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In vitro Cytotoxicity, Antimicrobial, Antioxidant Activities and HPLC Finger Print Analyses of the Extracts of *Ceiba insignis* Leaves Growing in Egypt

Abdel-Wanes Anter Abdel-Aziz[1]*, Mona A. Mohamed[1], Magda A. Abdallah[2], Rasha Shaaban[3], Nadia S. Mohamed[1], Nehal M. Elwan[2]

[1]Medicinal Chemistry department, [3]Biochemistry and Molecular Biology department -Theodor Bilharz Research Institute, Kornaish El-Nile, 12411 Warrak El-Hadar, Imbaba (P.O. 30), [2]Chemistry Department, Faculty of Science, Cairo University, Giza

ABSTRACT

Polyphenols - rich plants can be used to reduce health disorder resulting from cancer and infectious diseases. **Methodology:** Different solvent extracts of *Ceiba insignis* leaves were evaluated for their *in vitro* cytotoxic, antimicrobial, and antioxidant activities, while their chemical profiles were investigated via high‑performance liquid-chromatography (HPLC) -fingerprint analyses. **Results:** Showed that methanol, butanol, and dichloromethane extracts of *Ceiba insignis* leaves exhibited a moderate cytotoxicity against HepG2 with IC_{50} values of 98.54, 75.38, 40.71 µg/ml respectiviely, while petroleum ether and water exhibited weak activity with IC_{50} values of 118.15 and 170.03 µg/ml respectively and very weak activity was recorded with ethyl acetate extract with IC_{50} value of 924.05µg. Moreover, methanol, dichloromethane, petroleum ether, and ethyl acetate exhibited strong antimicrobial activities with inhibition zones (20-25 mm), (15-30 mm), (14-16 mm), and (16-28 mm) respectively, while butanol and water exhibited low to moderate activity with inhibition zones (0-18 mm), and (0-16 mm) respectively. On the other side, strong DPPH antioxidant scavenging activity was recorded within petroleum ether extract with IC_{50} (24.72 µg/ml), while the least antioxidant activity was recorded within ethyl acetate with IC_{50} (97.50 µg/ml). HPLC finger-print analyses revealed the presence of major compounds; syringic acid in dichloromethane extract, gallic acid, chlorogenic acid & syringic acid in ethyl acetate extract, and naringenin & gallic acid in methanol, butanol and water extracts, this finding provides an insight into the usage of the tested species as a source of naturally occurring antioxidant, cytotoxic and antimicrobial agents.

Key words: Antimicrobial, antioxidant (DPPH), *Ceiba insignis*, cytotoxicity, HPLC- finger print

INTRODUCTION

In Egypt, family Bombacaeae is represented by the two genera *Bombax and Ceiba*, which are mainly used for decoration and shading due to their large branches and bright flowers [1]. *Ceiba* is the name of the genus of about 20 large trees in tropical and subtropical regions [2]. *Ceiba* is mainly cultivated for its gorgeous flowers, because it blooms in autumn, adding a little 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 [3]. These plants are traditionally used for many health disorders, e.g., headache, fever, diabetes, diarrhea, parasitic infections, peptic ulcer and rheumatism [4]. 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 [5, 6]. Free radicals are a kind of high-energy unstable reactive substances that contain odd numbers of electrons, which can penetrate human cells and tissues and cause abnormal cell growth [7]. Overproduction and accumulation of reactive species within the human

*Corresponding author e-mail: waneis_science@yahoo.com.; (Abdel-Wanes Anter Abdel-Aziz).
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body lead to a phenomenon recognized as oxidative stress that initiates several health disorders like cancer, cardiovascular, and inflammation. The destructive effects of such phenomenon can be diminished via utilizing naturally occurring antioxidant agents as free radical scavengers [8]. Most of the anti-cancer drugs and chemotherapeutics that have been developed come from medicinal plants as natural sources [9]. The strong resistance of some pathogenic microbial strains to the existing antibiotics is still a great challenge for scientists, so scientists seek to discover new types of antibiotics from safe natural sources like medicinal plants, microbial extracts, and marine organisms [10]. Moreover, several naturally occurring bioactive compounds have been reported for their antimicrobial effects against microbial infections [11].Recently, pathogenic microbial strains have become resistant to antibiotics much faster than ever before. Although human medicine has made great progress, infectious diseases caused by bacterial and fungal infections are still a major threat to public health [12]. In the past thirty years, the resistance of microorganisms to antibacterial agents has increased dramatically. This situation has stimulated the development of new antibacterial agents, thereby effectively treating infectious diseases [13]. Therefore, this matter continued until the era when potential antimicrobial agents were identified from natural resources. Therefore, this research evaluated the in vitro antibacterial, cytotoxicity, and antioxidant activities for six extracts of *Ceiba insignis* grown in Egypt, as well as (HPLC) fingerprint analyses.

MATERIALS AND METHODS

Chemicals and reagents

2, 2- Diphenyl-1-picrylhydrazyl (DPPH) radical, ascorbic acid, Dimethyl sulfoxide (DMSO) and all standards phenolic compounds used were purchased from Sigma‑Aldrich (Steinheim,Germany), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) bio basic Canada INC

Plants materials

The leaves of *Ceiba insignis* were collected from the Zoo Garden, Giza, Egypt in April, 2018 and identified via Dr. Threase Labib, the Consultant in Botanical Garden (Orman) and National Gene Bank. Voucher specimens (No. C17/3/21) was kept at the herbarium of the garden

Extraction and fractionation

Leaves dry powder of *Ceiba insignis* (1000 g) were separately macerated in 90 % Methanol at room temperature for 3 days. The resulting extracts were concentrated via rotatory evaporator (Buchi, Switzerland) at 45°C, the crude 90 % methanol extracts were successively fractionated using petroleum ether, dichloromethane, ethyl acetate, *n*-butanol and water

Cytotoxicity evaluation

Determination of sample cytotoxicity on HepG2 cells (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). The 0.1 ml of each dilution was tested in different wells leaving 3 wells as control, receiving only maintenance medium. The plate was incubated at 37°C and examined. The Cells were checked for any physical signs of toxicity, e.g. partial or complete loss of the monolayer, rounding, shrinkage, or cell granulation by inverted microscope. The MTT solution was prepared (5mg/ml in pbs) (bio basic canada inc). The 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 (37C, 5% CO2) for 1-5 hours to allow the MTT to be metabolized then 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 (1), 150 rpm for 5 minutes, to thoroughly mix the formazan into the solvent then read optical density at 560 nm and subtract background at 620 nm. Optical density 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, as follows: $IC_{50} \leq 20$ 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 in compare with the control

Antimicrobial activity evaluation (disc agar plate assay)

The cup plate method as described by Collens and Lyne's [16]; was used to estimate the antimicrobial activities of six different solvent extracts [17]. Four different test microbes; *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans and Aspergillus niger* were selected to evaluate the antimicrobial activities as representatives of G + ve bacteria, G − ve bacteria, yeast and fungal groups. Since the micro‑organisms were obtained from the Northern Utilization Research and Development Division, United State Department of Agriculture, Peoria, Illinois, USA.

2,2‑Diphenyl‑1‑picrylhydrazyl radical scavenging activity (DPPH)

The scavenging activity of the stable DPPH free radical was determined according to the method described by **Nogueira** [18] 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 anti-radical 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 absorbance of the control and of the test sample after 20 min, respectively [19]

Separation and quantification of phenolic compounds

The HPLC analyses was carried out using an Agilent 1260 series and the separation was carried out by using Eclipse C18 column (4.6 mm x 250 mm). The mobile phase consisted of water and 0.05% trifluoroacetic acid in acetonitrile 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 [20]

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 IC_{50} 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 as seen in Table (1) , Figures (1-7), Graph (1-6) , the fractions showed a moderate cytotoxicity against HepG2 methanol (IC_{50} = 98.54 **µg**) , butanol (IC_{50} = 75.38 **µg**) , dichloromethane (IC_{50} = 40.71 **µg**) & and weak toxicity in water (IC_{50}=170.03 **µg**) & Petroleum ether (IC_{50}=118.15 **µg**), and very low toxicity in ethyl acetate (IC_{50} =924.05 **µg**), So we conclude that, the most cytotoxic fraction against HepG2 is methylene chloride fraction as seen in figure (7) & graph (6). When O.D of HepG2, *C.i* methanol, *C.i* water, *C.i* petroleum ether, *C.i* butanol, *C.i* CH2Cl2, and *C.i* Ethyl acetate in the studied concentration are as Mean ± SE; the data were analyzed t test. While the Viability and Toxicity are represented as % percent.

Table 1: Cytotoxicity evaluation (MTT) using 6 concentrations 1000, 500, 250, 125, 62.5, 31.25 µg/ml of 6 different solvent extracts of *Ceiba insignis* leaves against HepG2

ID	Conc. µg/ml	O.D			Mean of O.D	S.E	Viability %	Toxicity %	IC50 µg
HepG2	1:02	0.352	0.341	0.378	0.357	0.01097	100	0	
C.i methanol	1000	0.035	0.025	0.031	0.030333	0.002906	8.497	91.503	98.54 **moderate**
	500	0.041	0.036	0.052	0.043	0.004726	12.0448	87.955	
	250	0.056	0.084	0.062	0.067333	0.008511	18.861	81.139	
	125	0.121	0.109	0.117	0.115667	0.003528	32.400	67.600	
	62.5	0.245	0.282	0.265	0.264	0.010693	73.950	26.0504	
	31.25	0.341	0.326	0.348	0.338333	0.006489	94.771	5.229	
C.i water	1000	0.023	0.025	0.021	0.023	0.001155	6.442	93.557	170.03 **weak**
	500	0.06	0.042	0.058	0.053333	0.005696	14.939	85.0607	
	250	0.123	0.114	0.116	0.117667	0.002728	32.960	67.0401	
	125	0.185	0.197	0.173	0.185	0.006928	51.821	48.179	
	62.5	0.263	0.274	0.284	0.273667	0.006064	76.657	23.343	
	31.25	0.336	0.342	0.358	0.345333	0.006566	96.732	3.268	
C.i petroleum ether	1000	0.019	0.018	0.019	0.018667	0.000333	5.229	94.771	118.15 **weak**
	500	0.09	0.02	0.019	0.043	0.023502	12.0448	87.955	
	250	0.075	0.056	0.064	0.065	0.005508	18.207	81.793	
	125	0.156	0.168	0.153	0.159	0.004583	44.538	55.462	
	62.5	0.321	0.299	0.318	0.312667	0.006888	87.582	12.418	
	31.25	0.357	0.362	0.35	0.356333	0.00348	99.813	0.187	
C.i butanol	1000	0.023	0.024	0.022	0.023	0.000577	6.442	93.557	75.38 **moderate**
	500	0.021	0.019	0.026	0.022	0.002082	6.162	93.838	
	250	0.056	0.063	0.07	0.063	0.004041	17.647	82.353	
	125	0.099	0.105	0.1	0.101333	0.001856	28.385	71.615	
	62.5	0.178	0.195	0.188	0.187	0.004933	52.381	47.619	
	31.25	0.242	0.269	0.267	0.259333	0.008686	72.642	27.358	
C.i CH2Cl2	1000	0.019	0.02	0.019	0.019333	0.000333	5.415	94.584	40.71 **moderate**
	500	0.023	0.018	0.019	0.02	0.001528	5.602	94.398	
	250	0.02	0.018	0.018	0.018667	0.000667	5.229	94.771	
	125	0.036	0.041	0.05	0.042333	0.004096	11.858	88.142	
	62.5	0.095	0.092	0.108	0.098333	0.00491	27.544	72.456	
	31.25	0.219	0.205	0.216	0.213333	0.004256	59.757	40.243	
C.i Ethyl acetate	1000	0.124	0.156	0.157	0.145667	0.010837	40.803	59.197	924.05 **Non-toxic**
	500	0.321	0.346	0.338	0.335	0.007371	93.838	6.162	
	250	0.358	0.342	0.366	0.355333	0.007055	99.533	0.467	
	125	0.359	0.348	0.349	0.352	0.003512	98.599	1.400	
	62.5	0.364	0.353	0.356	0.357667	0.003283	100.186	0	
	31.25	0.355	0.355	0.361	0.357	0.002	100	0	

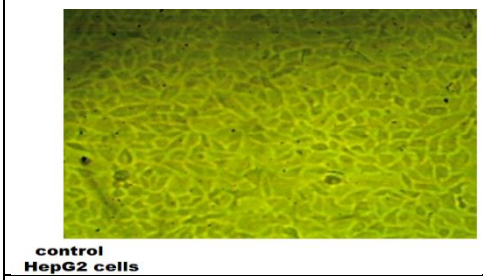


Figure 1: The control HepG2 cells and photo taken by inverted microscope with specifications; objective lense (10X)

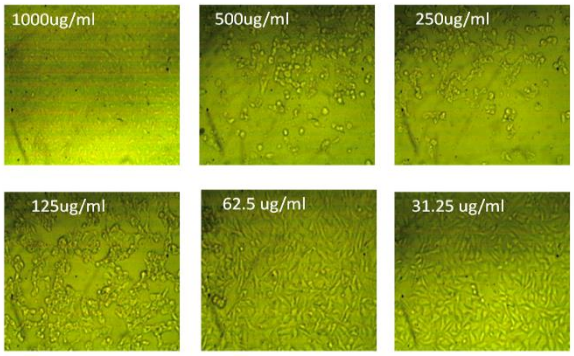
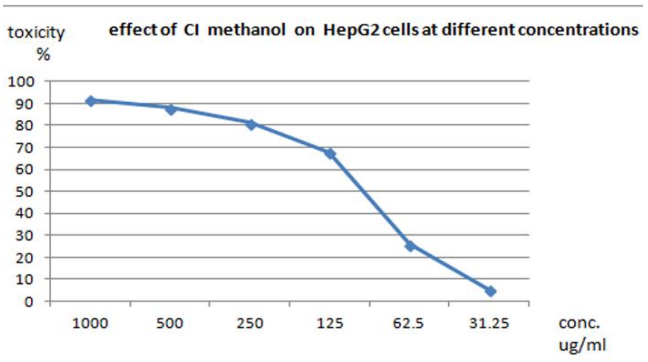


Figure 2: Effect of methanol extract of *Ceiba insignis* on HepG2 cells at different concentrations



Graph 1: Variation of toxicity percentage with different concentration of methanol extract on HepG2 cells

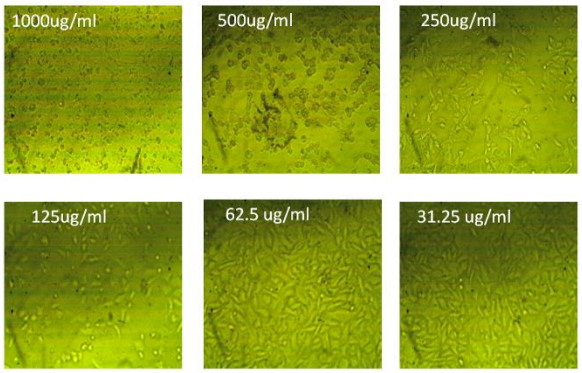
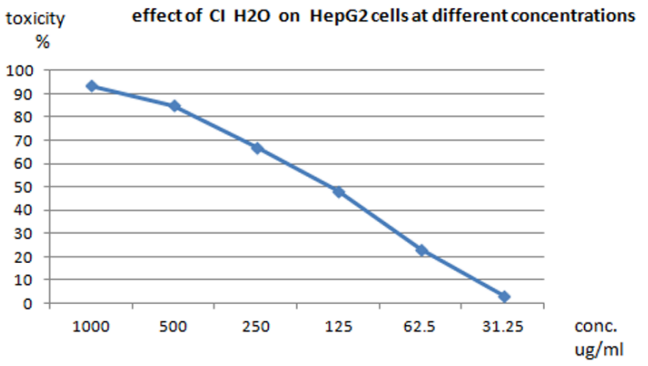


Figure 3: Effect of water extract of *Ceiba insignis* on HepG2 cells at different concentrations



Graph 2: Variation of toxicity percentage with different concentration of water extract on HepG2 cells

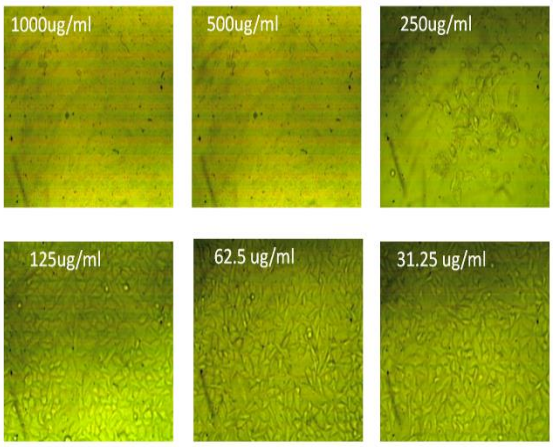
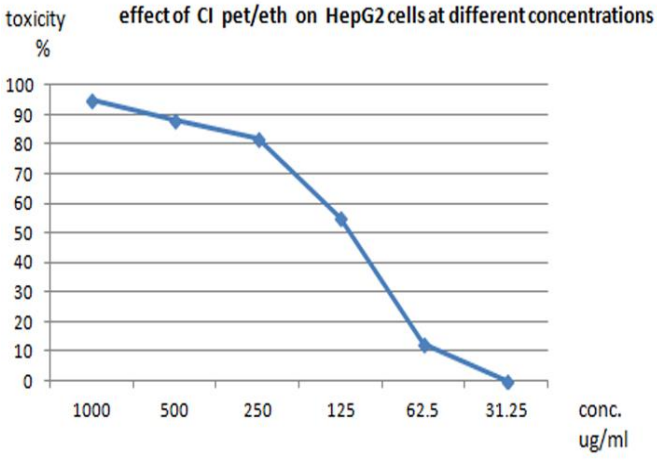


Figure 4: Effect of petroleum ether extract of *Ceiba insignis* on HepG2 cells at different concentrations



Graph 3: Variation of toxicity percentage with different concentration of petroleum ether extract on HepG2 cells

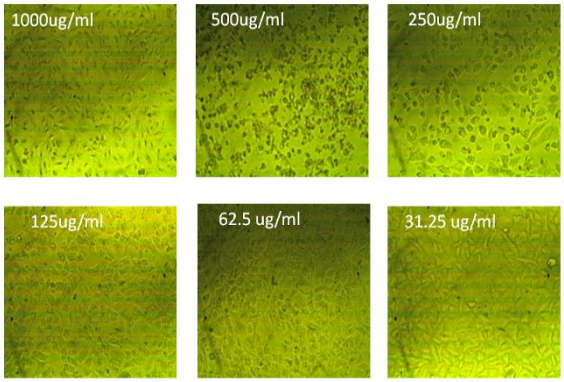
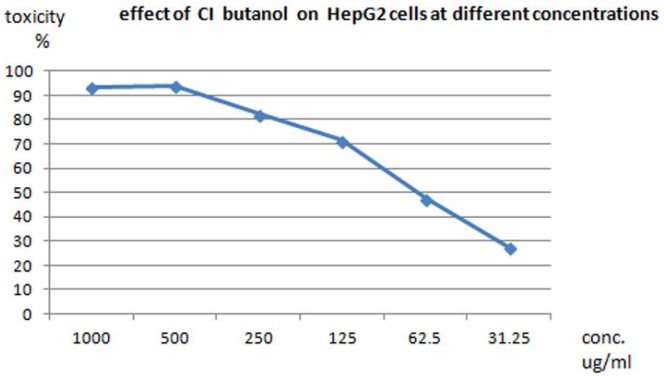


Figure 5: Effect of butanol extract of *Ceiba insignis* on HepG2 cells at different concentrations



Graph 4: Variation of toxicity percentage with different concentration of butanol on HepG2 cells

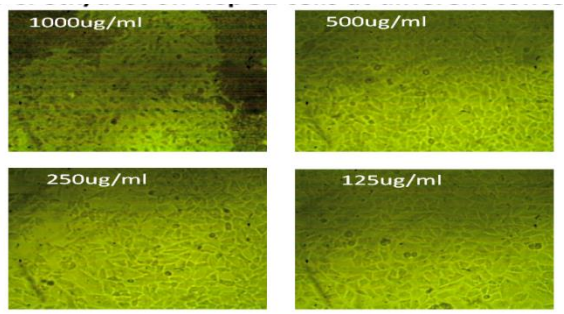
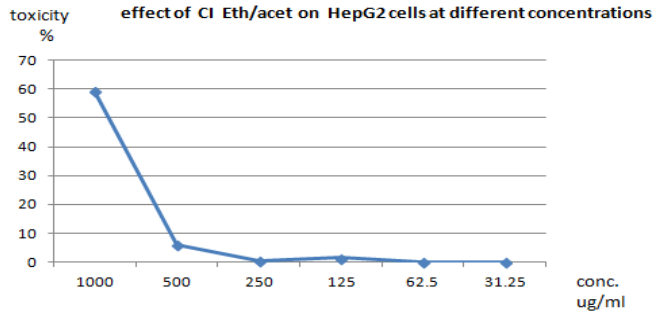


Figure 6: Effect of ethyl acetate extract of *Ceiba insignis* on HepG2 cells at different concentrations



Graph 5: Variation of toxicity percentage with different concentration of ethyl acetate extract on HepG2 cells

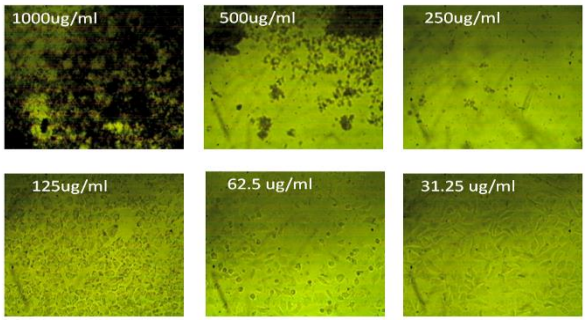
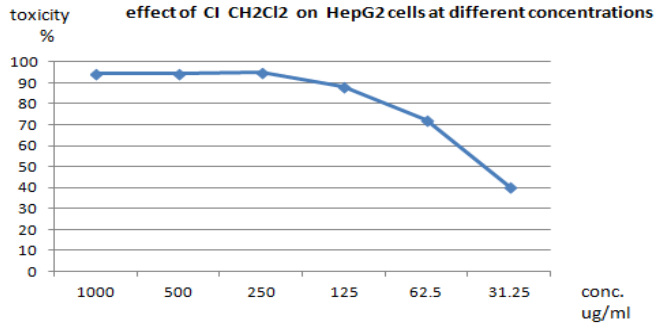


Figure 7: Effect of dichloromethane extract of *Ceiba insignis* on HepG2 cells at different concentrations



Graph 6: Variation of toxicity percentage with different concentration of dichloromethane extract of on HepG2 cells

Antimicrobial activity

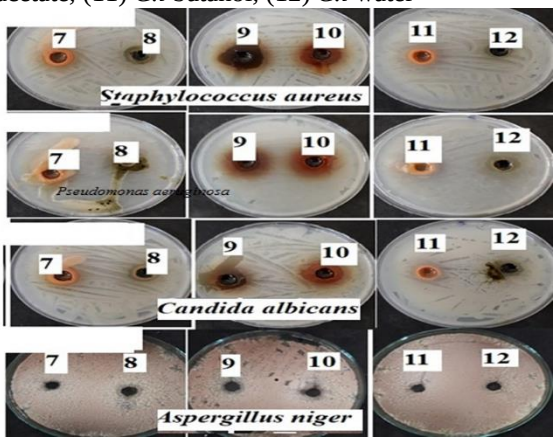
The results in Table (2) & Figure (8), revealed that methanol extract showed high activity toward the four different test microbes; *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans and Aspergillus niger* with inhibition zones (25, 20, 23, 25 mm) respectively. The petroleum ether extract showed moderate activity toward *Staphylococcus aureus, Candida albicans & Aspergillus niger* with inhibition zones (16, 15, 16 mm) respectively, while low activity on *Pseudomonas aeruginosa* with inhibition zone (14 mm). The dichloromethane extract showed very high activity toward *Staphylococcus aureus, Candida albicans* & *Aspergillus niger* with inhibition zones (28, 25, 30 mm) respectively, while moderate activity toward *Pseudomonas aeruginosa* with inhibition zones (16mm). The butanol extract showed moderate activity only on *Pseudomonas aeruginosa* with inhibition zone (18 mm), while on *Staphylococcus aureus, Candida albicans* showed low activity with inhibition zones *(14 mm)* and no activity on

Aspergillus niger. The water extract showed moderate activity only toward *Pseudomonas aeruginosa* with inhibition zone (16 mm), while low activity on *Staphylococcus aureus*, *Candida albicans* with inhibition zones (14,13 mm) respectively while no activity toward *Aspergillus niger*. The activities of the above mentioned extracts were compared with two standard antibiotics the first one, Penicillin G was used at 50 µg per disk with inhibition zones *Staphylococcus aureus* (19mm), *Pseudomonas aeruginosa* (20mm), *Candida albicans* (23mm), while the second one, Griseofulvin was used as specific antifungal antibiotic at 50 µg per disk with inhibition zone *Aspergillus niger* (29mm)

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

Serial no	Extract name	Clear zone (ϕmm)			
		Staphylococcus aureus	***Pseudomonas aeruginosa***	***Candida albicans***	*Aspergillus niger*
7	methanol	25	20	23	25
8	petroleum	16	14	15	16
9	methylene	30	15	26	23
10	ethyl acetate	28	16	25	20
11	butanol	14	18	14	0
12	water	14	16	13	0
standard	Penicillin G[1]	19	20	23	0
	Griseofulvin[2]	0	0	0	29

Figure 8: The antimicrobial inhibition zones (mm) of the 6 different solvent extracts of *C.i against* four pathogenic microbial strains; *Staphylococcus aureus* (first row), *Pseudomonas aeruginosa* (second row), *Candida albicans* (third row), *and Aspergillus niger* (fourth row), since (7) *C.i* methanol, (8) *C.i* petroleum, (9) *C.i* dichloromethane, (10) *C.i* ethyl acetate, (11) *C.i* butanol, (12) *C.i* 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 [6]. 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. The result in Table (3) revealed that the IC_{50} varied from 24.72 to 97.5 µg/ml, and the results are in the order, petroleum ether (**IC_{50}** = 24.72) > dichloromethane (**IC50** = 31.02) > water (**IC_{50}** = 78.22) > methanol (**IC_{50}** = 96.08) > *n*-butanol (**IC_{50}** = 96.36) > ethyl acetate (**IC_{50}** = 97.5) µg/ml. so the most antioxidant fraction is petroleum ether.

Extract	**DPPH (IC_{50})[1] [µg/ml]**
	Ceiba Insignis
methanol	96.08 ± 0.20
Petroleum ether	24.72 ± 3.45
dichloromethane	31.02 ± 0.99
Ethyl acetate	97.50 ± 0.31
water	78.22 ± 2.10
n-butanol	96.36 ± 0.32
Ascorbic acid	7.60 ± 0.85

Table 3: Free radical scavenging antioxidant activities (DPPH) of the 6 different solvent extracts of *C.i* 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 [21]. Among them, high-performance liquid chromatography (HPLC) has been the most widely used technology for identifying differences in chemical compositions among medicinal herbal samples [24]. Owing to the moderate toxicity against HepG2 in fractions methanol, Butanol, dichloromethane and weak toxicity in fractions water & Petroleum ether and very low toxicity in ethyl acetate, and strong antimicrobial activities in fractions methanol, dichloromethane, petroleum ether, ethyl acetate and moderate antimicrobial in fractions of butanol & water, and also antioxidant activity with IC_{50} values varied from 24.72 to 97.5 µg/ml, and most antioxidant fraction was petroleum ether. The mentioned extracts were subjected to further and rutin were the major standards while others standards are minor. In dichloromethane extract; syringic acid is the major standard while other standards are minors with absence of pyrocatechol acid. In methanol extract; both of naringenin & gallic acids are the major standards while others are minors with absence of pyrocatechol. In water extract; both of narginen & gallic acids are the major standards while the other standards are minor with absence of catechin, pyro catechol, rutin, ferulic acid, taxifolin and kaempferol. In ethyl acetate extract; both of gallic acids, chlorogenic acid and syringic acid, are the major standards while others are the minor with absence of ellagic acid. In petroleum ether extract; all standards are minor with absence of pyro catechol and ellagic acid. Due to the existence of characteristic structural standards for effective free radical scavenging, phenolic compounds show strong antioxidant potential, heavy hydroxylation mode, extended conjugation system and keto group [22, 23]. Although these compounds have antimicrobial effects, they have specific modes of action, such as cell wall damage

Standard		
	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 polyphenolic standard

Dichloromethane extract			
	Area	Conc. (µg/ml	Conc .(µg/g)
Gallic acid	87.69	8.80	478.02
Chlorogenic acid	72.83	5.73	311.38
Catechin	1.32	0.16	8.59
Methyl gallate	2.18	0.03	1.52
Coffeic acid	158.36	6.07	329.65
Syringic acid	905.52	38.32	2082.7
Pyro catechol	0.00	0.00	0.00
Rutin	61.77	8.17	444.16
Ellagic acid	5.26	0.36	19.63
Coumaric acid	700.05	11.83	642.82
Vanillin	50.30	1.07	58.13
Ferulic acid	141.64	4.42	240.33
Naringenin	218.12	11.78	640.16
Taxifolin	411.39	30.26	1644.6
Cinnamic acid	747.06	7.51	407.94
Kaempferol	23.84	0.89	48.14

Table 5: area under the peak of extract CH_2CL_2 against 16 polyphenolic standards

Methanol extract			
	Area	Conc. µg/ml	Conc. (µg/g)
Gallic acid	810.67	81.31	4517.45
Chlorogenic acid	228.93	18.01	1000.48
Catechin	402.39	48.29	2682.87
Methyl gallate	160.11	2.06	114.41
Coffeic acid	71.88	2.75	152.96
Syringic acid	631.90	26.74	1485.71
Pyro catechol	0.00	0.00	0.00
Rutin	316.77	41.91	2328.19
Ellagic acid	245.63	16.86	936.68
Coumaric acid	300.62	5.08	282.18
Vanillin	10.38	0.22	12.26
Ferulic acid	155.19	4.85	269.18

Naringenin	10476.	565.7	31430.8
Taxifolin	14.66	1.08	59.91
Cinnamic acid	8.67	0.09	4.84
Kaempferol	201.42	7.48	415.75

Table 6: area under the peak of CH3OH extract against 16 polyphenolic standards

ethyl acetate extract			
	Area	Conc. µg/ml	Conc. (µg/g)
Gallic acid	1155.04	115.86	6365.74
Chlorogenic	1185.92	93.29	5125.75
Catechin	788.97	94.69	5202.55
Methyl	1620.22	20.84	1145.05
Coffeic acid	574.80	22.02	1209.70
Syringic acid	2530.15	107.08	5883.49
Pyro catechol	17.25	1.17	64.44
Rutin	1323.26	175.06	9618.74
Ellagic acid	0.00	0.00	0.00
Coumaric	3763.36	63.59	3493.69
Vanillin	450.14	9.57	525.94
Ferulic acid	706.73	22.07	1212.38
Naringenin	1654.79	89.36	4909.93
Taxifolin	541.99	39.87	2190.60
Cinnamic	392.38	3.94	216.62
Kaempferol	74.81	2.78	152.73

Table 7: area under the peak of ethyl acetate extract against 16 polyphenolic standards (HPLC)

Water extract			
	Area	Conc. (µg/ml	Conc. (µg/g)
Gallic acid	850.22	85.28	5537.7
Chlorogenic acid	60.98	4.80	311.50
Catechin	0.00	0.00	0.00
Methyl gallate	52.04	0.67	43.46
Coffeic acid	50.04	1.92	124.46
Syringic acid	121.43	5.14	333.71
Pyro catechol	0.00	0.00	0.00
Rutin	0.00	0.00	0.00
Ellagic acid	96.02	6.59	427.98
Coumaric acid	38.83	0.66	42.60
Vanillin	11.40	0.24	15.73
Ferulic acid	0.00	0.00	0.00
Naringenin	1785.8	96.44	6262.0
Taxifolin	0.00	0.00	0.00
Cinnamic acid	2.56	0.03	1.67
Kaempferol	0.00	0.00	0.00

Table 8: area under the peak of H2O extract against 16 polyphenolic standards (HPLC)

Petroleum ether extract			
	Area	Conc. (µg/ml)	Conc. (µg/g)
Gallic acid	18.95	1.90	125.0
Chlorogenic	39.08	3.07	202.2
Catechin	3.20	0.38	25.30
Methyl gallate	10.79	0.14	9.13
Coffeic acid	1.99	0.08	5.01
Syringic acid	27.88	1.18	77.64
Pyro catechol	0.00	0.00	0.00
Rutin	8.71	1.15	75.83
Ellagic acid	0.00	0.00	0.00
Coumaric acid	17.45	0.29	19.40
Vanillin	8.10	0.17	11.33
Ferulic acid	12.25	0.38	25.16
Naringenin	46.90	2.53	166.6
Taxifolin	0.47	0.03	2.29
Cinnamic acid	327.99	3.30	216.8
Kaempferol	23.90	0.89	58.43

Table 9: area under the peak of petroleum ether extract against 16 polyphenolic standard (HPLC)

Butanol extract			
	Area	Conc.	Conc.(µg/
Gallic acid	1490.	149.46	7992.54
Chlorogenic	1155.	90.93	4862.77
Catechin	733.9	88.08	4710.26
Methyl	294.9	3.79	202.90
Coffeic acid	3.67	0.14	7.51
Syringic	3011.	127.46	6816.24
Pyro	219.4	14.93	798.20
Rutin	1128.	149.24	7980.58
Ellagic acid	330.7	22.70	1214.11
Coumaric	482.8	8.16	436.22
Vanillin	564.4	12.00	641.82
Ferulic acid	977.8	30.53	1632.69
Naringenin	1622	875.95	46842.49
Taxifolin	134.5	9.90	529.24
Cinnamic	112.8	1.13	60.66
Kaempferol	108.3	4.03	215.28

Table 10: area under the peak for butanol extractagainst 16 polyphenolic standard (HPLC)

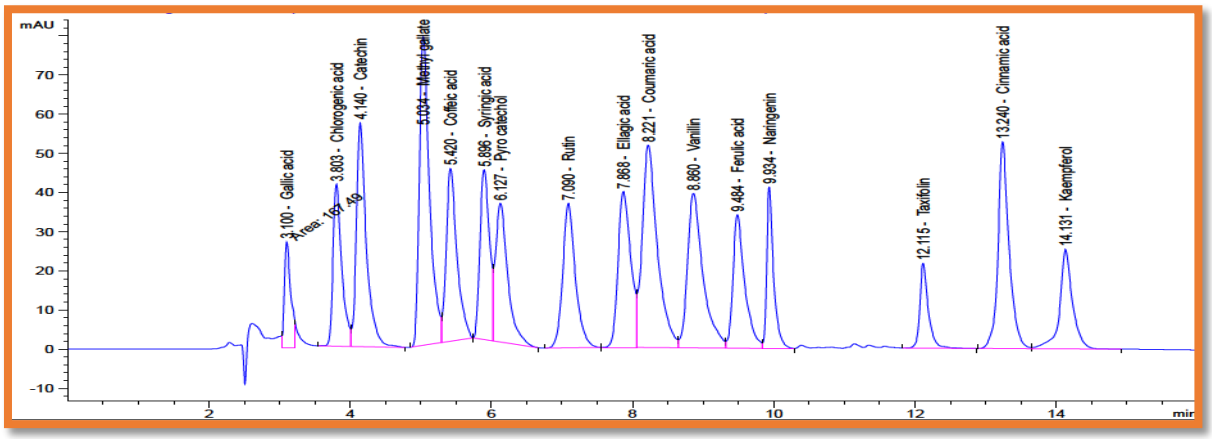


Figure 9: High performance liquid chromatography chromatogramof 16 standard phenolic compounds; ; gallic acid (1), chlorogenic acid (2), catechin (3), methyl gallate (4), coeffic acid (5), syringic acid (6), pyrocatechol (7), rutin (8), and ellagic acid (9), coumaric acid (10), vanillin (11), ferulic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

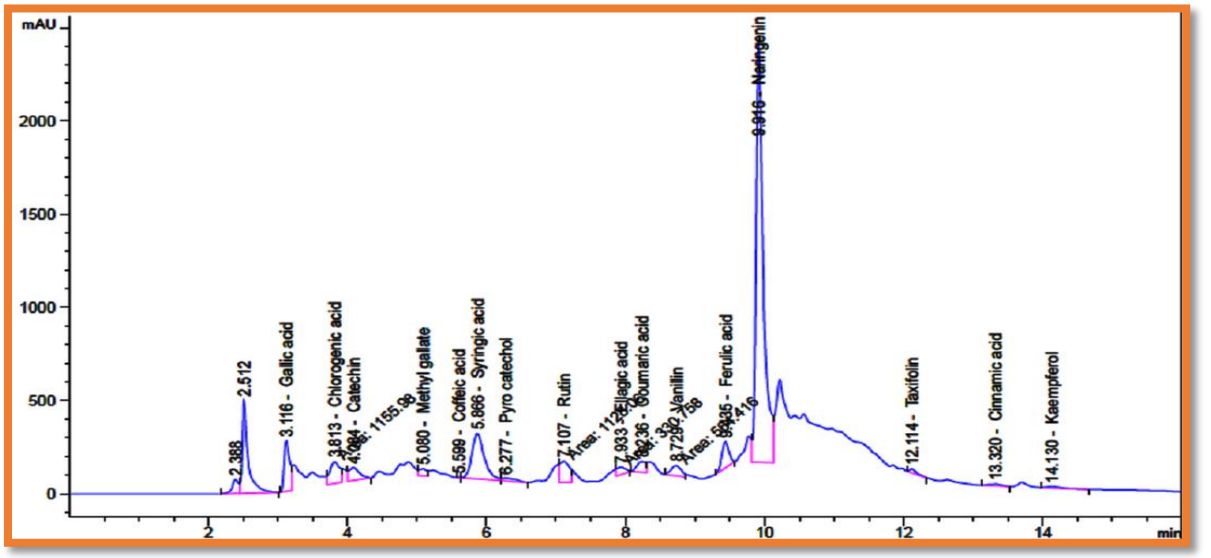


Figure 10: High‑performance liquid chromatography‑finger print chromatogram of butanol extract

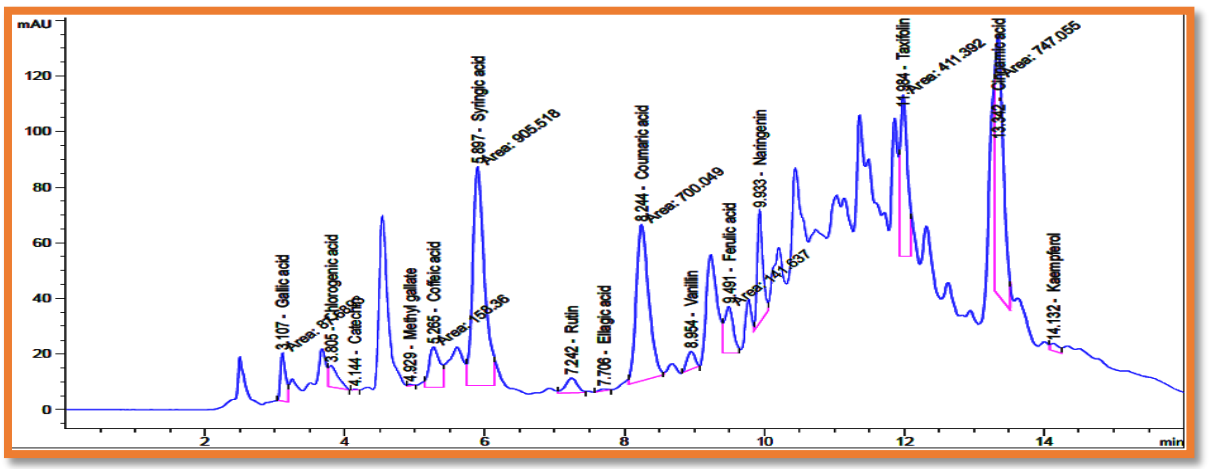


Figure 11: High‑performance liquid chromatography‑fingerprint chromatogram of dichloro-methane extract

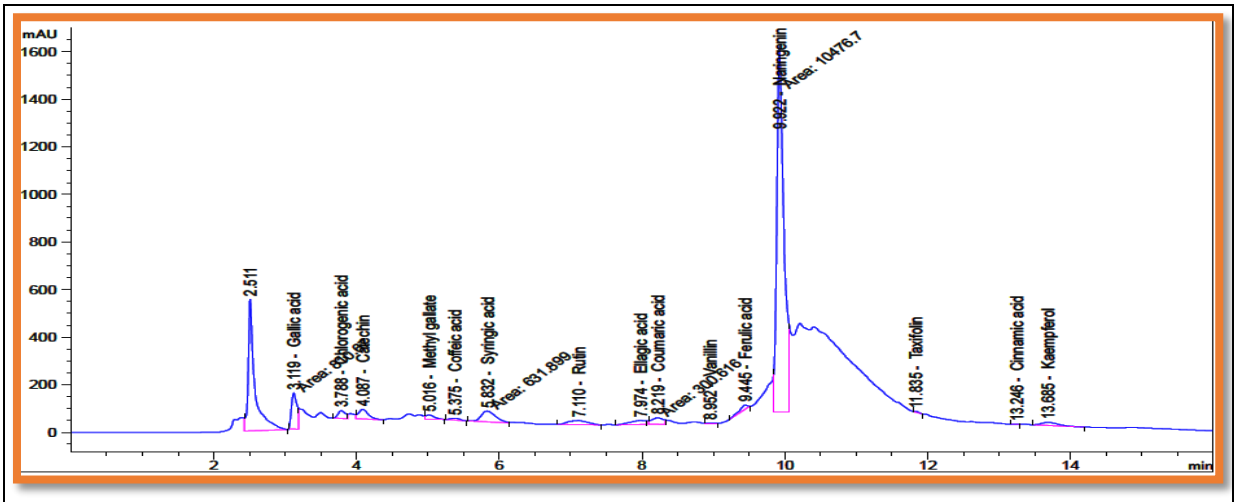


Figure 12: High-performance liquid chromatography-fingerprint chromatogram of methanol extract

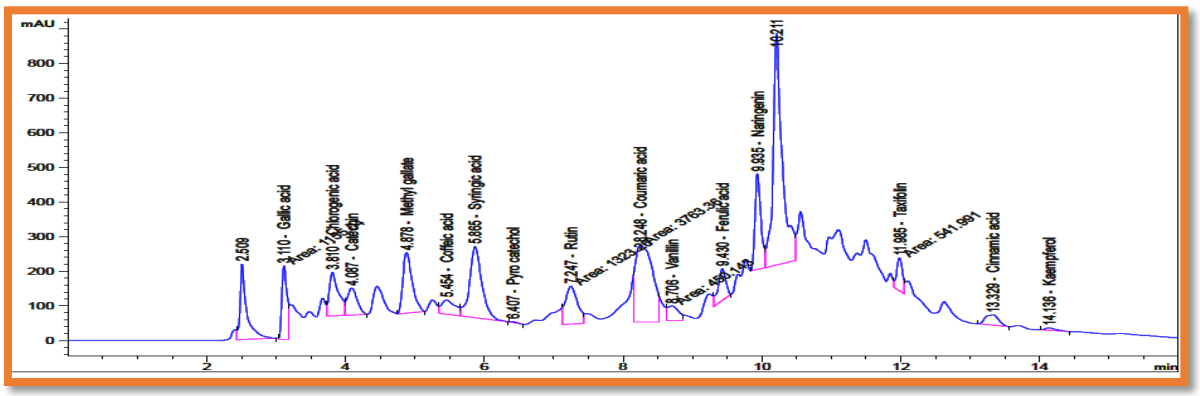


Figure 13: High-performance liquid chromatography-fingerprint chromatogram of ethyl acetate extract

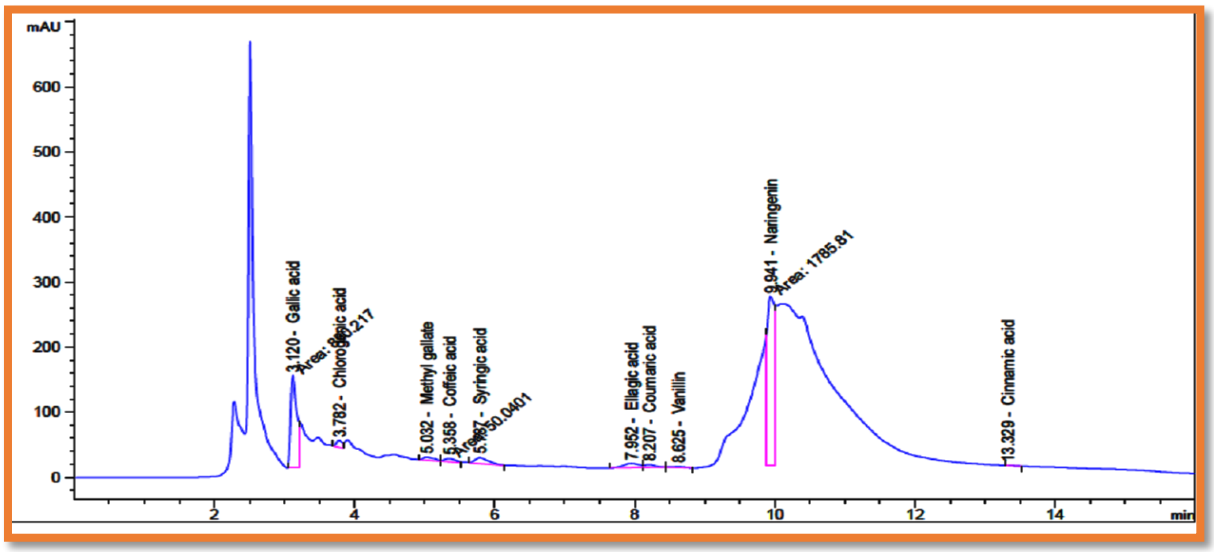


Figure 14: High-performance liquid chromatography-fingerprint chromatogram of water extract

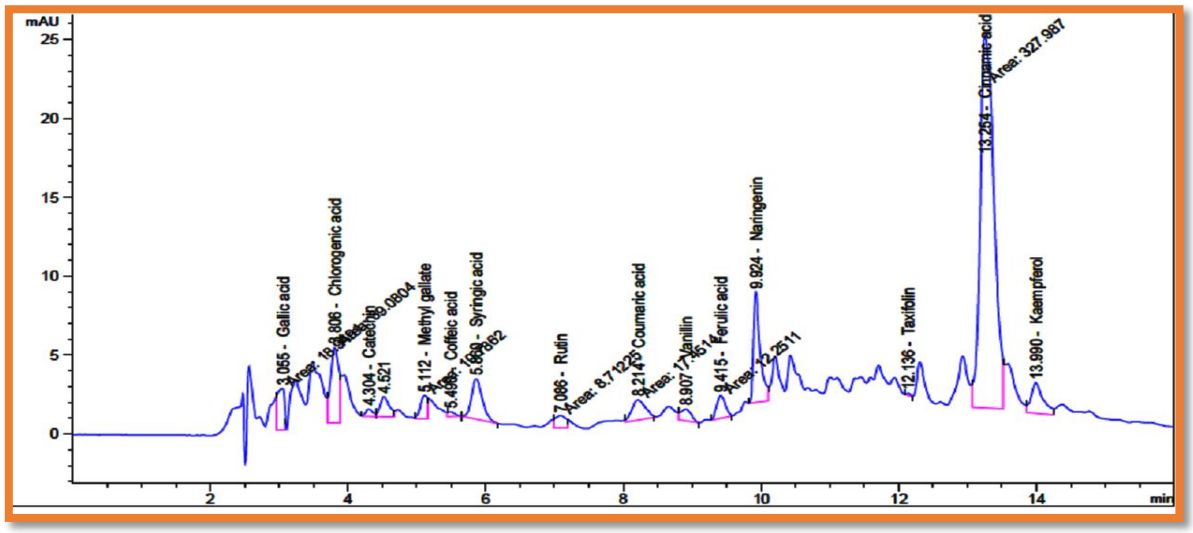


Figure 15: High-performance liquid chromatography-fingerprint chromatogram of petroleum ether extract

CONCLUSION: the extracts of *Ceiba insignis* leaves collected from zoo garden in Giza - Egypt, showed a moderate toxicity against HepG2 in fractions dichloromethane, methanol, butanol & and weak cytotoxicity in petroleum ether and water while very low toxicity in ethyl acetate, also showed strong antimicrobial activities toward the four tested microbes in fractions methanol, dichloromethane, petroleum ether, ethyl acetate and moderate antimicrobial activities in fractions of butanol and water, also showed strong antioxidant activity in fractions petroleum ether, dichloromethane, and the least antioxidant activity is fraction ethyl acetate. HPLC finger print is done for all fractions and result revealed that in dichloromethane fraction only syringic acid is major only while others are minor, also in methanol extract; its found both of naringenin and gallic acid only major and others are minor. In ethylacetate fractions both of gallic acid, cglorogenic acid and syringic acid are majors and others are minor, in water extract both of naringenin and gallic acid are the major while others are minor, in petroleum ether extract all standards are minor, in butanol extract both of naringenin, rutin and gallic acid are the majors and others are minor, 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.

Abbreviations Used: HPLC: High-performance liquid chromatography; IC_{50}: Median inhibitory concentration; DPPH: 2, 2-Diphenyl-1-picrylhydrazyl; G+ve: Gram-positive; G−ve: Gram-negative; IP: Inhibition percentage; RP-HPLC: Reversed phase-high performance liquid chromatography; DAD: Diode array detection; NCI: National Cancer Institute*; C.i Ceiba insignis*, DMSO: dimethyl sulfoxide

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There are no conflicts of interest

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