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THE BIOLOGICAL ACTIVITY OF *CONOCARPUS ERECTUS* EXTRACTS AND THEIR APPLICATIONS AS CYTOTOXIC AGENTS

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Abstract

Essential oils are found to have multiple active components which can show *in vitro* cytotoxic action against various cancerous cell lines. This study reports the *in vitro* cytotoxic effects of the essential oil from *Conocarpus erectus* (Combretaceae) growing wild in Egypt. Water-distilled essential oil of *C. erectus* was examined for its cytotoxic effects using a modified brine shrimp and MTT assays. Fresh leaves aerial part of *C. erectus* was subjected to hydro distillation using a Clevenger-type apparatusvolatile to obtain its volatile oil. Cytotoxicity of the essential oil was measured against HepG2 cancer cells and brine shrimps larva. The essential oil 50% cytotoxic concentrations were found to be 33µg/ml and 8.7µg/ml against brine shrimp and human liver carcinoma HepG2 cell line, respectively; thus the volatile oil displayed good cytotoxic action against the human tumor cell line. Moreover, *C. erectus* methanol extract was very effective; it exhibited cytotoxic activity against brine shrimp larva within IC_{50} value of 15µg/ml. The investigation from GC Mass, led to the identification of 12 constituents, representing 97.53% of the total oil, of which the major chemical constituents were identified by gas chromatography mass spectrometry as being rich in 3-(2,2 dimethylpropylid ene)bicyclo[3.3.1]nonane-2,4-dione (**3**) (67.12%), (decanoic acid derevatives (**11**) (7.77%), 22-tritetracontanone (**12**) (6.03%), 1-octanol, 2-butyl- (**2**) (5.51%) and oleic acid (**6**) (4.33%). This is the first report on anticancer potential and separation of essential oils from *C. erectus.* The findings of this study necessitate the need for further consideration of this essential oil in anti-neoplastic chemotherapy.

Keywords: *Conocarpus erectus, cytotoxicity, HepG2 cells, MTT assay, volatile oil, phytochemistry*

Introduction

In 2018, 1,735,350 new cancer cases and 609,640 cancer deaths are projected to occur in the United States (1). Many people don't really know what cancer is? If a normal cell demonstrate, on the inside of the cell is the nuclei, that contains DNA and that DNA contains genes that control the action of the cell. The one of those important actions is how a cell makes a copy of itself. If a normal cell placed in culture it will simply make a copy of itself and if there's room those cells will make copies of themselves and they will keep doing that until they fill up that area. If one of those cells dies then an adjacent cell is going to jump into that cell cycle and it is going to fill that hole. That how we went from a zygote to the trillions of cells that are inside our body. Natural products sometime have therapeutic benefit but better as it is more effective and cause fewer side effects in treating diseases than the synthetic one. As mentioned before that secondary metabolism are group of organic compounds that are produced from the plant which are alkaloids, triterpenes, monotriterpense, flavonoids, essential oils, sterols, tannins and saponins each one has its own role in secondary metabolism that can affect in anti-proliferation of cancer cells (2-5). According to world Health organization (WHO) the best source to obtain a variety of drugs is from medicinal plants. About 80% of individuals from developed countries use traditional medicines which have derived from natural plant products (6-8).

Conocarpus erectus, its family combretaceae R. Br and its genus is conocarpus L. (9). According to Hussein, 2016; that *Conocarpus erectus* leaves showed high free radical scavenging activity toward DPPH radical between (29.87-79.33) and antibacterial activity was found to be present in n-butanol extract (10) (Hussein, 2016).

In addition, the essential oils are the products of extraction of a plant species, so they are more concentrated and may exhibit higher toxicity than the original plant (11,12). In the present study, we focus on assessment of the essential oils cytotoxicity extracted from *C. erectus*, against brine shrimps and HepG2 cell line liver cancer and GC Mass investigation for the oil constituents. Moreover we carry out a phytochemical screening for *C. erectus* methanol extract.

Methods

General

Plant collection

The leaves of *Conocarpus erectus* of Combretaceae family was collected at March from desert Egypt - Alexandria road and identified by Prof. Dr. Wafaa Amer, Professor of Plant Taxonomy, Faculty of Science, Cairo University. Voucher specimen no. CE 1 has been deposited at the Herbarium of Medicinal Chemistry Department, Theodor Bilharz Research Institute, Giza, Egypt.

Methanol extraction from *Conocarpus erectus*

Dry powdered leaves about 200 g were soaked for 3 days with 85% methanol, and then filtered off and the extract were evaporated using a rotary vacuum evaporator to dryness in vacuum. The consecutive partition fraction of methanol crude extract of leaves was then collect it and starts to use in preliminarily phytochemical screening.

Chemical and reagents

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.

Extraction of essential oil

The methods of extraction of essential oils from plants considerably have an effect on the chemical constituents and composition of the essential oil. The foremost acceptable and convenient technique to concentrate the targeted biologically active compound into the essential oil ought to be chosen. If the activity is predicated on a mixture, not one compound, then all the active parts of components should be targeted from the extract. Since, in general, most constituents of essential oils are tiny, volatile and lipophilic; a key thought is the need to separate these compounds from aqueous plant materials. Many ways have been developed, and we review only the most recent reports describing strategies accustomed extract essential oils. In follow-up studies, complete structural determination, specially the relative and absolute stereochemical assignments, is vital for a complete understanding of the active compounds and their structure-activity-relationships.

Gas Chromatography (GC)

GC are able to the best resolution of the essential oils, however there are some vital limitations with regards to preceding scale separations. Typically, because the sample capability is magnified, the resolution of the chromatographical separation is reduced. On a research lab scale, equipment is accessible that allows 24-hour automated and unattended separations. It is also can accustomed to determine the essential oil constituents by exploitation database libraries of each retention indices and mass spectral fragmentation patterns.

The used system was a mix between gas chromatography and mass spectrometry carried out on a GC/MS System. 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.

Isolation of the essential oil

The aerial fresh leaves of *Conocarpus erectus* were sliced into small pieces at room temperature. About (1000 g) were separately subjected to hydrodistillation using a clevenger-type apparatus for 3 hours. After decanting and drying the oil over anhydrous sodium sulfate and collected by ether, the oil was recovered.

Preliminarily phytochemistry

i. Tests for sterols and triterpenes

About 10 ml of each extract in ethanol were evaporated to dryness. The residue was dissolved in 20 ml of chloroform solution and filtered. The filtrate was subjected to the following tests. The following test called Salkowski test about 5 ml of the chloroform solution, an equal volume of sulphuric acid were added carefully. Formation of a red color mentions the presence of sterols and /or triterpenes (13).

ii. Test of saponins

About 10 gm of each extract was shaken with distilled water and filtered. This filtrate was shaken strongly and allowed to stand for about five minutes. The presence of voluminous froth indicates the presence of saponins (14).

iii. Test for carbohydrates and glycosides

1 gm of each extract was mixed with 10 ml of 50 % aqueous ethanol. 5 ml of each extract in ethanol were mixed with 0.5 ml of ethanolic α-naphthol solution followed by one ml of H_2SO_4 poured carefully on the wall of the test tube. The appearance of a violet ring between the two layers indicates the presence of carbohydrates and /or glycosides (15).

iv. Test for alkaloids

10 gm of each extract was mixed with 100 ml of dilute hydrochloric acid. The acidic extract was filtered, and converted to alkaline with ammonium hydroxide solution, followed by extraction with chloroform. The chloroform extract was evaporated till dryness and the extract was dissolved in 2 ml of HCl solution. The formation of very faint brown precipitate with Wagner's reagent and very slight precipitate with Mayer's reagent confirming the presence of nitrogen bases (16).

v. Test for tannins

About 2 gm of each extract was added to 20 ml of 50 % aqueous ethanol and filtered. Add few drops of ferric chloride solution; if a green color obtained confirming the probability of existence of tannins (17).

vi. Test for flavonoids

5 gm of each extract sample was soaked overnight with 150 ml of 1% hydrochloric acid solution and filtered. The filtrate was subjected to flavonoid compounds tests as follows. About 10 ml of the filtrate was converted to alkaline with NaOH solution. The appearance of a yellow color denotes that the presence of flavonoids. About 5 ml of the filtrate was mixed with 5 ml of HCl acid solution and few pieces of magnesium metal were added. The formation of a red color indicates the presence of flavonoid (18).

Cytotoxicity assay

Primary test for cytotoxic activity by using brine shrimp (larva).

Brine shrimp lethality bioassay method was utilized for pharmacological screening (19). The eggs of brine shrimp, *Artemia salina* leaches were taken in a conical flask and seawater (prepared by dissolving 38 gm of NaCl in one liter of distilled water) was added to it. Two days were allowed to hatch the shrimp and to become matured as nauplii. Measured toxicity of each sample was tested at 100, 150, 200, 250, 300, 350, 400, and 450 ppm in 10 ml seawater with 1% DMSO (v/v).

From each of these test solutions 100 µl were added to the prepared numbered glass test tubes containing 5 ml of sea water and 10 shrimp (larvae) nauplii and by using a Pasteur pipette take 10 living nauplii then were put to each of the tubes. After 24 hours, the test tubes were observed with a magnifying glass and number of survived nauplii in each test tube was counted. From this data, the percent of the lethality mortality of brine shrimp nauplii was calculated for each concentration in µg/ml according to Reed-Muench method (20).

Antitumor assay

Antitumor assay is also called MTT assay (tetrazolium) its consider one of the most widely used assay in determine the preliminary anticancer activity of both natural products and both synthetic derivatives.

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% CO_2 and were sub cultured two to three times a week.

For antitumor assays, the tumor cell lines were suspended in medium at concentration $5x10^4$ cell/well in 96-well tissue culture plates, and 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 plates 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 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 (5, 21).

Statistical analysis

The experimental results were expressed as mean ± standard deviation (SD) of three replicates. Where applicable, the data were subjected to one-way analysis of variance (ANOVA). Microsoft Excel 2010 statistical package was used for all analyses.

Results

The phytochemical screening showed positive results for triterpenes/steroids, alkaloids and/or nitrogenous bases, carbohydrates and/or glycosides, volatile oil, flavonoids, saponins, tannins.

Table **1**:- Phytochemical screening of *C. erectus* leave methanol extract.

Test	*C. erectus*
Volatile oil	Positive
Saponins	Positive
Carbohydrates and/or glycosides	Positive
Sterols and/or triterpenes	Positive
Alkaloids and/or nitrogenous bases	Positive
Tannins	Positive
Flavonoids	Positive

Identification of compounds

GC-mass spectrometry (GC-MS) is most frequently and effectively used to identify the essential oil constituents by using database libraries of both retention indices and mass spectral fragmentation patterns. The steam-distilled oil derived from the galls of *Conocarpus erectus* was analyzed by chromatography and spectral techniques, these being identified using GC-MS. The investigation led to the identification of 12 constituents, representing 97.53% of the total oil such as sesqueterpenes, steroid derivative, alkaloid and dialdehyde. The oil was found to be rich in 3-(2,2 dimethylpropylid-ene)bicyclo[3.3.1]nonane-2,4-dione (**3**) (67.12%), (decanoic acid derevatives (**11**) (7.77%), 22-tritetracontanone (**12**) (6.03%), 1-octanol, 2-butyl- (**2**) (5.51%) and oleic acid (**6**) (4.33%). The other main constituents characterized were, 1-decanol, 2-methyl- (**1**), (22R)-6á,11á,21-trihydroxy-16à,17à propylmethylenedioxypregna-1,4-diene-3,20-dione (**4**), Iso-velleral (**5**), Benzene, (1-butyloctyl)- (**7**) as well as (5á)pregnane-3,20á-diol, 14à,18à-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-,diacetate (**8**) (table 2).

Cytotoxicity assay

Brine shrimp lethality assay

Overall, high-polarity solvent (methanol) was very effective in extracting more antioxidant compounds so it exhibited cytotoxic activity against brine shrimp larva within IC_{50} value of 15µg/ml (fig. 1), while the volatile oil showed cytotoxic activity of 33µg/ml (fig. 2).

In recent years, substantial attention has been targeted on characteristic present in naturally occurring substances capable of inhibiting, retarding, or reversing the method of period of time carcinogenesis. In the present study, we have a tendency to examined whether or not the essential oil of *C. erectus* has any potential in cancer chemoprevention using HepG2 human liver cells. A modified MTT assays is used to measure the cytoxicity. The essential oil displayed good sensible cytotoxic action towards the human tumor cell line. The cytotoxic effects of *C. erectus* oil were tested using HepG2 cells. Shows the cytotoxic values against tumor cell lines treated with totally different concentrations of *C. erectus* essential oil.

The results showed that the IC_{50} value for tumor cell lines treated at a concentration of 8.7µg/ml (fig. 3). According to the American Cancer Institute, and National Cancer Institute (NCI) the standards of cytotoxic activity for the crude extract is an IC_{50}< 20 µg/ml (22). Thus *C. erectus* essential oil showed IC_{50} fall among the (NCI) criteria, therefore this extract thought about as a promising anticancer potential.

Thus, the antioxidant constituents of *C. erectus* could be chargeable for its antitumor activity; it can be play indirect part as cancer interference by protects cells from the damage caused by free radicals.

The general meaning of Cancer chemo is by using either chemical or dietary component to inhibit, block or reverse the development of cancer in normal or pre-neoplastic tissues. An outsized range of potential chemo-preventive agents are known, and that they operate by mechanisms directed in the least major stages of carcinogenesis. Essential oil constituents have an awfully totally different mode of action in microorganism (bacterial) and eukaryotic cells. For microorganism cells, they're having strong robust antiseptic properties, whereas in eukaryotes they modify programmed cell death (apoptosis) and

differentiation, interfere with the posttranslational modification of cellular proteins, and inhibit or induce some hepatic detoxifying enzymes. Therefore, essential oils might induce terribly totally different effects in prokaryotes and eukaryotes. Based on this study, *C. erectus* essential oil is reported to be cytotoxic. Some reports support the link between toxicity and inhibitor (antioxidant activity). In spite of the restrictions of all *in vitro* studies with relevancy in vivo impact, the current results measure terribly promising as way as anti-neoplastic chemotherapy is concerned. This additional form a firm basis for future research. Although all *in vitro* experiments hold limitations with relation to potential *in vivo* effectiveness, the results of this study are attention relating to the potential antineoplastic chemotherapy and kind a basis for future research. Even supposing this essential oil may not be ideal for the treatment of human cancers, the oil tested actually deserves further investigation.

When the body is below a particular pressure, it will turn out excess free radicals (23). However, excess production of free radical can exacerbate oxidative injury to proteins, lipids and DNA and even cause to cell death (24, 25).

The higher susceptibility of the tested Gram-positive bacteria than Gram-negative bacteria to *C. erectus* extracts is consistent with previous studies on the antibacterial activity of natural products (26-28).

Most of those compounds are familiar to exhibit numerous pharmacological activities. Essential oil influenced the recovery of phytocompounds and also the highest pharmacological activities of the volatile oil may be related to the presence of further bioactive compounds. According to a previous report, decanoic acid (capric acid) relieved inflammatory protein production (TNF-α and IL-6) and connected gene expression (*NF-ϰB*, *TNF-α*, *IFN-γ*), eased oxidative stress (GSSG/GSH ratio, H_2O_2, and malondialdehyde), and increased oxidative stress-related gene expression (*SOD1* and *GCLC*) in cyclophosphamide-treated IPEC-J2 cells (29). Capric acid exerts bactericidal and anti-inflammatory activities against *P. acnes*. The anti-inflammatory drug result part could affect the inhibition of NF-kB activation and also the phosphorylation of MAP kinases (30).

The end result of bioactivity of hexa-tetracontane compounds with varied concentrations; 400mg/mL, 500 mg/mL, 600mg/mL with incubation periode of 1 – 7 days, represent that the diameter of the inhibition zone is at the height of the microorganism (bacterial) growth underneath the concentration of 400 mg/ml and incubation period of 7 days and the diameter of inhibition zone is 21,33 mm (metric linear) with great distinction compared to the opposite concentrations (31). Acoording to Martin-Moreno *et al,* 1994 says that, the consumption of oleate in olive oil has been related to a decreased risk of breast cancer (32). The isovelleral represent a cytotoxic effect against ECA and L cells were in range 2µg/ml, additionally it showed 100% hemolysis of bovine erythrocyte at a concentration of 100 µg/ml (33).

However, as present below for isovelleral, the presence of each organic compound (aldehyde groups) seems to be important for the mutagenic agent activity of unsaturated dialdehydes within the Salmonella/microsome assay, The chance to correlate any biological activity of unsaturated dialdehydes with as an example structural parameters like distances and angles between the organic compounds (aldehyde groups), and chemical properties like lipophilicity, could cause the invention of fascinating structure-activity relationships for these biologically fascinating compounds (34). A compound shows an exhibited cytotoxicity against the human hepatoma cell line Bel-7402 is called Peragnan compounds, with IC_{50} values of 9.33, 11.02 and 18.68 µM, severally. In addition, one amongst them exhibited promising antibacterial drug activity against *Pseudomona puido*, with a MIC value of 31 nM, which is approximately 5-fold less assailable than antibiotic (MIC = 156 nM) (35). Nevertheless, additional research analysis efforts are needed to isolate, characterize, and measure these compounds from *C. erectus* leaves to validate their numerous pharmacological importance.

Conclusion

Even though essential oils are most typically utilized in our daily life as fragrance and flavoring materials, and are major elements in fruits, vegetables, beans and alternative plant food products, the particular

role within the maintenance of our health continues to be below investigation. A major issue within the study of the biologically active essential oils is that there markable biological activity usually doesn't depend upon just one active compound. Additionally, the essential oils are typically mixtures of structurally terribly similar compounds and, therefore, the biological activity of essential oils cannot essentially be explained with a straightforward, single molecule - single activity approach. From the antecedently mentioned results, it may be concluded that the *C. erectus* essential oil could also be exploited as a health-promoting agent that can handily notice its applicable therapeutic applications. The results of this study deserve attention to attainable anti-neoplastic chemotherapy and type a basis for future analysis. Thus, this plant will function as a replacement natural supply for getting several therapeutically numerous metabolites against various diseases.

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References

1. Siegel RL, Miller KD, Jemal A (2018). Cancer Statistics, Surveillance and Health Services Research, American Cancer Society.
2. Murthy HN, Lee E, Paek K (2014). Production of secondary metabolites from cell and organ cultures: strategies and approaches for biomass improvement and metabolite accumulationin chloroplasts and their functions. Faculty of Life Science and Biotechnology. 141: 391-396.
3. Hamed MM, Mohamed MA, Ibrahim MT (2015). Phytochemical investigation and cytotoxic characterization of bioactive constituents from *Conyza dioscoridis*. International Journal of Pharmacognosy and Phytochemical Research. 7(5): 948-955.
4. Hamed MM, Mohamed MA, Ibrahim MT (2016). Cytotoxic activity assesment of secondary metabolites from *Tecomaria capensis* v. *aurea*. International Journal of Pharmacognosy Phytochemical Research. 8(7): 1173-1182.
5. Hassanein H , El-Ahwany E, Salah F, Hammam O, Refai L, Hamed M (2010). Extracts of five medicinal herbs induced cytotoxicity in both hepatoma and myloma cell lines. Cancer Sciences Therapy. 3(10): 239-243.
6. Nagarajan M, Rajasekaran S, Ganesh SK (2013). Antibacterial activity of *Lawsonia inermis* L. International Journal of Modern Biology Medicine. 4: 169-175.
7. Abdel-Gwad MM, Anwar F, Refahy LA, Hamed MM, El-Amin SM. (2000a). Structure elucidation of the separated contents of *Datura innoxia* having molluscicidal activity. Egypt Journal of Biomedical Science. 6:150-158.
8. Abdel-Gwad MM, Anwar F, Refahy LA, Hamed MM, El-Amin SM (2000b). Separation and identification of triterpenoid saponins having molluscicidal activity from *Pittosporum tobira*. Journal of Drug Research Egypt. 23: 1-10.
9. Bashir M, Uzair M, Chaudhry BA (2015). A review of phytochemical and biological studies on *Conocarpus erectus* (Combretaceae). Pakistan journal of pharmaceutical research. 1(1): 1-8.
10. Hussein RA (2016). Evaluation antioxidant and antibacterial activities of n-butanol fraction of *Conocarpus erectus* L. leaves extract. International Journal of Pharmaceutical and Medical Research. 4(6): 394-400.
11. Bisset NG. Herbal drugs and phytopharmaceuticals – A handbook for Practice on a Scientific Bases**.** London/Stuttgart, Medpharm/CRC Press (1994).
12. Hamed MM (2007). Volatile oils from *Pelargonium zonale* and its cytotoxic effect on tumor cells. Bulletin Faculty of Pharmacy Cairo University. 45(3): 297-300.

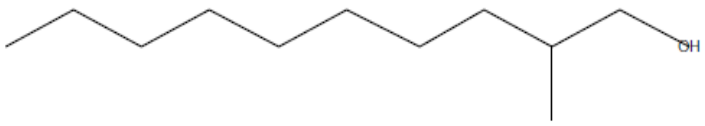
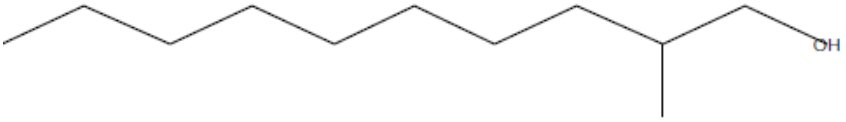
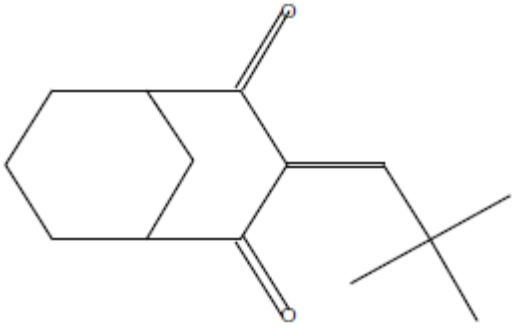
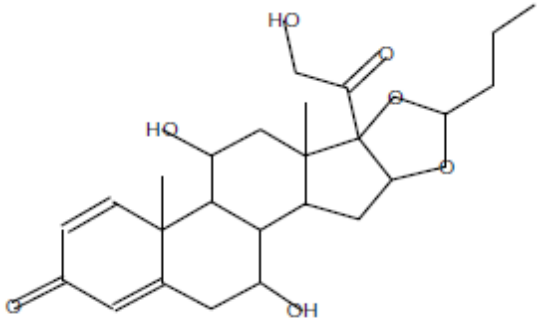
13. Shmidt J (1964). "Organic Chemistry" Oliver and Bayed, Edinburgh and London, 8th Ed. 318-673.
14. Harbone JB (1973). Phytochemical Methods: A Guide to Modern Technique of Plant Analysis, 2nd ed. Chapman and Hall: New York, NY.
15. Sofowora A (1993). Medicinal Plants and Traditional Medicinal in Africa. 2nd Ed. Sunshine House, Ibadan, Nigeria: Spectrum Books Ltd; Screening Plants for Bioactive Agents. 134-156.
16. Shellard EJ (1957). "Practical Plant Chemistry" Pitman Medicinal Publishing Co., LTD, London. 53: 54.
17. Gonzalez EE, Delgado JN (1962). Phytochemical investigation of *Amphipterygium adstringens*. Journal of Pharmaceutical Sciences. 51: 786-790.
18. Marby TT, Markhan KR, Thomas MB. The systematic identification of flavonoids. Springer Verlag, New York. 1970; 46-54.
19. Meyer BN, Ferrign RN, Putnam JE, Jacobson LB, Nicholas DE, McLaughlin JL (1982). Brine shrimp: a convenient general bioassay for active plant constituents. Planta Medica. 45: 31-34.
20. Ipsen J, Feigi P. Bancroft's Introduction to Biostatistics, 2nd ed., Harper & Row, New York, Chap. 1970; 15.
21. Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunology Methods. 65: 55-63.
22. Boik J (2001). Natural compounds in cancer therapy. Oregon Medical Press, Minnesota, USA.
23. Stohs SJ (2011). The role of free radicals in toxicity and disease. Journal of basic and clinical physiology and pharmacology. 6(3-4): 205-228.
24. Selvi MT, Thirugnanasampandan R, Sundarammal S (2012). Antioxidant and cytotoxic activities of essential oil of *Ocimum canum* Sims. from India. Journal of Saudi Chemical Society online.
25. Maridonneau PI, Harpey C (2012). Effect of trimetazidine on membrane damage induced by oxygen free radicals in human red cells. British Journal of Clinical Pharmacology. 20(2): 148-151.
26. Ceylan E, Fung DYC (2004). Antimicrobial activity of spices. Journal of Rapid Method Automat Microbiology. 12: 1-55.
27. Lopez P, Sanchez C, Batlle R, Nerin C (2005). Solid and vapor-phase-antimicrobial activities of six essential oils: susceptibility of selected food-borne bacterial and fungal strains. Journal of Agriculture and Food Chemistry. 53: 6939-6946.
28. Smith-Palmer A, Stewart J, Fyfe L (1998). Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. Letters in Applied Microbiology. 26: 118–122.
29. Lee SI, Kang KS (2017). Function of capric acid in cyclophosphamide-induced intestinal inflammation, oxidative stress, and barrier function in pigs. Scientific Reports. 7: 16530.
30. Huang W, Tsai T, Chuang L, Li Y, Zouboulis CC, Tsai P (2014). Anti-bacterial and anti-inflammatory properties of capric acid against *Propionibacterium acnes*: A comparative study with lauric acid. Journal of Dermatological Science. 73 232–240.
31. Nurdin M, Usman (2017). Bioactivity test of compound (hexa-tetra contana) from sponge (*Callyspongia pseudoreticulata*) as antibacterial of withered disease on potato plant (*Ralstonia solanacearum*). International Journal of Pharma Bio Sciences. 8(4): 183-186.
32. Martin-Moreno JM, Willett WC, Gorgojo L, Banegas JR, Rodriguez-Artalejo F, Fernandez-Rodriguez JC, Maisonneuve P, Boyle P (1994). "Dietary fat, olive oil intake and breast cancer risk". International Journal of Cancer. 58(6): 774-780.
33. Anke H, Sterner O (1991). Planta Medica. 57: 344.
34. Sterner O, Carter RE, Nilsson LM (1987). Structure-activity relationships for unsaturated dialdehydes 1. The mutagenic activity of 18 compounds in the Salmonella/microsome assay. Mutation Research. 188: 169-174.

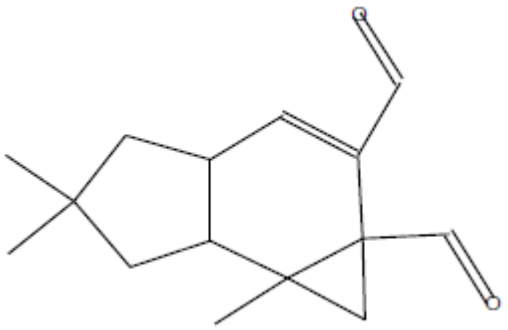
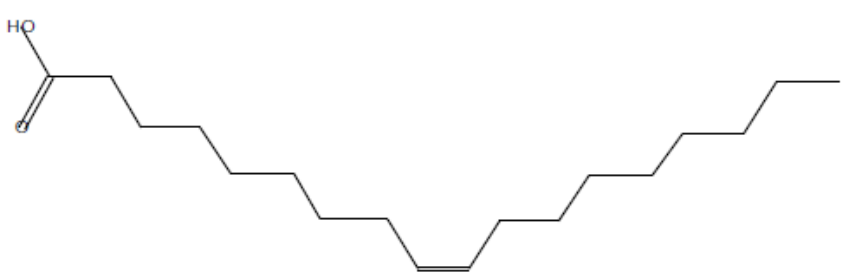
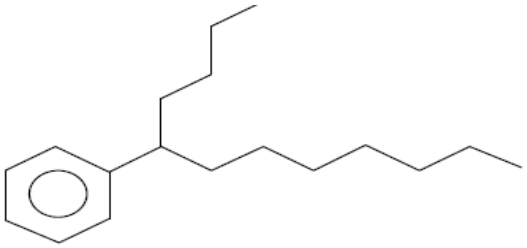
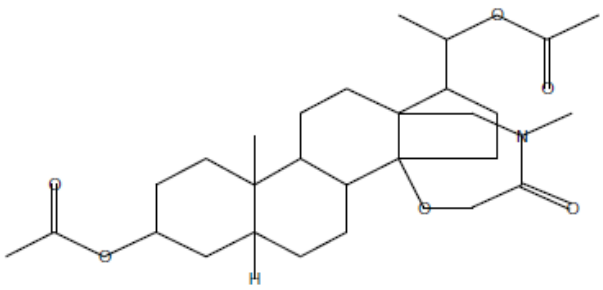
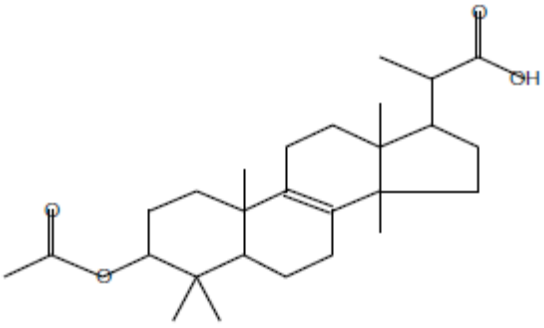
35. Zhao H, Shao C, Li Z, Han L, Cao F, Wang C (2013). Bioactive pregnane steroids from a South China sea Gorgonian *Carijoa* sp. Molecules. 18(3): 3458-3466.

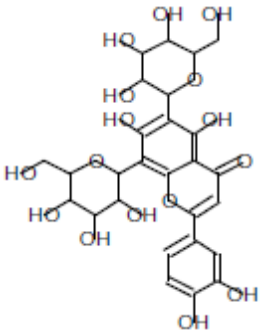
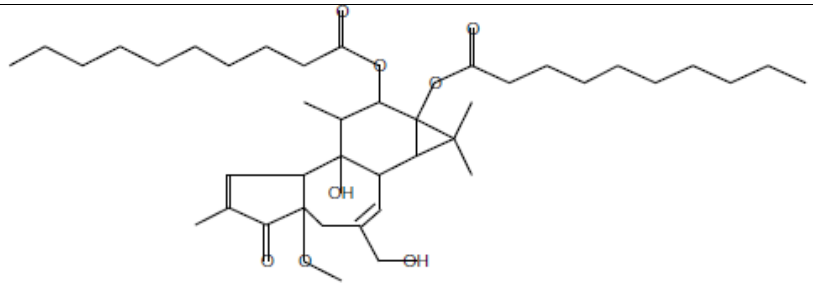
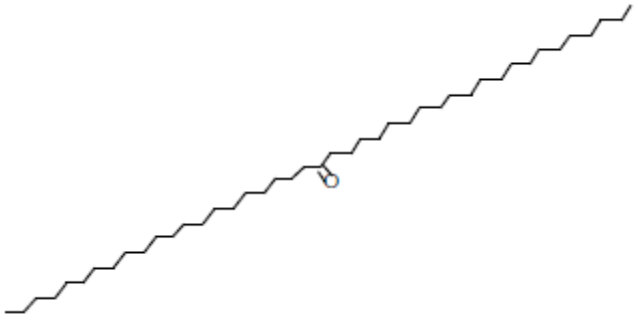
Table 2: Results of GC/MS analyses of *Conocarpus erectus* leaves oil.

No.	Identified Compounds	M.F.	R_t	Area %
1	1-Decanol, 2-methyl-	$C_{11}H_{24}O$	22.59	3.50
2	1-Octanol, 2-butyl-	$C_{12}H_{26}O$	25.69	5.51
3	3-(2,2 dimethylpropylid-ene)bicyclo[3.3.1]nonane-2,4-dione	$C_{14}H_{20}O_2$	34.41	67.12
4	(22R)-6á,11á,21-Trihydr-oxy-16à,17à propylmeth-ylenedioxypregna-1,4-diene-3,20-dione	$C_{25}H_{34}O_7$	35.75	1.02
5	Iso-velleral	$C_{15}H_{20}O_2$	37.27	0.69
6	Oleic acid	$C_{18}H_{34}O_2$	38.81	4.33
7	Benzene, (1-butyloctyl)-	$C_{18}H_{30}$	39.47	0.61
8	(5á)Pregnane-3,20á-diol, 14à,18à-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-,diacetate	$C_{28}H_{43}NO_6$	48.48	0.30
9	Propanoic acid, 2-(3-acetoxy-4,4,14 trimethyla ndrost-8-en-17-yl)-	$C_{27}H_{42}O_4$	51.85	0.11
10	Lucenin 2	$C_{27}H_{30}O_{16}$	53.74	0.54
11	Decanoic acid, 1,1a,1b,4,4a,5,7a,7b,8,9-decahydro-4a,7b-dihydroxy-1,1,6,8-t etramethyl-5-oxo-3-[[(1-oxodecyl)oxy]methyl]-9aH-cyclopropa [3,4]benz[1,2-e]azulene-9,9a-diyl ester,[1aR-(1aà,1bá,4aá,7aà,7bà,8à,9á,9 aà)]-	$C_{50}H_{82}O_9$	58.11	7.77
12	22-Tritetracontanone	$C_{43}H_{86}O$	58.16	6.03
Total %				97.53%

Table 3: Structures of essential oil compounds from *C. erectus*.

Structure	Number
	1
	2
	3
	4

	5
	6
	7
	8
	9

	10
	11
	12

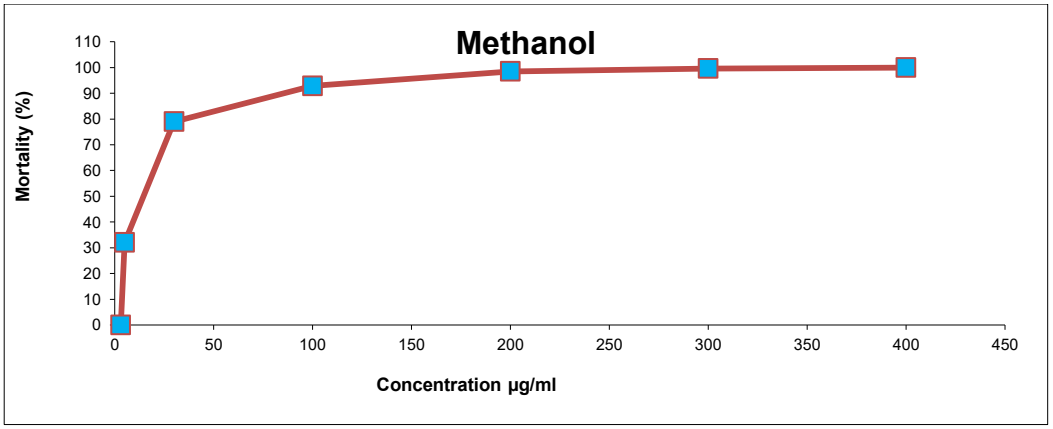


Fig. 1: Brine shrimp bioactivity assay of *C. erectus* methanol extract.

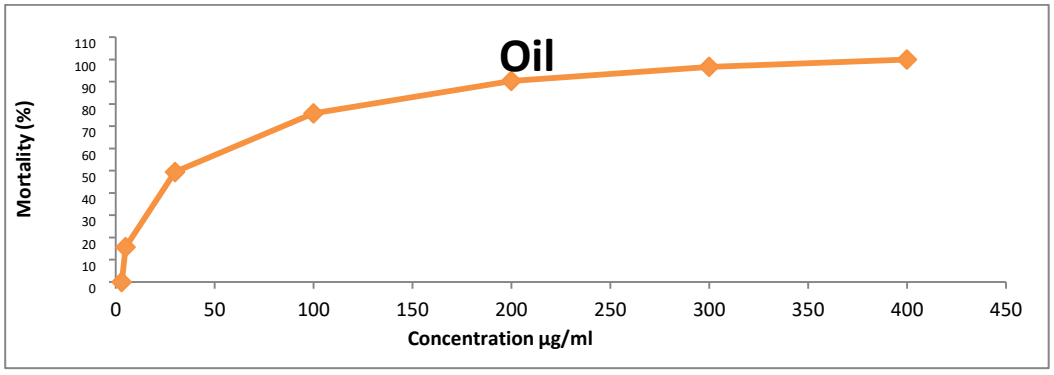


Fig. 2: Brine shrimp bioactivity assay of *C. erectus* volatile oil.

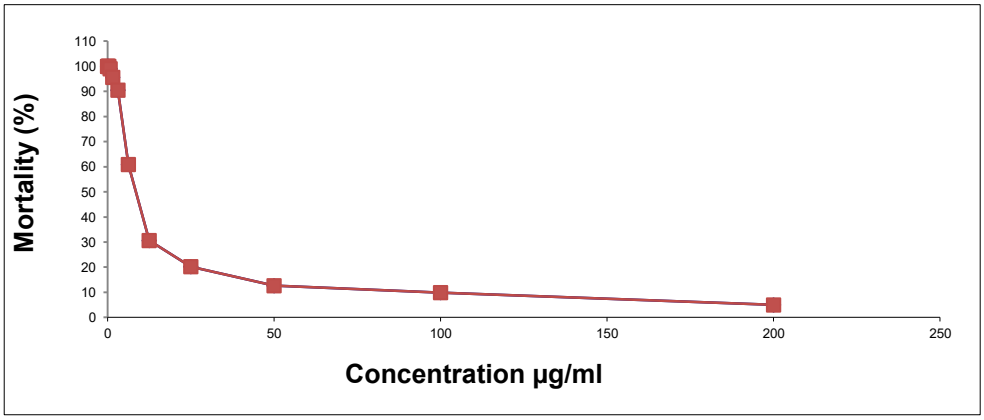


Fig. 3: The cytotoxic activity of *C. erectus* against HepG2 liver cancer cells.