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STUDIES OF THE BIOLOGICAL ACTIVITY OF *CASSIA FISTULA*

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Abstract

Cancer has been the most dreadful disease from ages till now. However, with the passage of time it been more understood its mechanism, types, some causing factors, and way of its cell duplication. By the progress of science there are now more than one available method for treatment, diagnosis or prevention of cancer. But this progress did not reach till now cheap, fast and effective specific targeting treatment for all types or even one type. For this aim, the field of searching for alternative natural compounds extracted from plants to replace those expensive non effective targeting compounds has been wider and advanced. One of familiar plant families is Leguminosae specifically class of *Cassia fistula* plant. This type of plant has been examined to ensure its biological activity and by detecting and analysis it show anticancer activity against liver cancer cell line; hepatocellular carcinoma HepG2. *C. fistula* volatile oil extract was able to inhibit proliferation for HepG2 cancer cell lines at 3.05 ± 0.08µg/ml. The methanolic and oil extracts of *Cassia fistula* showed good brine shrimp larvicidal activity with lethality concentration (LC_{50}) of 15 and 55 µg/mL, respectively. Finally using GC-MS, the essential oil compounds were identified, whereas 39 compounds produces and accounted for 90.46% of the total oil, the major compound was compound sulfurous acid; cyclohexyl-methyl octadecyl ester (21.62%).

Keywords: *Fabaceae, essential oil, HepG2 treatment, Cassia fistula, phytochemical.*

Introduction

"Humans have been plagued by diseases throughout the history of civilization" (Laford *et al*., 1988). In ancient time, at lack of research evolution, it was the begging of asking questions to understand the mutation definition, causes, cases and how to deal with it. The next worldwide dreaded disease at that time was cancer what encourages the United States Senate to assign specific team from researchers and scientists to conquest cancer and it was the first financial solved issue for researching at that time. (Lowe-Kenty *et al*., 2011).

Nowadays it is more clear the main difference in definition either in the known structure between "Cancer" and "Tumor". Generally "cancer" is said for the whole disease. However, the mass of cells that start to divide and grow in an abnormal way and the repair mechanism cannot able to force it to its regular form; it forms what known scientifically by tumor. The differentiation character between tumor and cancer is the ability for this mass of cells to move from its original or primary part of tissue or organ to another place inside the body by the aid of circulatory system, what is scientifically known as "metastases". Any tumor mass has the option of metastases now it should be called "Cancer". (Balachandram *et al*., 2005)

One from cancer types that widely spread is liver cancer, its tumor where the primary origin forms in liver tissue. Liver is the largest organ inside our bodies as it considers the sixth most common cancer worldwide, liver cancer has more than one type according the type of cancerous cells, but Hepatocellular Carcinoma is the most common liver cancer known. Hepatocellular Carcinoma considers more than 90% of all cancer liver.

While the using traditional methods for treatment have a lot of side effects and disadvantages; chemotherapy and clinical drugs have targeting issue as they do not target only the cancer cell and force it to apoptosis but it effect all normal cells throughout this process, radiotherapy also more than side effect as changing in color of skin due to exposing to high radiation that sure effect other organs all this in addition to their high cost.

Using of plants in the new medical and pharmacological investigation is the alternative way of treatment to replace those methods (Abdel-Gwad, 2000a; Abdel-Gwad, 2000b). After the wide variety of existence effective natural compounds that have significant antioxidant, anticancer and chemo preventive activity, all studies were directed to report and list all effective phytocompound with reference cases (Hamed, 2007; Hassanein *et al*., 2010; Irshad *et al*., 2010; Hamed, 2015; Hamed, 2017).

One of popular and effective medical family in plant is The Fabaceae, Fortunately one of effective class that has anticancer potential and chemo-prevention activities is *C. fistula* that rich by magnificent compounds (Basker *et al*., 2012). This plant has been experimented for many tests to ensure its safety on the normal cell that was used in this study as brine shrimp larva and detect its cytotoxicity against various cancer cell lines.

Materials and Methods

1. Plant Collections:

The leaves of *Cassia fistula* family Fabaceae was collected at October from EL-Orman National Garden and identified by Prof. Dr. Wafaa Amer, Professor of Plant Taxonomy, Faculty of Science, Cairo University. Voucher specimen no. CF 1 has been deposited at the Herbarium of the Medical Chemistry Department, Theodor Bilharz Research Institute, Giza, Egypt.

2. Experimental Material and Chemicals:

The used solvents and reagents used in this study were all analytically graded as; hydrocholoric acid, ammonium hydroxide, citric acid, salicylic acid, mercuric choloride, diethyl ether, sodium sulfate, sulfuric acid, chloroform, acetic anhydrides, ammonium solution, magnesium powder, sodium hydroxide, ferric chloride, α-naphthol, and ethanol. Other solvents/reagents used for brine shrimp assay were saline (Instant Oceanic, Marine land Labs, USA) and brine shrimp's eggs (Artemia Inc., California). Also for MTT assay other chemicals were used as Dimethyl sulfoxide (DMSO), trypan blue dye (obtained from Sigma St. Louis, Mo., USA), Fetal Bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin and 0.25% Trypsin-EDTA (obtained from Lonza Belgium). Cell lines that used were from the American Type Culture Collection (ATCC, Rockville, MD) as it was Hepatocellular carcinoma cell line HepG-2. All solvents, materials and acids were fetched from Merck Chemical Company and Sigma-Aldrich Company.

3. Essential Oil GC/MS Analysis:

The used system was a combination between gas chromatography and mass spectrometry carried out on a GC/MS System: Thermo Scientific TRACE 1310 Gas Chromatography attached with ISQLT single quadrupole Mass Spectrometer detector, under specific conditions: Column: DBS-MS, 30m; 0.25mm ID (J&W Scientific), Ionization mode: EL, Ionization voltage: 70ev, Temperature Program: 40 °C (5 min)-275 °C (5 min) AT 5 °C/min, Detector Temperature: 300 °C, Injector temperature: 300 °C, Carrier gas: Helium; Flow 1 and Searched library: WILEY & NIST MASS SPECIRAL DATA BASE. This work was done at The Regional Center for Mycology & Biotechnology, Al-Azhar University, Cairo, Egypt.

4. Extraction procedures:

Leaves of *Cassia fistula* (1 KG) were crushed, dried and collected in a container in aim to start extraction process at room temperature. Extraction process was done with 85% methanol for several times then filtrated by using Whatman filter paper NO.1.

Evaporation mechanism of filter papers to reach dryness was done by rotary evaporator in vacuum at 50 ℃ to have 30gm of brownish viscous residue. The resulted methanol extract was tested for variety of phytochemical screening and availability/cytotoxicity test using brine shrimp eggs as a normal cell and HepG2 cell lines (hepatocellular carcinoma cell lines) as a cancer cells.

4.1. Hydro-Distillation process for oil extraction:

Extraction and manufacture of essential oil is performed by simple and cheap process called Hydro-Distillation process. Advantages of using specifically this process in addition to its low cost but also it consume less stream, less consumed time, and higher oil yield. In this stage leaves of *Cassia fistula* (2 KG) were sliced into small pieces and collected in the Clevenger apparatus, placed in boiling water (5 liters). The main purpose of using heat and boiling water is to break down and burst the cell structure of the plant that will aid to releasing the essential oils. In order to collect the resulting oil, molecule of oil carried out by the stream in a long pipe channels through a cooling tank to help molecules to return in its liquid form, the process that last for 4 hours. By using diethyl ether and since oil is lighter than water that will appear floating on the surface of water it will be easy to extract the oil from the mixture (Hamed, 2007).

Subsequently, the collected oil was then sent to National Cancer institute to examine the cytotoxicity of the extracted compounds. Moreover it was also sent to be analyzed with Gas chromatography to identify its ingredient.

5. Cytotoxicity and Cell viability assays:

In order to detect and determine if the oil extraction is firstly safe to be used on normal cells and has no side effect there was a primary check test done using brine shrimps larva.

After the incubation period IC_{50} test was measured using probit analysis by assessing the confidence intervals to 95%, to identify that inhibitory concentration (IC_{50}) for all test tubes. Any LC_{50} less than 100pm was pointed to be considered as active, while any LC_{50} less than 1000 µg/mL pointed to be toxic, non-toxic value starting from any LC_{50} greater than 1000 µg/ml.

5.1. Brine Shrimp Lethality Assay (BSLA):

By obtaining of brine shrimp eggs they were added to sea salt water and incubated for eggs hatching in a plastic container with dark cover to prevent any light passages to reach the required hatching conditions in addition to the light normal partial. The eggs were added directly to the dark side of the plastic container; once those eggs hatched they will move spontaneously to the light side that associated with lamp above it. Stage of hatching and maturation of eggs took 48 hour to be completed with product known now as larva. 4ml of the pre-prepared seawater was added to facilitate toxicity/viability measurements. The mixture solution that contains seawater was divided into seven test tubes. One tube is for control testing, the rest of the seven tubes were measured for toxicity at 3, 50, 30,100, 200, and 400 ppm in 10ml seawater solution added to methanol (1%). In order to evaporate the added methanol as it has negative and harmful effects on the normal cells, each test tube was well mixed and immersed in the water bath at 60°C for 5 min. In each test tube 10 larva were added. After one day of incubation for the uncovered test tube, number of viable larvae was counted and recorded for the analysis (the data were analyzed and LC_{50} values calculated according (Ipsen and Feigi, 1970; Hamed *et al*., 2016).

5.2. Antitumor assays:

The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50µg/ml gentamycin. The cells were maintained at 37°C in a humidified atmosphere with 5% CO2 and were subcultured two to three times a week.

For antitumor assays, the tumor cell lines were suspended in medium at concentration $5x10^4$ cell/well in Corning® 96-well tissue culture plates, then incubated for 24 hr. The tested compounds were then added into 96-well plates (three replicates) to achieve twelve concentrations for each compound. Six vehicle controls with media or 0.5 % DMSO were run for each 96 well plate as a control. After incubating for 24 h, the numbers of viable cells were determined by the MTT test. Briefly, the media was removed from the 96 well plate and replaced with 100 µl of fresh culture RPMI 1640 medium without phenol red then 10 µl of the 12 mM MTT stock solution (5 mg of MTT in 1 mL of PBS) to each well including the untreated controls. The 96 well plates were then incubated at 37°C and 5% CO_2 for 4 hours. An 85 µl aliquot of the media was removed from the wells, and 50 µl of DMSO was added to each well and mixed thoroughly with the pipette and incubated at 37°C for 10 min. Then, the optical density was measured at 590 nm with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as [(ODt/ODc)]x100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (IC_{50}), the

concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each conc. using Graphpad Prism software (San Diego, CA. USA) (Mosmann, 1983; Hassanein *et al.,* 2010).

6. Preliminary phytochemical screening for *C. fistula*:

The dry powder and extracts of *C. fistula* leaves were subjected separately to the following phytochemical tests.

6.1. Test for saponins:

10gm from the leaves extract was added to distilled water. This mix was strongly shaken then left for 5 minutes to know if this plant contains saponins or not. Appearance of white bubbles after left period have been finished was an indicator for the presence of saponins in the plant (Harbone, 1973).

6.2. Test for carbohydrates and glycosides:

1 gm of extraction was mixed with 10ml of 50% ethanol in test tube, then the mixed ethanol solution transferred into another tube with 0.5 ml of ethanolic α-naphthol solution, a drops of 1ml sulfuric acid was added to the wall of the test tube to appear violent ring, appearing of this ring is a clear indicator for the presence of carbohydrates and glycosides in the plant (Sofowora, 1993).

6.3. Test for sterols and Triterpenes:

After mixing 10gm of leave extract with 20 ml of chloroform, some drops of sulfuric acid was added on the wall of test tube. Appearing of red ring is a positive indicator for presence of triterpenes.

6.3.1. Salkowski test

5 ml of the chloroform solution and an equal amount of sulphuric acid were added carefully on the wall of test tube. Appearing of a red color is a positive indicator of sterols and /or triterpenes.

6.3.2. Liebermann-Burchard test

Evaporate about 5 ml of the chloroform solution of extract to a small volume then added to 1 ml of acetic anhydride and some drops of sulfuric acid about 2 ml were added on the wall of test tube. Reddish brown ring at the junction between the two layers is a positive indicator for the presence of unsaturated sterols and/or triterpenes (Shmidt, 1964).

7. Test for Alkaloids:

By adding 100ml of dilutes HCl on 10gm of leave extract mixed with ammonia solution (needed for naturalization) that followed by extraction using chloroform. The extract was filtrated and evaporated till it become dry, then dissolved in 2ml of HCl. It supposed to show slight precipitate in the bottom. Disappearing for this white precipitate is an also indicator that the plant doesn't contain alkaloids (Shellard, 1957).

8. Test for Tannins:

10gm of leaves extract was added to 20ml of 50% ethanol and ferric chloride solution. Green blue color appearance was the indicator for existence of tannins in the leaves (Gonzalez and Delgado, 1962).

9. Test for flavonoids:

150ml of 1% HCl was added to 5gm of leaves extract followed by adding of magnesium metal powder. Formation of a red color was a positive indicator to presence of flavonoids (Marby *et al*., 1970).

Statistical analysis

The data was subjected to analysis of variance (ANOVA), SPSS (Version 17). Statistical analysis between groups was performed using 1- way ANOVA. The analysis was based on at least three replications of every experiment that produced quantitative data.

Results

Preliminary phytochemical screening for *C. fistula* revealed the presence of oils, saponins, carbohydrates and/or glycosides, sterols and/or triterpenes, tannins, flavonoids and absence of alkaloids (Table 1). The evaluation for cytotoxicity was applied *in vitro* and the observed results from the methanol extract detect the presence of a huge amount of bioactive compounds. The extract was examined using MTT assay (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide).

However, to determine the activity for the oil extract from the *C. fistula* plant the extract was send to the National Cancer Institute to test either this oil has anticancer activity or not. And after observation of the oil on more than one type of cell lines, oil extraction of *C. fistula* has shown a significant effect on hepatocellular carcinoma cell line (HepG2) (Hamed *et al*., 2010).

After observation, the oil extract was able to inhibit proliferation of HepG2 cancer cell lines at (3.05 ± 0.08µg/ml) compared to Doxorubicin which exhibited antitumor activity at (4 µg/ml) as a control (Fig.1) (Hamed *et al*., 2010). According to the guidelines of the American National Cancer Institute (NCI) stated that the activity limit for extract at 50% inhibition time (IC_{50}) of cell proliferation after 72 hours should be less than 30 µg/ml. However, when the IC_{50} of the extract is less than 20 µg/ml it considers having highly level of cytotoxicity (fig.1). In this study, the results showed a cytotoxic effect of oil extract of *C. fistula* on HepG2 cancer cell lines. Although the results also showed IC_{50} value lower than that stated and specified by NCI to determine it as anticancer agents.

The observed lethality of *C. fistula* extracts to brine shrimps indicated the presence of potent cytotoxic and probably antitumor components of this plant. According to Meyer *et al*., crude plant extract is active if it has an LC_{50} value of less than 1000 µg/ml while inactive if it is greater than 1000 µg/ml (Meyer *et al*., 1982; Gupta *et al*., 1996).

The degree of lethality was directly proportional to the concentration of the extract. Maximum mortalities (100%) were observed at a concentration of 400 µg/mL in both extracts. Based on the results, the brine shrimp lethality of *C. fistula* extracts were found to be concentration-dependent. The observed lethality of *C. fistula* extracts to brine shrimps indicated the presence of potent cytotoxic and probably antitumor components of this plant. The methanolic and oil extracts of *Cassia fistula* tested showed good brine shrimp larvicidal activity with lethality concentration (LC_{50}) of 15 and 55 µg/mL, respectively (Fig.2 and 3). Analyses of the essential oils from the leaves of *C. fistula* were carried out using hydrodistillation method, (Table 2) shows representative data for the major constituents in its leaf essential oils, these being identified using GC-MS. Accordingly, 39 compounds were identified and accounted for 90.46% of the total. Of the mixture of alkaloid, sesquiterpenelactone, monoterpene, pseudoguaianolide, stearaldehyde, steroid, flavonoids, alcohols, fatty acid, alkenes and alkanes, the main constituents present were the sulfur containing compound sulfurous acid, cyclohexyl-methyl octadecyl ester (21.62%), 3-(2,2- dimethylpropylid-ene)bicyclo[3.3.1]nonane-2,4-dione (20.72%), 2,6-di(t-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one (10.13%), hexadecanoic acid (CAS) (3.99%) and pivaloylacetone, enol (2.96%). The remaining constituents of leaf oils were, octadecanoic acid, 3-hydroxy-2-tetradecyl-, methyl ester (CAS) (2.65%), 2,5-cyclohexadiene-1,4-dione, 2,6-bis(1,1 dimethyleth-yl)-(2.62%), l-(+)-Ascorbic acid 2,6-dihexadecanoate (2.63%), Cholestan-3-one, cyclic 1,2-ethanediyl acetal, (5à)- (CAS) (0.48%). Interestingly, although the alkaloid compounds, Ibogamine-18-carboxylic acid, 16,17-didehydro-9,17-dihydro-9, 20-dihydroxy-12-methoxy-, methyl ester (20S)- and dichotine, 19-hydroxy-11-methoxy-, 2-acetate were present in *C. fistula* leaf tissue in percent of (0.62%) and (0.25%), respectively also, flavonoid compounds; quercetin-7,3',4'-trimethoxy (0.23%), and Lucenin 2 (0.36%) were detected (Fig. 4), these perhaps being an indicator of a molecular marker for *C. fistula* biological importance. This IC_{50} value was observed to have effect anticancer activity on HepG2 liver cancer cell lines to be considers as a new promising anticancer potential agent. This result is suggested to be due to presence of specific phytochemicals in the extract of *Cassia fistula*, as there was previous study showed that presence of flavonoids in the extract causes inhabitation for liver cancer cells. From those several compound are; phenolic acids that is expermintally known for anticancer potential by causing apoptosis in tumor cells as a result for blocking the DNA synthesis in the cell (Morris 1999; Yuenyongsawad *et al.* 2013), lectin that found in legumes as a phytoconstituent has the ability to bind with glycoconjugates which present specifically on tumor cells, saponin's anticancer activity is dynamic by interfering with the replication of DNA, and as a result it prevents sequence the proliferation of cancer cells that leads to tension decrease of the aqueous solution that known as lyobipolar property (Isil *et al.*, 2015), and tannins are another phytochemical compound which is found affluently in *C. fistula* that have the antioxidant activity and cancer-prevention activity. These activities could be performed by tannins as they are able to bind to the former of metallic ions and protein of the cells that force it to apoptosis cycle. N-hexadecanoic acid and it showed significant cytotoxicity against human colorectal carcinoma cells (HCT-116) with an IC_{50} value of 0.8 µg/mL (Ravi and Krishnan, 2017). Baader *et al.*, reported that; ascorbic acid (AA) was found to be cytotoxic to neuroblastoma cells *in vitro* and *in vivo* (Baader *et al.*, 1994). Steroids are biologically active constituents, they are considered to be a part of plants' defense systems, and as such have been included in a large group of protective phyto-compounds found in plants named phytoprotectants (Morrissey and Osbourn 1999; Gus-Mayer *et al.*, 1994).

Steroids play critical roles in a number of disorders, including malignancies like prostate cancer, where steroid production inside and outside the tumour promotes cancer cell aggressiveness (Lubik *et al.*, 2016).

Moreover, several alkaloids exhibit significant biological activities, such as the relieving anticancer effects (Li *et al.*, 2007). Hamed *et al.*, says; a flavonoid compound quercetin exhibited inhibitory activity against colon carcinoma cells (HCT-116) with IC_{50} value of, 10 µg/ml (Hamed *et al.*, 2015).

Finally we strongly can attribute the potent cytotoxic activity of *C. fistula* essential oil to its constituents from alkaloids, sesquiterpene-lactone, steroid and flavonoids.

Taken together, this data revealed a potential use for the *C. fistula* oils for medicinal applications.

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Table 1: Phytochemical screening of *Cassia fistula* leaves.

Test Name:	*Cassia fistula*
Test for volatile oil	Positive
Saponins	Positive
Carbohydrates and/or glycosides	Positive
Sterols and/or Triterpenes	Positive
Alkaloids and/or nitrogenous bases	Negative
Tannins	Positive
Flavonoids	Positive

Table 2: Results of GC/MS analyses of *Cassia fistula* leaves.

Peak No.	R_t	Area %	M.F.	Identified Compounds
1	8.97	0.7	$C_6H_{12}O$	3-Hexen-1-ol, (E)-
2	12.93	1.05	$C_8H_{24}O_4Si_4$	Cyclotetrasiloxane, octamethyl-
3	13.02	2.96	$C_8H_{14}O_2$	Pivaloylacetone, enol
4	19.55	0.26	C_9H_9NO	2,6-Dimethylphenyl isocyanate
5	21.83	1.7	$C_{18}H_{34}O_2$	9-Octadecenoic acid (Z)- (CAS)
6	23.14	1.38	$C_{14}H_{30}O$	1-Tetradecanol (CAS)
7	24.56	1.8	$C_9H_{18}O_2$	Nonanoic acid (CAS)
8	26.37	1.03	$C_{22}H_{30}N_2O_3$	Aspidospermidin-17-ol, 1-acetyl-16-methoxy- (CAS)
9	26.78	0.56	$C_{12}H_{24}N_2O_3$	Pent-4-enoic acid, 2-(2-hydroxy-3-isobutoxypropyl)-, hydrazide
10	27.57	0.95	$C_{15}H_{24}O$	4,6-di-tert-Butyl-m-cresol
11	27.91	2.62	$C_{14}H_{20}O_2$	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1 dimethylethyl)-
12	28.05	0.78	$C_{15}H_{24}O_2$	Baimuxinal
13	28.38	10.13	$C_{15}H_{24}O_2$	2,6-di(t-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one
14	29.20	20.72	$C_{14}H_{20}O_2$	3-(2,2- dimethylpropylid-ene)bicyclo[3.3.1]nonane-2,4-dione
15	30.14	0.65	$C_{15}H_{22}O_2$	1-methyl-6-(1-oxoethyl)-3-oxo-4-prop-2-ylidenecyclo-nonene
16	33.41	21.62	$C_{25}H_{50}O_3S$	Sulfurous acid, cyclohexyl-methyl octadecyl ester
17	34.05	0.37	$C_{10}H_{13}NO_3$	L-Serine, O-(phenylmethyl)-(CAS)
18	34.28	0.35	$C_{19}H_{26}O_6$	Isochiapin B
19	35.21	0.77	$C_{13}H_{14}O_4$	5-Acetyl-6-formyl-7 aceto-xymethylenecyclo [2.2.1]hept-2-ene

20	35.72	0.28	$C_{18}H_{30}D_6O$	2,2,3,3,4,4 Hexadeutero octadecanal
21	36.17	0.23	$C_{18}H_{16}O_7$	Quercetin-7,3',4'-trimethoxy
22	36.26	0.26	$C_{12}H_{10}FN_5$	1H-Purin-6-amine, [(2-fluorophenyl)methyl]- (CAS)
23	36.63	0.7	$C_{18}H_{36}O$	2-Pentadecanone, 6,10,14-trimethyl- (CAS)
24	37.25	1.06	$C_{16}H_{22}O_4$	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
25	37.35	0.48	$C_{29}H_{50}O_2$	Cholestan-3-one, cyclic 1,2-ethanediyl acetal, (5à)- (CAS)
26	37.55	0.46	$C_{18}H_{34}D_2O$	2,2-Dideutero octadecanal
27	38.28	2.65	$C_{33}H_{66}O_3$	Octadecanoic acid, 3-hydroxy-2-tetradecyl-, methyl ester (CAS)
28	38.38	2.94	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester (CAS)
29	39.00	0.17	$C_{21}H_{36}$	14-á-H-Pregna
30	39.19	0.79	$C_{22}H_{23}NO_6S$	2-Demethylthiocolchicine Formate
31	39.95	0.96	$C_{19}H_{38}O_4$	Hexadecanoic acid, 2,3-dihydroxypropyl ester (CAS)
32	40.10	2.63	$C_{38}H_{68}O_8$	l-(+)-Ascorbic acid 2,6-dihexadecanoate
33	40.36	3.99	$C_{16}H_{32}O_2$	Hexadecanoic acid (CAS)
34	42.23	1.36	$C_{19}H_{38}O_2$	Methyl stearate
35	43.25	0.50	$C_{35}H_{68}O_5$	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester (CAS)
36	43.57	0.49	$C_{24}H_{42}O_7$	L-Ascorbic acid, 6-octadecanoate
37	48.44	0.62	$C_{22}H_{28}N_2O_5$	Ibogamine-18-carboxylic acid, 16,17-didehydro-9,17-dihydro-9, 20-dihydroxy-12-methoxy-, methyl ester, (20S)-
38	49.16	0.36	$C_{27}H_{30}O_{16}$	Lucenin 2
39	50.00	0.25	$C_{25}H_{30}N_2O_9$	Dichotine, 19-hydroxy-11-methoxy-, 2-acetate
Total %		90.46%		

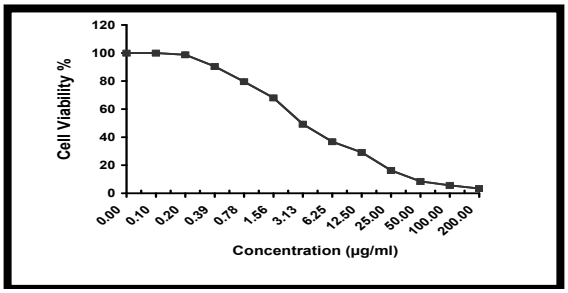

Fig.1: The cytotoxic activity of *C. fistula* against HepG2 liver cancer.

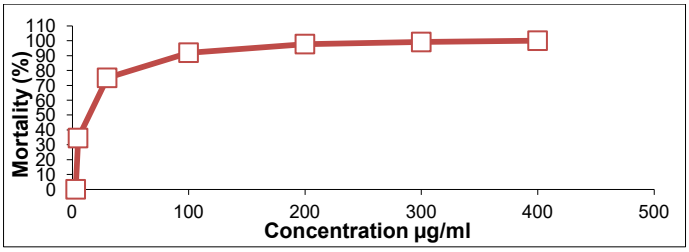

Fig.2: Brine shrimp lethality assay of *C. fistula* methanol extract.

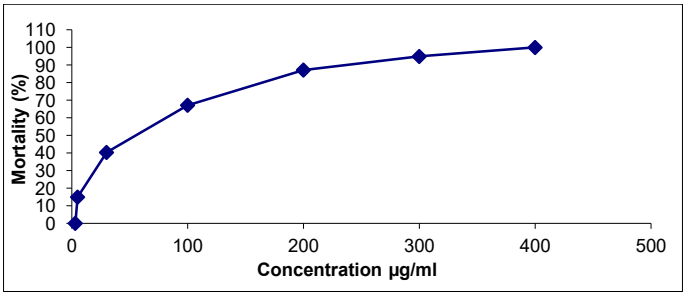

Fig.3: Brine shrimp lethality assay of *C. fistula* oil extract.

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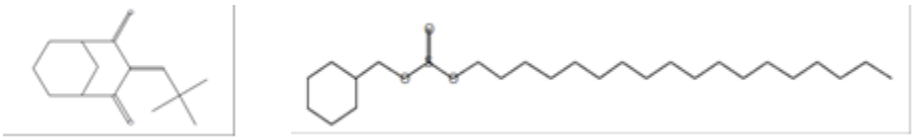

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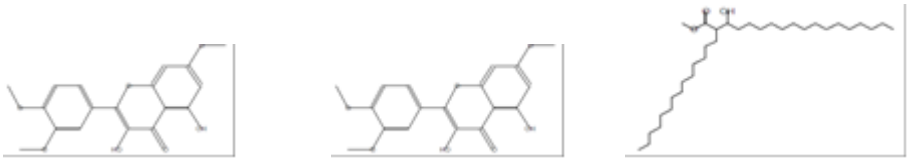

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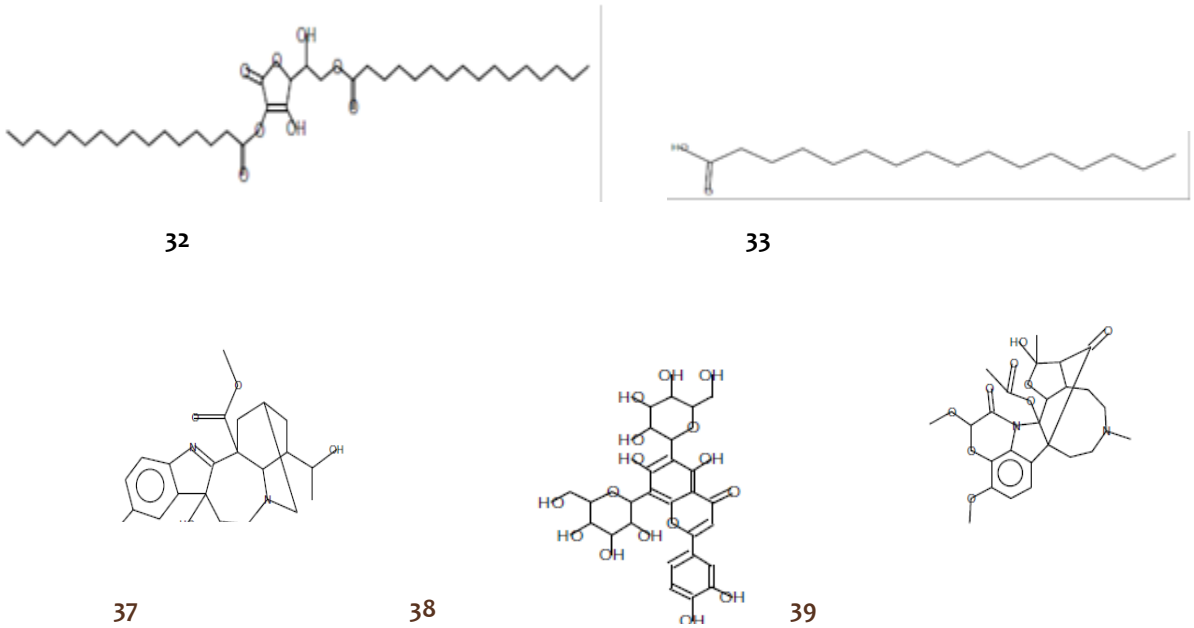

Fig. 4: *C. fistula* oil compounds structure.