicity, Antimicrobial and Antioxidant Activities of the Extracts of *Ceiba speciosa* Growing in Egypt

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Abstract

Background: Cancer diseases and microbial resistance are serious health diseases related to oxidative stress and infectious diseases. The risk can be reduced by using plants rich in polyphenols. **Methodology:** Different solvent extracts from leaves of *Ceiba speciosa* (*C.s*) were evaluated for their biological and chemical activities. Also, the chemical profiles were investigated via high‑performance liquid-chromatography (HPLC) -fingerprint analyses. **Results:** the leaves from *Ceiba speciosa* collected from the Zoo garden in Egypt, showed a moderate cytotoxicity against HepG2 in extracts*;* petroleum ether, ethyl acetate, dichloromethane, while weak-cytotoxicity in butanol, methanol extracts and non-cytotoxicity in water extract. Moreover, high antimicrobial activities were showed within dichloromethane, petroleum ether extracts, while a moderate antimicrobial activities were showed in methanol, ethyl acetate extracts, and low antimicrobial activities were showed in both of butanol and water extracts. On the other side, a strong antioxidant activities were recorded within extracts; dichloromethane and methanol while the least antioxidant activity was recorded within water extract. High-performance liquid-chromatography (HPLC) -fingerprint analyses is done for all fractions, this finding provides an insight into the usage of the tested species as a source of naturally occurring cytotoxic, antimicrobial and antioxidant agents

Key words: Antimicrobial, antioxidant (DPPH), Bombacae, *Ceiba speciosa*, Cytotoxicity, High‑performance liquid chromatography‑fingerprint analyses

Introduction

Bombacaceae is family of flowering plants, contains about 28 genera and about 200 species [1] .In Egypt, Bombacaeae is represented by two genera, *Bombax* and *Ceiba* which are cultivated mostly for ornamental and shade purposes due to their large branches and brightly colored flowers [2]. *Ceiba* is the name of a genus of about 20 species of large trees found in tropical and subtropical areas [3]. *Ceiba* is mainly cultivated for its ornamental brilliant flowers since it blooms during autumn, adding a touch of color at the time when most blooms are fading. It is also cultivated for the silky fiber (or floss) that is obtained from the ripened seeds, so named as "silk floss tree". Additionally, because of its twisted shape, it is sometimes nicknamed as "the drunken tree [4]. These plants are traditionally used for many health disorders, e.g., headache, fever, diabetes, diarrhea, parasitic infections, peptic ulcer and rheumatism [5]. Biologically, it was reported that some *Ceiba* species possess wide range of useful anti-inflammatory, hepato-protective, cytotoxic, antioxidant and hypoglycemic with high safety margins [6] .On the other hand, a limited number of *Ceiba* species including *C. speciosa* and *C. crispiflora* were subjected to some phytochemical analyses that provided a number of flavonoids, anthocyanins, sterols, triterpenes and carbohydrates [7].

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Free radicals are highly energetic unstable reactive species containing odd electrons that able to penetrate cells and tissues of our human bodies led to abnormal cell growth which known by mutation [8].Moreover, the high accumulation rate of such harmful species in the human body is known by oxidative stress which is the starting point of cancer disease. The plant containing antioxidant compounds can be used to overcome such phenomena [8]. The plant-derived naturally occurring compounds are considered as good chemotherapeutic anticancer agents [9]. Plants are considered a vital source of the bioactive chemical ingredients used for the treatment of many diseases especially cancer [10]. Most of the developed anticancer drugs and chemotherapeutic agents were derived from medicinal plants as natural sources [11]. Recently, the resistance of the pathogenic microbial strains against antibiotics develops much faster than ever. Infectious diseases caused by bacterial and fungal infections are still a major threat to public health, despite the tremendous progress in human medicine [12]. The past three decades have seen a dramatic increase in microbial resistance to antimicrobial agents. Such situation stimulates the development of new antimicrobial agents to treat the infectious disease in an effective manner [13]. So this matter continued to an era to identify the potential antimicrobial agent from the natural resources. Therefore, our research evaluate the *in vitro* antimicrobial, cytotoxic and antioxidant activities of *Ceiba speciosa* growing in Egypt as well as high-performance liquid chromatography (HPLC)-finger print analyses.

Experimental

Chemicals and reagents

2, 2- Diphenyl-1-picrylhydrazyl (DPPH) radical, ascorbic acid, dimethyl sulfoxide (DMSO) and all the 16 standard phenolic compounds were purchased from Sigma-Aldrich (Steinheim, Germany), while 3 -(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) was purchased from BIO BASIC CANADA INC (Toronto, Canada)

Plants materials

The leaves of *Ceiba speciosa* were collected from the Zoo Garden, Giza, Egypt in July, 2019 and identified via Dr. Threase Labib, Consultant in Orman Botanical Garden and National Gene Bank. Voucher specimens (No. C15/2/21) was kept at the herbarium of the garden.

Extraction and fractionation

Leaves dry powder of *Ceiba speciosa* (500 g) were separately macerated in 85% Methanol at room temperature for 7 days. The resulting extracts were concentrated via rotatory evaporator (Buchi, Switzerland) at 45°C, the crude 85% methanolic extracts were successively fractionated by using petroleum ether, dichloromethane, Ethylacetate, n-butanol and water

Cytotoxicity evaluation

Determination of cytotoxicity on HepG2 cells by using (MTT protocol) [14] **.**The 96 well tissue culture plate was inoculated with 1 X 10^5 cells / ml (100 µl / well) and incubated at 37°C for 24 hours to develop a complete monolayer sheet. Growth medium was decanted from 96 well micro titer plates after confluent sheet of cells were formed, cell monolayer was washed twice with wash media. Two-fold dilutions of tested sample were made in RPMI medium with 2% serum (maintenance medium). 0.1 ml of each dilution was tested in different wells leaving 3 wells as control, receiving only maintenance medium. Plate was incubated at 37°C and examined. Cells were checked for any physical signs of toxicity, e.g. partial or complete loss of the monolayer sheet by using inverted microscope, rounding, shrinkage, or cell granulation. MTT solution was prepared (5mg/ml in PBS) (BIO BASIC CANADA INC). 20 µl MTT solutions were added to each well. Place on a shaking table, 150 rpm for 5 minutes, to thoroughly mix the MTT into the media. Incubate (37 ºC, 5% CO2) for 1-5 hours to allow the MTT to be metabolized. Dump off the media. (Dry plate on paper towels to remove residue if necessary. Resuspend formazan (MTT metabolic product) in 200 µl DMSO. Place on a shaking table, 150 rpm for 5 minutes, to thoroughly mix the formazan into the solvent. Read optical density at 560 nm and subtract background at 620 nm. Optical density (OD) 570 nm should be directly correlated with cell quantity.it was found according to the National Cancer Institute (NCI), the criteria and the conditions of cytotoxic activity for the crude extract IC_{50} against HepG2 growth based on U.S. National Cancer Institute (NCI) and (MTT protocol) , as follows: IC_{50} ≤ 20 µg/ml = highly cytotoxic, IC_{50} 21-100 µg/ ml = moderately cytotoxic, IC_{50} 101-200 µg/ml = weakly cytotoxic and IC_{50}> 501 µg/ml = not cytotoxic [15] . The isolated fractions were evaluated for their in vitro cytotoxic potentiality against HepG2. Six Concentrations 1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml and 31.25 µg/ml of each were used to determine the cytotoxic effect on HepG2 cells in compare with the control

Antimicrobial activity evaluation

The samples were prepared by dissolving 2mg in 2ml of DMSO and 100µl (containing 100µg) was used in this test. The antimicrobial activity of different samples was investigated by the agar cup plate

method. Four different test microbes namely: *Staphylococcus aureus* (G+ve), *Pseudomona aeruginosa* (G-ve), *Candida albicans* (yeast) and *Aspergillus niger* (fungus) were used. Nutrient agar plates were heavily seeded uniformly with 0.1ml of 10^5-10^6 cells/ml in case of bacteria and yeast. A Czapek-Dox agar plate seeded by 0.1ml the fungal inoculum was used to evaluate the antifungal activities. Then a hole (1cm diameter) was made in media by gel cutter (Cork borer) in sterile condition. Then one drop of melted agar was poured into hole and allowed to solidify to make a base layer. After that specific amount of tested sample (0.1 ml) was poured into the hole. Then plates were kept at low temperature (4°C) for 2-4 hours to allow maximum diffusion. The plates were then incubated at 37°C for 24 hours for bacteria and at 30ºC for 48 hours in upright position to allow maximum growth of the organisms. The antimicrobial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter (mm). The experiment was carried out more than once and mean of reading was recorded [16-19]. The micro‑organisms were obtained from Northern Utilization Research and Development Division, United State Department of Agriculture, Peoria, Illinois, USA.

2, 2‑Diphenyl‑1‑picrylhydrazyl radical scavenging activity

The scavenging activity of the stable DPPH free radical was determined according to the method described by Algfri *et al*. (2019) [20] with some modifications. Briefly, the reaction medium contained 2 mL of 100 mM DPPH purple solution in methanol and 2 mL of plant extract, ascorbic acid was used as standard. The reaction mixture was incubated in the dark for 20 min and the absorbance was recorded at 517 nm. The assay was carried out in triplicate. The decrease in absorbance on addition of test samples was used to calculate the antiradical activity, as expressed by the inhibition percentage (%IP) of DPPH radical, following the equation: %IP = (Ac − As)/Ac × 100; where Ac and As are the absorbances of the control and of the test sample after 20 min, respectively [21].

Separation and quantification of phenolic compounds

HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using Eclipse C18 column (4.6 mm x 250 mm i.d., 5 µm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate 1 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–5 min (80% A); 5-8 min (60% A); 8-12 min (60% A); 12-15 min (85% A) and 15-16 min (82% A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 10 µl for each of the sample solutions. The column temperature was maintained at 35 °C. Phenolic compounds were assayed by external standard calibration at 280 nm. All values were the mean of two injections [22]

RESULTS AND DISCUSSION

Cytotoxicity

All extract fractions were presented as mean ± standard deviation for three measurements as shown table (1). Microsoft excel was used to calculate $P<0.05$ (A small p-value (typically ≤ 0.05) indicates strong evidence against the null hypothesis, so we reject the null hypothesis. A large p-value (> 0.05) indicates weak evidence against the null hypothesis, so we fail to reject the null hypothesis) for each fraction against the control (+ve). The dose-response curves were plotted to enable the calculation of IC50 for each sample. Statistical analysis of the count of viable HepG2 cells grown in serial dilutions of the effect of the extracted fractions compared to control. It was found the extracts petroleum ether, ethyl acetate, and methylene chloride of *Ceiba speciosa* showed moderate cytotoxicity. While n- butanol and methanol extracts of *Ceiba speciosa* showed weak cytotoxicity while water extract of *Ceiba speciosa* has not cytotoxic effect. So we conclude that, the most cytotoxic extract of *Ceiba speciosa* against HepG2 is dichloromethane IC_{50} = 57.3 **µg.** the effects on cells are present in figures from (2 to 7), while variations of toxicity with concentrations are present in praphs from (1 to 6)

Antimicrobial activity

The results in Table (2) & figure (8), revealed that for *Staphylococcus aureus;* only *Ceiba speciosa* dichloromethane extract showed high antimicrobial toward with inhibition zone (23 mm***)*** while both of methanol, petroleum ether and ethyl acetate showed moderate activity with inhibition zones in the manner; (methanol, petroleum ether, ethyl acetate) (16, 16, 17 mm) respectively, on the other hand butanol extract showed weak activity with inhibition zone (12 mm) and water extract showed no any activity at all with inhibition zones (0 mm). For *Pseudomonas aeruginos;* both of petroleum, and dichloromethane extracts showed high activity with inhibition zones in the manner; (petroleum ether, dichloromethane) (19, 22 mm) respectively while both of (methanol, ethyl acetate) extracts showed moderate activity with inhibition zones (18,18 mm) respectively on the other hand both of (butanol, water) extracts show no activity with inhibition zone (0,0 mm) respectively. For *Candida albicans* both of (methanol, petroleum ether and dichloromethane) extracts showed high activity with inhibition zones (20, 19, 21) respectively**.** For

Aspergillus niger only dichloromethane extract showed high activity with inhibition zones (21 mm) while (methanol, petroleum ether, and water) extracts showed moderate activity with inhibition zones (15,18,15 mm) respectively , on the other hand both of (ethyl acetate and butanol) extracts showed moderate activity with inhibition zones (14, 13 mm) respectively.)

ID	Conc µg/ml	O.D			Mean O.D	ST.E	Viability %	Toxicity %	IC50 µg
HepG2	1:2	0.352	0.341	0.378	0.357	0.01097	100	0	
***C.s* petelum Ether extract**	1000	0.019	0.02	0.021	0.02	0.000577	5.602240896	94.3977591	74.35 **moderate**
	500	0.02	0.016	0.023	0.019667	0.002028	5.508870215	94.49112979	
	250	0.021	0.028	0.022	0.023667	0.002186	6.629318394	93.37068161	
	125	0.095	0.082	0.079	0.085333	0.00491	23.90289449	76.09710551	
	62.5	0.153	0.168	0.192	0.171	0.011358	47.89915966	52.10084034	
	31.25	0.301	0.284	0.279	0.288	0.006658	80.67226891	19.32773109	
***C.s* ethyl acetate extract**	1000	0.021	0.028	0.027	0.025333	0.002186	7.096171802	92.9038282	79.73 **moderate**
	500	0.019	0.021	0.022	0.020667	0.000882	5.78898226	94.21101774	
	250	0.018	0.026	0.022	0.022	0.002309	6.162464986	93.83753501	
	125	0.093	0.071	0.088	0.084	0.006658	23.52941176	76.47058824	
	62.5	0.194	0.173	0.206	0.191	0.009644	53.50140056	46.49859944	
	31.25	0.311	0.302	0.309	0.307333	0.002728	86.08776844	13.91223156	
***C.s* n-butanol extract**	1000	0.018	0.019	0.02	0.019	0.000577	5.322128852	94.67787115	446.11 **weak**
	500	0.132	0.145	0.129	0.135333	0.00491	37.90849673	62.09150327	
	250	0.295	0.306	0.307	0.302667	0.003844	84.7805789	15.2194211	
	125	0.341	0.369	0.351	0.353667	0.008192	99.06629318	0.933706816	
	62.5	0.357	0.344	0.356	0.352333	0.004177	98.69281046	1.307189542	
	31.25	0.37	0.352	0.349	0.357	0.006557	100	0	
***C.s* water extract**	1000	0.142	0.163	0.157	0.154	0.006245	43.1372549	56.8627451	954.99 **Non-toxic**
	500	0.346	0.323	0.358	0.342333	0.010269	95.89169001	4.108309991	
	250	0.362	0.347	0.353	0.354	0.004359	99.15966387	0.840336134	
	125	0.359	0.351	0.355	0.355	0.002309	99.43977591	0.56022409	
	62.5	0.364	0.342	0.362	0.356	0.007024	99.71988796	0.280112045	
	31.25	0.358	0.362	0.347	0.355667	0.004485	99.62651727	0.373482726	
***C.s* dicholoromethane extract**	1000	0.019	0.017	0.018	0.018	0.000577	5.042016807	94.95798319	57.3 **moderate**
	500	0.019	0.02	0.019	0.019333	0.000333	5.415499533	94.58450047	
	250	0.02	0.018	0.019	0.019	0.000577	5.322128852	94.67787115	
	125	0.056	0.074	0.069	0.066333	0.005364	18.58076564	81.41923436	
	62.5	0.142	0.116	0.124	0.127333	0.007688	35.66760037	64.33239963	
	31.25	0.253	0.264	0.247	0.254667	0.004978	71.33520075	28.66479925	
C.s Methanol extract	1000	0.023	0.018	0.025	0.022	0.002082	6.162464986	93.83753501	410.37 **weak**
	500	0.115	0.108	0.105	0.109333	0.002963	30.62558357	69.37441643	
	250	0.294	0.306	0.316	0.305333	0.00636	85.52754435	14.47245565	
	125	0.347	0.326	0.359	0.344	0.009644	96.35854342	3.641456583	
	62.5	0.362	0.357	0.343	0.354	0.005686	99.15966387	0.840336134	
	31.25	0.349	0.362	0.361	0.357333	0.004177	100.0933707	0	

Table 1: Cytotoxicity evaluation using different concentrations 1000, 500, 250, 125, 62.5, 31.25 µg/ml, of different solvent extracts of *Ceiba speciosa* (*C.s*) leaves against HepG2

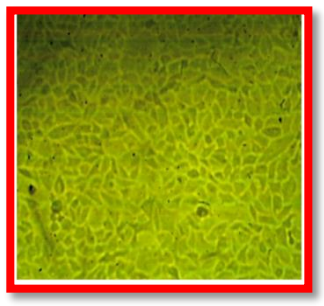


Figure 1: show the control HepG2 cells without effect of any *Ceiba speciosa* extract taken by inverted microscope with specifications; objective lense (10X)

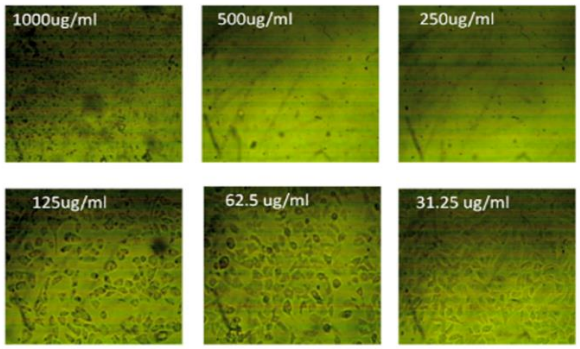
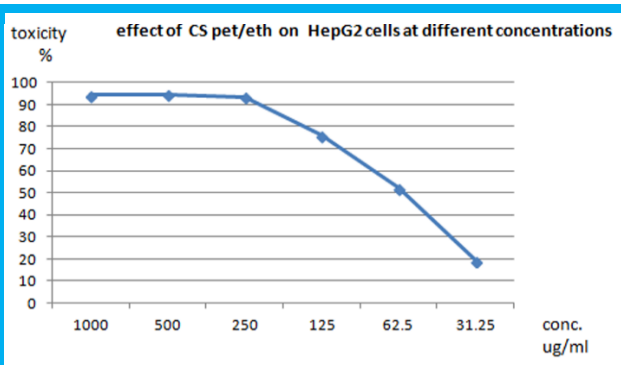


Figure 2: show effect of petroleum ether extract of *Ceiba speciosa* on HepG2 cells at different concentrations



Graph 1: show toxicity percentage with different concentration of petroleum ether extract of *Ceiba speciosa* on HepG2 cells

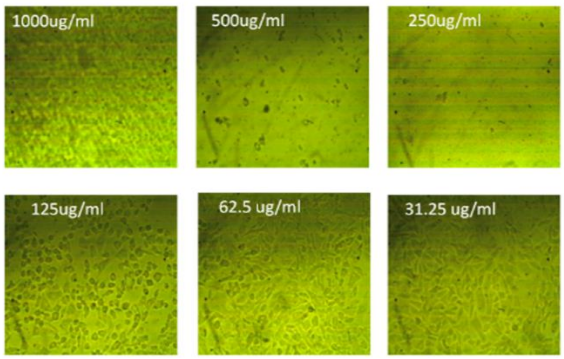
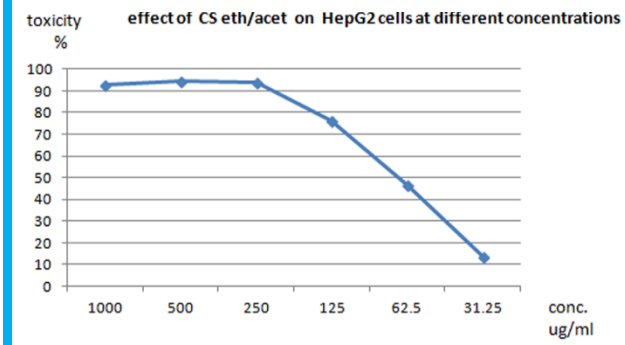


Figure 3: show effect of ethyl acetate extract of *Ceiba speciosa* on HepG2 cells at different concentrations



Graph 2: show toxicity percentage with different concentration of ethyl acetate extract of *Ceiba speciosa* on HepG2 cells

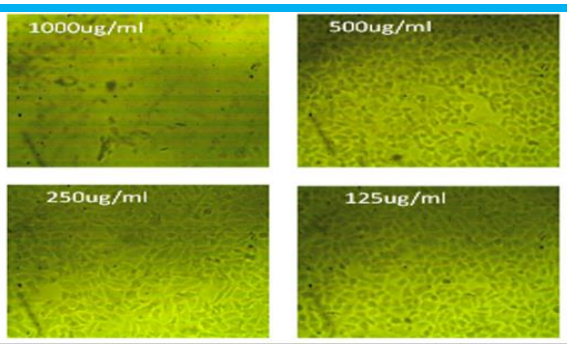
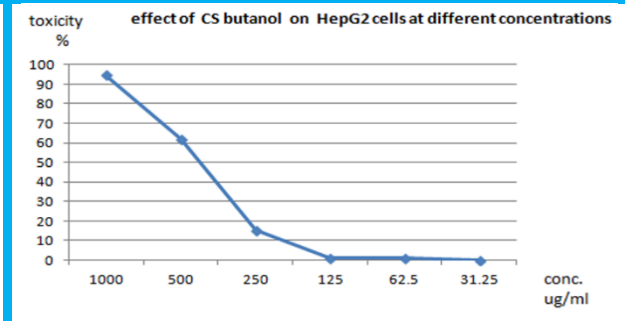


Figure 4: show effect of n- butanolextract of *Ceiba speciosa* on HepG2 cells at different concentrations



Graph 3: show toxicity percentage with different concentration of n- butanol extract of *Ceiba speciosa* on HepG2 cells

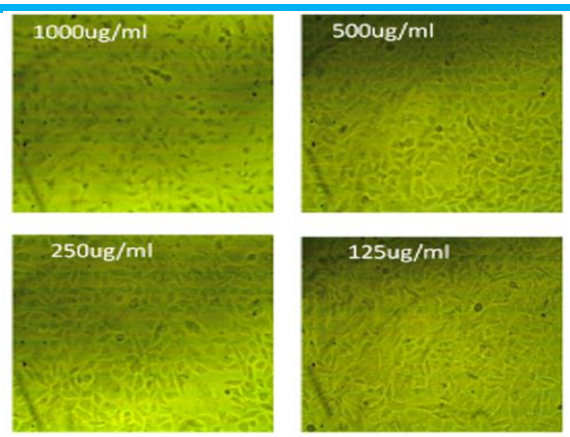
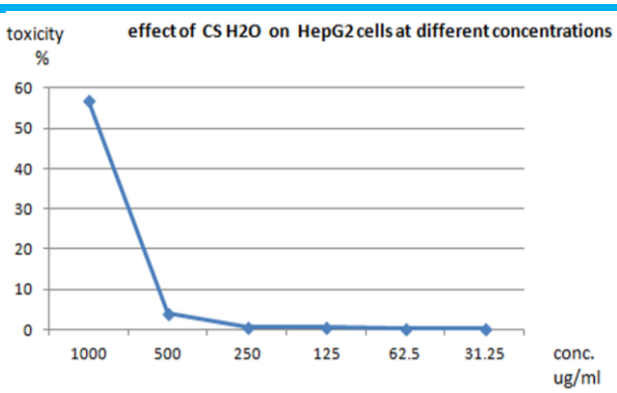


Figure 5: show effect of water extract of *Ceiba speciosa* on HepG2 cells at different concentrations



Graph 4: show toxicity percentage with different concentration of water extract of *Ceiba speciosa* on HepG2 cells

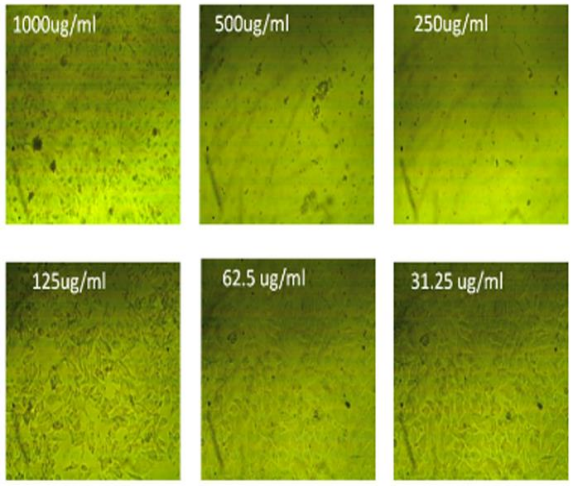
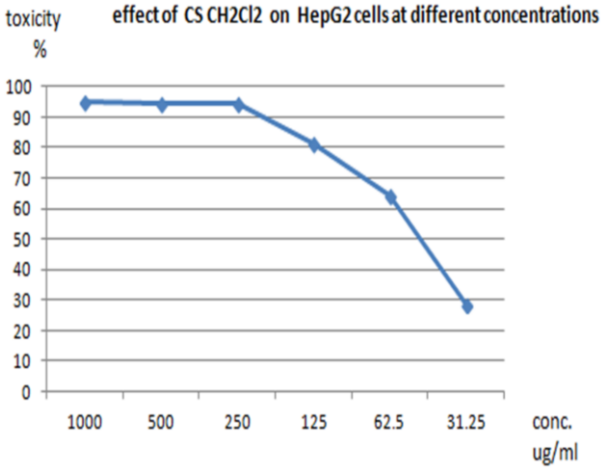


Figure 6: show effect of dichloromethane extract of *Ceiba speciosa* on HepG2 cells at different concentrations



Graph 5: show toxicity percentage with different concentration of dichloromethane extract of *Ceiba speciosa* on HepG2 cells

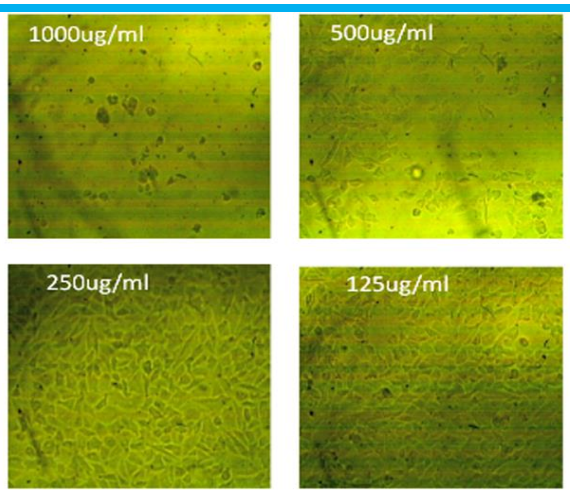
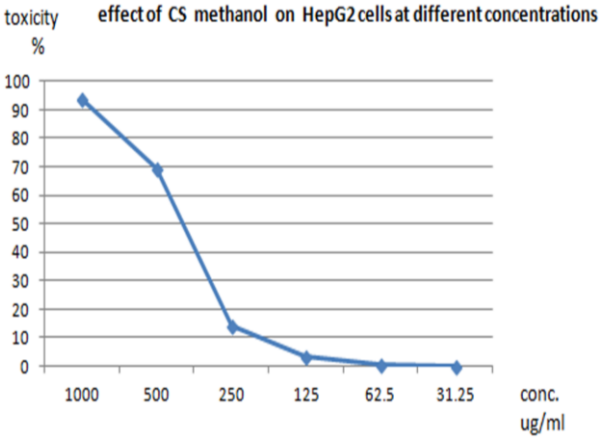


Figure 7: show effect of methanol extract of *Ceiba speciosa* on HepG2 cells at different concentrations



Graph 6: show toxicity percentage with different concentration of methanol extract of *Ceiba speciosa* on HepG2 cells

Serial no	Sample name	Clear zone (ϕmm)			
		Staphylococcus aureus	*Pseudomonas aeruginosa*	*Candida albicans*	*Aspergillus niger*
1	C.S methanol	16	18	20	15
2	C.S petroleum	16	19	19	18
3	C.S CH_2Cl_2	23	22	21	21
4	C.S ethyl acetate	17	18	16	14
5	C.S butanol	12	0	15	13
6	C.S water	0	0	13	15
Standard	Penicillin G[1]	19	20	23	0
	Griseofulvin[2]	0	0	0	29

Table 2: *In vitro* antimicrobial activity of 6 different extracts of *C.s* leaves on *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger using* Penicillin G[1]& Griseofulvin[2] as standards

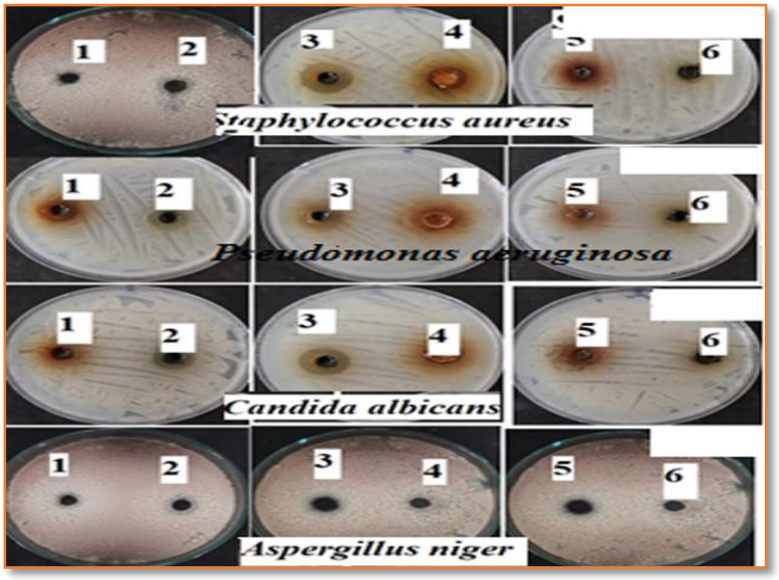


Figure 8: The antimicrobial inhibition zones (mm) of the different solvent extracts from *C. s against* four pathogenic microbial strains; *Staphylococcus aureus* (first row), *Pseudomonas aeruginosa* (second row), *Candida albicans* (third row), *and Aspergillus niger* (fourth row),1- *C.s* methanol, 2- *C.s* petroleum, 3- *C.s* dichloromethane, 4- *C.s* ethyl acetate, 5- *C.s* butanol, 6- *C.s* water

Free radical antioxidant activity (2, 2-diphenyl-1-picrylhydrazyl assay)

The DPPH radical is a stable chromogen widely used to assess the antioxidant potentials of extracts, fractions or pure isolates derived from medicinal plants [23], Moreover, the *in vitro* DPPH model is based on the characteristic absorption at 517 nm (purple in color), which decreases significantly when exposed to Radical-scavengers (due to hydrogen atoms transfer from antioxidant sample to the DPPH radical to become DPPH-H with yellow color [24]. For the IC_{50}values for *Ceiba speciosa* leaves, table (3) were varied from 12.37 to 78.76 µg/ml, and the results are in the order, CH_2Cl_2 (12.37) > MeOH (15.48) > EtOAC (27.07) > *n*-BuOH (59.68) > Pet. ether (60.97) >H_2O (78.76) µg

Extract sample	DPPH (IC_{50})[1] [µg/ml]
	Ceiba Speciosa
methanol	15.48 ± 3.80
Petroleum ether	60.97 ± 2.29
Dichloro methane	12.37 ± 4.52
Ethyl acetate	27.07± 1.72
water	78.76 ± 2.26
n-Butanol	59.68 ± 4.46
Ascorbic acid	7.60 ± 0.85

Table 3: Free radical scavenging antioxidant activities (2, 2 -diphenyl-1-picrylhydrazyl) of the different solvent extracts of *Ceiba speciosa* leaves

High performance liquid chromatography - fingerprint analyses

Fingerprint analyses approach are widely used to identify the chemical composition and relative proportions of phenolic compounds in different medicinal plant extracts due to their simplicity and reliability [25]. Among them, high-performance liquid chromatography (HPLC) has been the most widely used technology for identifying differences in chemical compositions among medicinal herbal samples [26]. Owing to the high *in vitro* antimicrobial activity of the ethyl acetate, methanol, methylene chloride and petroleum ether extracts of *C.s* and also the high *in vitro* antioxidant activity of the *C.s* dichloromethane & methanol extracts compared to the rest of the tested extracts, and also high cytotoxicity of *C.s* petroleum, ethyl acetate, and methylene chloride extracts compared to others extracts so, the mentioned extracts were subjected to further phytochemical investigations via HPLC-fingerprint analyses aiming to identify their chemical constituents and to correlate the obtaining activities with these chemical ingredients. sixteen standard phenolic compounds namely; gallic acid (1), chlorogenic acid (2), catechin (3), methyl gallate (4), coeffic acid (5), syringic acid (6), pyrocatechol (7), rutin (8), and ellagic acid (9), coumaric acid (10), vanillin (11), ferulic acid (12), naringenin (13), taxifolin (14), cinnamic acid (15), kaempferol (16) were used in this study Table (4-10) and Figures (9- 15). The results revealed that the above mentioned standards were present in the tested extracts in different proportions. In *C.s* petroleum extract narginen & gallic acid are the major standards while others standards are minor. In *C.s* ethyl acetate coumaric acid, taxifolin & gallic acid are major's standards while other standards are minors, in dichloromethane extract of *C.s* cinnamic & gallic acids are the major's standards while others are minors. In methanolic extract of *C.s* narginen & gallic acids are the major standards while the other standards are minor. Many authors have been reported in the correlation between the existence of phenolic compounds in medicinal plant extracts and their biological activities. The phenolic compounds exhibited potent antioxidant potential due to the presence of the characteristic structural criteria for effective free radical scavenging activity like; heavy hydroxylation pattern, extended conjugation system and ketonic groups [27-30] . While for the antimicrobial action these compounds have specific modes of actions like; cell walls damage [31-33]

(16) Polyphenolic Standards		
	Conc. (µg/ml)	Area
Gallic acid	16.8	167.49
Chlorogenic acid	28	355.95
Catechin	67.5	562.44
Methyl gallate	10.2	793.01
Coffeic acid	18	469.94
Syringic acid	17.2	406.41
Pyro catechol	29.2	429.39
Rutin	61	461.09
Ellagic acid	34.3	499.69
Coumaric acid	13.2	781.26
Vanillin	12.9	606.64
Ferulic acid	12.4	397.16
Naringenin	15	277.77
Taxifolin	13.2	179.44
Cinnamic acid	5.8	577.26
Kaempferol	12	322.98

Table 4: area under the peak for 16 standards polyphenolic standard`

Butanol extract			
	Area	Conc. (µg/ml)	Conc.(µg/g) extract
Gallic acid	1239.36	124.31	7968.80
Chlorogenic	2070.86	162.90	10442.43
Catechin	0.00	0.00	0.00
Methyl	929.84	11.96	766.67
Coffeic acid	822.26	31.49	2018.89
Syringic	4073.28	172.39	11050.45
Pyro	0.00	0.00	0.00
Rutin	586.40	77.58	4972.97
Ellagic acid	1824.87	125.26	8029.69
Coumaric	9091.18	153.60	9846.36
Vanillin	3.69	0.08	5.03
Ferulic acid	178.14	5.56	356.52
Naringenin	14811.80	799.86	51272.82
Taxifolin	105.60	7.77	497.94
Cinnamic	32.52	0.33	20.95
Kaempferol	0.00	0.00	0.00

Table 5: area under the peak and concentrations of the n- butanol extract of *Ceiba speciosa* against 16 polyphenolic standards

Dichloromethane extract			
	Area	Conc. (µg/ml)	Conc.(µg/g) extract
Gallic acid	138.74	13.92	869.78
Chlorogenic acid	46.94	3.69	230.76

Catechin	1.17	0.14	8.81
Methyl gallate	44.30	0.57	35.62
Coffeic acid	69.25	2.65	165.77
Syringic acid	415.68	17.59	1099.50
Pyro catechol	0.00	0.00	0.00
Rutin	29.47	3.90	243.70
Ellagic acid	32.47	2.23	139.30
Coumaric acid	381.49	6.45	402.85
Vanillin	6.69	0.14	8.89
Ferulic acid	148.90	4.65	290.56
Naringenin	111.57	6.02	376.55
Taxifolin	291.94	21.48	1342.20
Cinnamic acid	967.92	9.73	607.83
Kaempferol	160.93	5.98	373.69

Table 6: area under the peak and concentrations of the dichloro-methane extract of *Ceiba speciosa* against 16 polyphenolic standards

Methanol extract			
	Area	Conc. (µg/ml)	Conc.(µg/ g)
Gallic acid	929.72	93.26	5013.75
Chlorogenic acid	618.00	48.61	2613.67
Catechin	0.00	0.00	0.00
Methyl gallate	266.37	3.43	184.20
Coffeic acid	286.16	10.96	589.28
Syringic acid	1022.72	43.28	2327.04
Pyro catechol	0.00	0.00	0.00
Rutin	132.52	17.53	942.60
Ellagic acid	476.08	32.68	1756.94
Coumaric acid	2733.61	46.19	2483.15
Vanillin	0.00	0.00	0.00
Ferulic acid	139.55	4.36	234.24
Naringenin	4567.37	246.64	13260.43
Taxifolin	56.43	4.15	223.16
Cinnamic acid	86.15	0.87	46.54
Kaempferol	4.80	0.18	9.58

Table 7: area under the peak and concentrations of the methanol extract of *Ceiba speciosa* against 16 polyphenolic standards

Ethyl acetate extract			
	Area	Conc. (µg/ml)	Conc.(µg/g)
Gallic acid	732.26	73.45	4057.
Chlorogenic acid	428.68	33.72	1863.
Catechin	0.00	0.00	0.00
Methyl gallate	237.50	3.05	168.7
Coffeic acid	705.48	27.02	1492.
Syringic acid	1808.45	76.54	4228.
Pyro catechol	0.00	0.00	0.00
Rutin	29.38	3.89	214.7
Ellagic acid	118.53	8.14	449.5
Coumaric acid	6808.18	115.03	6355.
Vanillin	23.59	0.50	27.72
Ferulic acid	628.58	19.63	1084.
Naringenin	765.72	41.35	2284.
Taxifolin	1729.20	127.20	7027.
Cinnamic acid	884.41	8.89	490.9
Kaempferol	23.75	0.88	48.76

Table 8: area under the peak and concentrations of the ethyl acetate extract of *Ceiba speciosa* against 16 polyphenolic standards

Water extract			
	Area	Conc. (µg/ml)	Conc.(µg/g) extract
Gallic acid	1269.3	127.32	7073.51
Chlorogenic	196.52	15.46	858.84
Catechin	0.00	0.00	0.00
Methyl	78.16	1.01	55.85
Coffeic acid	247.27	9.47	526.18
Syringic acid	202.41	8.57	475.91
Pyro	0.00	0.00	0.00
Rutin	0.00	0.00	0.00
Ellagic acid	218.33	14.99	832.59
Coumaric	191.44	3.23	179.69
Vanillin	0.00	0.00	0.00
Ferulic acid	0.00	0.00	0.00
Naringenin	465.66	25.15	1397.01
Taxifolin	0.00	0.00	0.00
Cinnamic	0.00	0.00	0.00
Kaempferol	0.00	0.00	0.00

Table 9: area under the peak and concentrations of the water extract of *Ceiba speciosa* against 16 polyphenolic standards

Petroleum ether extract			
	Area	Conc. (µg/m	Conc.(µg/g) extract
Gallic acid	124.89	12.53	745.64
Chlorogenic	54.69	4.30	256.06
Catechin	0.00	0.00	0.00
Methyl	49.47	0.64	37.88
Coffeic acid	93.72	3.59	213.68
Syringic acid	187.56	7.94	472.49
Pyro catechol	0.00	0.00	0.00
Rutin	29.10	3.85	229.14
Ellagic acid	65.22	4.48	266.48
Coumaric	320.94	5.42	322.77
Vanillin	3.05	0.06	3.86
Ferulic acid	50.55	1.58	93.94
Naringenin	322.19	17.40	1035.63
Taxifolin	46.70	3.43	204.46
Cinnamic	137.08	1.38	81.98
Kaempferol	38.74	1.44	85.67

Table 10: area under the peak and concentrations of the petroleum ether extract of *Ceiba speciosa* against 16 polyphenolic standards

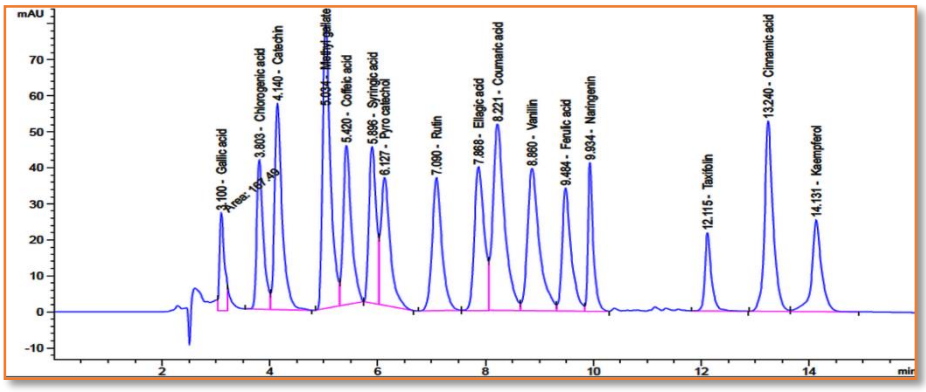


Figure 9: High performance liquid chromatography chromatogram of 16 standard phenolic compounds; ; gallic ac id (1), chlorogenic acid (2), catechin (3), methyl gallate (4), coeffic acid (5), syringic acid (6), pyrocatechol (7), rutin (8), and ellagic acid (9), coumaric acid (10), vanillin (11), ferulic acid (12), naringenin (13), taxifolin (14), cinnamic acid (15), kaempferol (16)

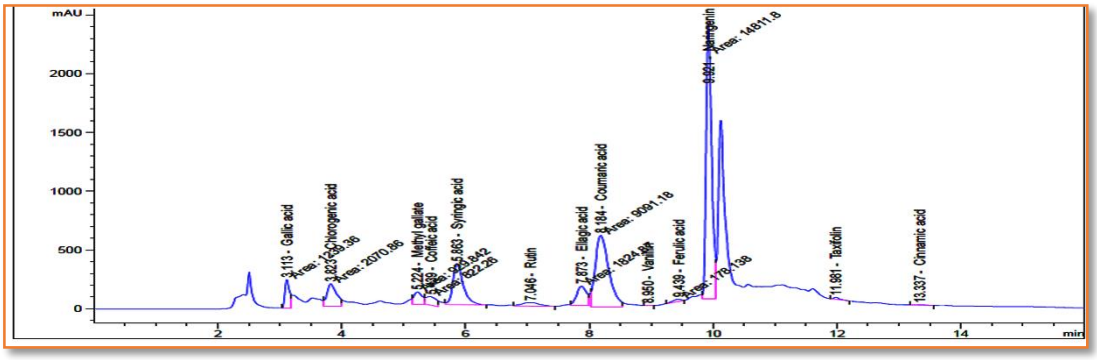


Figure 10: High-performance liquid chromatography-fingerprint chromatogram of butanol extract of *Ceiba speciosa leaves*

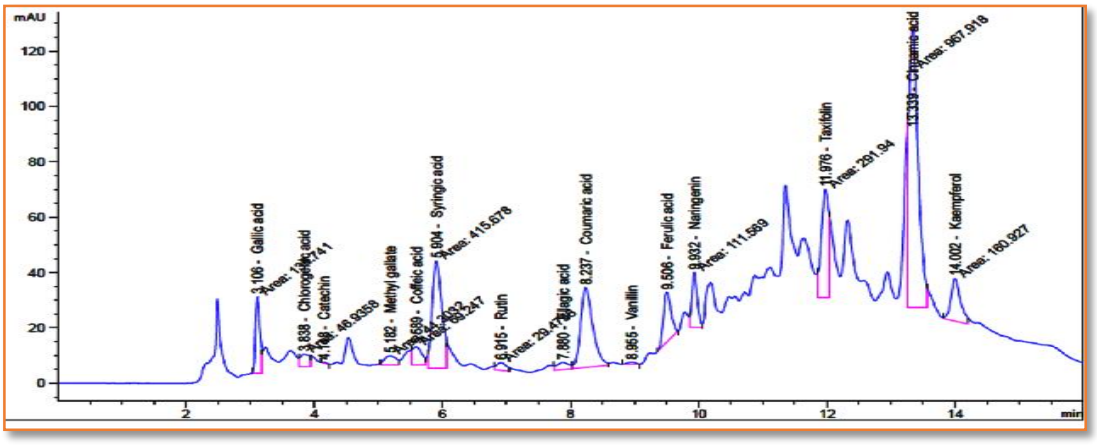


Figure 11: High-performance liquid chromatography-fingerprint chromatogram of dichloro-methane extract of *Ceiba speciosa leaves*

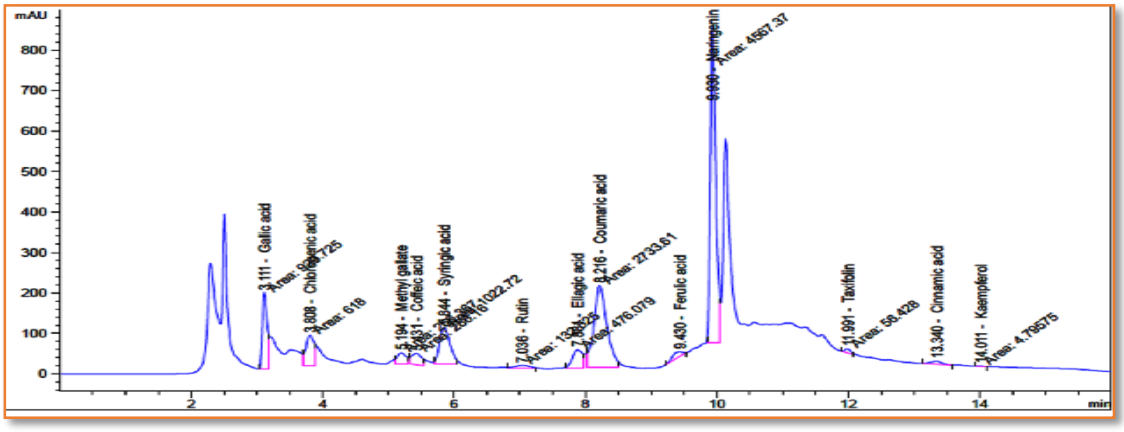


Figure 12: High-performance liquid chromatography-fingerprint chromatogram of methanol extract of *Ceiba speciosa leaves*

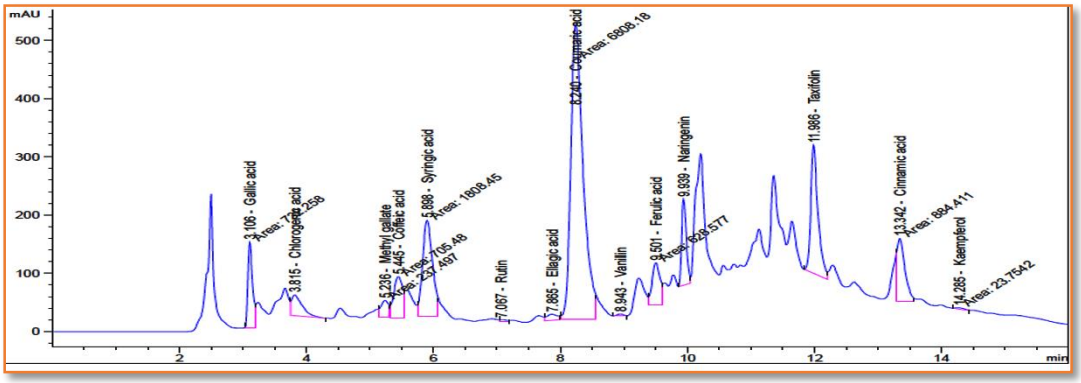


Figure 13: High-performance liquid chromatography-fingerprint chromatogram of ethyl acetate extract of *Ceiba speciosa leaves*

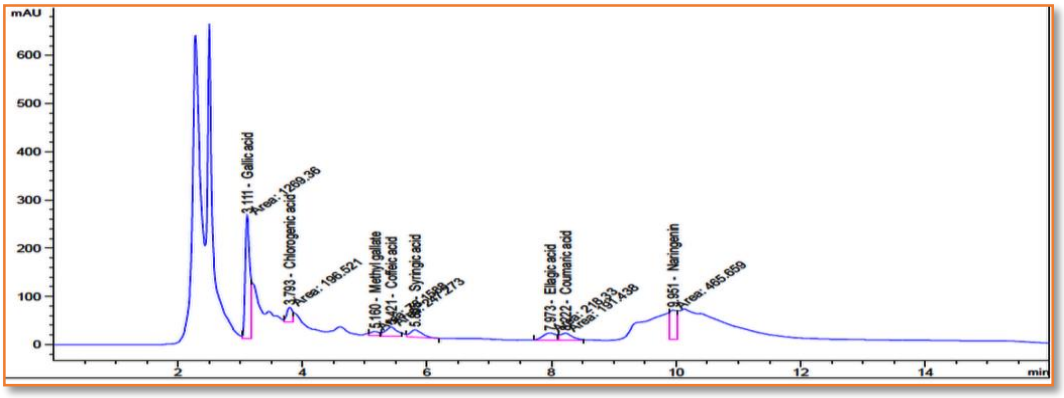


Figure 14: High-performance liquid chromatography-fingerprint chromatogram of water extract of *Ceiba speciosa leaves*

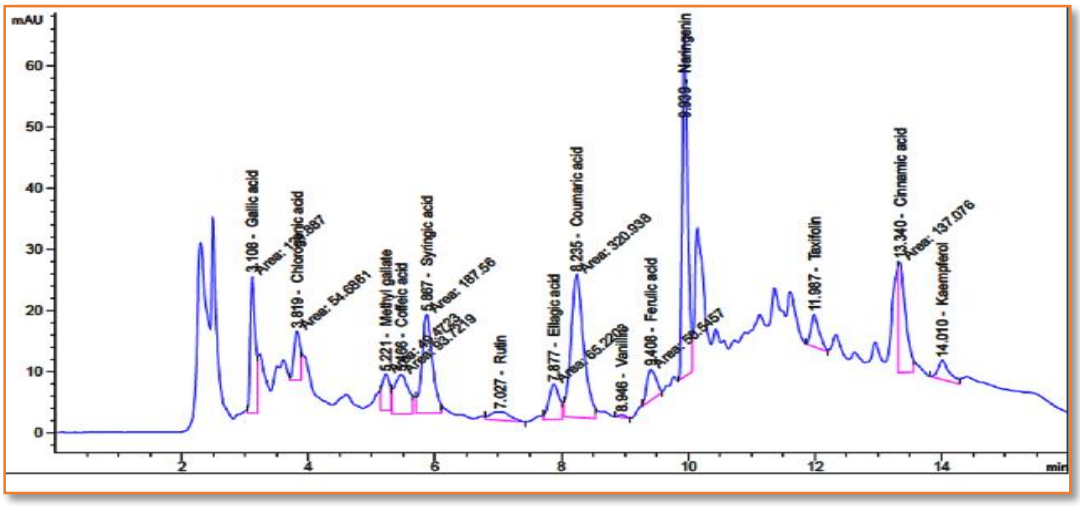


Figure 15: High-performance liquid chromatography-fingerprint chromatogram of petroleum ether extract of *Ceiba speciosa leaves*

CONCLUSION: Leaves from *Ceiba speciosa* collected from zoo garden in Egypt, showed a moderate cytotoxicity against HepG2 cells in extracts; petroleum ether (IC_{50} = 74.35 µg), ethyl acetate (IC_{50} = 79.73 µg), dicholoromethane (IC_{50} = 57.3 µg) and weak cytotoxicity in extracts; butanol (IC_{50} = 446.11 µg), methanol (IC_{50} = 410.37 µg), and non-cytotoxicity on water extract (IC_{50} = 954.99 µg). Antimicrobial activities done against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger*, it was found that inhibition zones varied according to type of extract since for methanol (15-20 mm), petroleum ether (16-19 mm), dicholoromethane (21-23 mm), ethyl acetate (14-18 mm), butanol (0-15) and water (0-15 mm), so the highest antimicrobial activity was found in dichloromethane extract. The antioxidant activity of extracts varied from 12.37 to 78.76 µg/ml, and the

results are in the order, dicholoromethane (12.37) > methanol (15.48) > ethyl acetate (27.07) > *n*-Butanol (59.68) > Petroleum ethet (60.97) >water (78.76) µg. HPLC finger print is done for all fractions, this finding provides an insight into the usage of the tested species as a source of naturally occurring cytotoxic and antimicrobial agents. Accordingly, we recommended the chromatographic isolation of the most promising extracts from the plant to identify its bioactive secondary metabolites.

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