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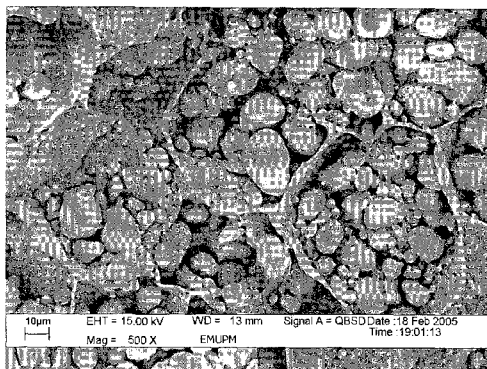


FIG. 1

(57) Abstract: The present invention relates to *Cola nitida*, an anticancer agent, the anticancer properties include antioxidant activities: FTC (31.61 ± 0.01 %), TBA (58.85 ± 0.11 %) and DPPH (78.21 ± 0.11 %); antioxidant minerals: oxygen (49.79 ± 0.36 %), carbon (47.56 ± 0.33 %), potassium (1.93 ± 0.15 %), phosphorus (0.43 ± 0.08 %) and magnesium (0.27 ± 0.05 %); total phenolic (68.41 ± 0.01 mg GAE/g sample) and total flavonoid (30.04 ± 0.04 mg RE/g sample) content.



COLA NITIDA (COLA NUT) AS AN ANTICANCER AGENT

FIELD OF INVENTION

The present invention relates to an anticancer agent. More particularly, the present invention relates to an anticancer agent which is *Cola nitida* (Cola nuts).

5 BACKGROUND OF INVENTION

Cancer is a group of disease which is characterized by uncontrolled cellular growth with local tissue invasion and or systematic metastasis. The number of new cancer cases has been increasing over the past nine decades. It is the largest single cause of death in both men and women, claiming over 6 million lives each year in the world. Cancer is the most
10 feared of all diseases. Unlike other diseases, cancer usually causes a slow death involving pain, suffering, mental anguish and a feeling of hopelessness.

In the year 2000, it has been estimated that there were over 10 million new cases, 6.2 million deaths and 22.4 million persons living with cancer. In terms of incidence, the most common causes are lung (12.3%), breast (10.4%) and stomach (8.7%). By 2020, the
15 World Health Organization estimated that 20 million new cases of cancer will be diagnosed each year.

Overall, 26089 cancer cases had been diagnosed among Malaysian people in Peninsular Malaysia in the year of 2002. From this amount, 11, 815 were men while the other 14, 274 were women. It was estimated that 10659 cases had not been registered. One in 5.5
20 Malaysian people were in the risk to get cancer in their lifetime. Nevertheless, if it was observed from the unregistered cases, the risk may be increased to 1 in 4 people. Cancer

is the major cause of death in Government Hospitals, which is 2.8 times higher than that of the heart diseases (16%).

There are various steps that have to occur before a tumor is formed. The process starts with an insult to the genetic cellular material (genotoxic events; known as initiation) and
5 then proceeds to the production of abnormal DNA (nongenotoxic events; known as promotion). The transformation of the altered cells may lead to the proliferation of cells with invasive (malignant) or noninvasive (benign) qualities. From a confined region of tissue, cancer spread to other tissue to develop secondary tumors or metastases and result in an ultimate cascading effect of the tumor cells. The process of carcinogenesis is
10 driven by multiple interactive factors. The external factors include chemicals, radiation and viruses, whereas the internal factors are hormones, immune condition and inherited mutation. Therefore, every one of us is at risk of developing cancer no matter at what age one is.

The most common causes of death due to cancer are cancers of the lung (17.8%),
15 stomach (10.4%) and liver (8.8%). In the present invention, *Cola nitida* had been screened to be effective towards liver cancer. Thus, the present invention is focused on the effect of *Cola nitida* on hepatocellular carcinoma.

Tumors of the liver are classified as being either primary (originating from the liver) or metastatic (spread from another body organ to the liver). Primary liver tumors may be
20 divided into those that are benign (not cancerous and remain in the liver) or malignant (cancerous and may be spread beyond the liver).

In the recent years, there has been growing interest in alternative therapies and therapeutic use of natural products, especially from plant derivative and in trend towards the use of natural substances which is also believed to have potential value as cancer chemopreventive or therapeutic agents.

- 5 The use of natural products together with their therapeutic properties is as ancient as human civilisation and, for along time, mineral, plant and animal products were the main source of drugs. However, the potential use of plants as a source of new drugs is still poorly explored. Of the estimated 250000 to 500000 plant species, only a small percentage has been investigated phytochemically and an even smaller percentage has
10 been properly studied in terms of their pharmacological properties.

Traditional medicine is well known for its nutritional value, as well as its ability to cure various ailments. In recent years various constituents have been found to provide protection against any disease including cancer. Any significant role by dietary intervention is encouraging and emerging as an acceptable approach for controlling the
15 cancer incidence worldwide.

Cola nitida is an astringent, bitter-sweet, anti-depressant herb that has a stimulating effect, especially on the heart. It contains alkaloid caffeine and phenolics, such as catechin, epicatechin and procyanidins. The *Cola nitida* tree is native to West Africa. It has been naturalized to South America, Central America, the West Indies, Sri Lanka, and
20 Malaysia. Related to cocoa, *Cola nitida* contains the methylxanthine alkaloids that also occur in coffee, cocoa, and tea. West Africans have been chewing *Cola nitida* for thousands of years. Its stimulant effects are the predominant application in the United States and Europe. In Africa, however, *Cola nitida* has been used as an appetite and thirst

suppressant, enabling soldiers who chew them to travel long distances without much food. Cola twigs, with an extremely bitter taste, are used to clean the teeth and gums. Cola maintains a strong cultural significance in West Africa, partly due to the fact that cola is a valuable commodity. It has been traded to other countries since at least the fourteenth century and it is used particularly by Islamic people, who, according to their religion, cannot drink alcohol, but desire a "social lubricant". Today, *Cola nitida* is exported worldwide. *Cola nitida* is also used in non-pharmaceutical preparations, including (at least formerly) cola-based beverages such as Coca Cola. It is on the GRAS (generally recognized as safe) list for food additives in the United States.

SUMMARY OF THE INVENTION

Accordingly, *Cola nitida* is an anticancer agent provided from its anticancer properties inclusive of antioxidant activities (FTC-TBA, DPPH), antioxidant minerals (oxygen, carbon, potassium, phosphorus and magnesium), total phenolic and total flavonoid content.

- 5 The present invention consists of several novel features and a combination of parts hereinafter fully described and illustrated in the accompanying description and drawings, it being understood that various changes in the details may be made without departing from the scope of the invention or sacrificing any of the advantages of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will be fully understood from the detailed description given herein below and the accompanying drawings which are given by way of illustration only, and thus are not limitative of the present invention, wherein:

- 5 FIG. 1 shows scanning electroscope (SEM) image of unprocessed (fresh) *Cola nitida* seed.

FIG. 2 shows cytotoxicity effect of *Cola nitida* on MCF-7, HepG-2 and Chang cell lines.

- FIG. 3(a) to (c) show flowcytometry analysis on effect of *Cola nitida* on HepG-2 hepatocellular carcinoma cell lines. Higher (20 µg/ml) and lower (10 µg/ml) concentrations
10 were compared with control to quantify the apoptotic cell. C illustrated the quantity of apoptotic cells.

- FIG. 4 shows body weight profile of rats for the 8-week of bioassay; N: Normal control, NCn: Normal + *Cola nitida*, C: Cancer control, CCn: Cancer + *Cola nitida* and CG: Cancer + Glycyrrhizin. *Carcinogens induced only to C, CCn and CG groups. Significance;
15 N^{abcde} , NCn^{abcde} , C^{abcd} , CCn^{abcde} and CG^{abcde} ; values with different superscript were significantly different at $p < 0.05$.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The present invention relates to an anticancer agent. More particularly, the present invention relates to an anticancer agent which is *Cola nitida*. Hereinafter, this specification will describe the present invention according to the preferred embodiments of the present invention. However, it is to be understood that limiting the description to the preferred
5 embodiments of the invention is merely to facilitate discussion of the present invention and it is envisioned that those skilled in the art may devise various modifications and equivalents without departing from the scope of the appended claims.

Plants are an important source of essential nutrients and beneficial components that are
10 crucial for healthy living. Knowledge about the beneficial effects of certain plants is mainly transmitted by personal communication; therefore, it remains unknown to the general population. Many studies need to be done to promote the usage and application of native plants. The phytochemical or chemical composition of plants can be characterized as major and minor constituents, depending on their general abundance in the plants. Major
15 constituents include carbohydrates lipids or fats, and proteins which are present at gram level in each 100 g portion of sample. Minor constituents include vitamins, secondary metabolites and minerals. When present, these constituents generally are found in the microgram to milligram range per 100 gram sample. Antioxidant vitamins, including polyphenol, pigments and flavonoids possess antimutagenic properties as well as blood
20 glucose decreasing activities. In the present invention, the antioxidant minerals such as calcium (Ca), magnesium (Mg), zinc (Zn) and copper (Cu) were determined by using atomic absorption spectrophotometer (GBC, Model 908 AA, USA) taken from the ash solution of *Cola nitida*. The surface morphological structure and biological composition of

Cola nitida was viewed and investigated under a variable pressure scanning electron microscope (VPSEM) equipped with energy dispersive x-ray (EDX) microanalysis (VPSEM model LEO VP-1455). VPSEM enables unprocessed samples to be viewed and examined. In the present invention, point and identification (ID) was used which enabled specific part of the sample to be viewed and the minerals content identified. Six replicates of unprocessed (fresh) *Cola nitida* seed were observed for their surface morphological structure under scanning electron microscope (SEM). The seed had to be cut into a number of 1 cm³ slice and was stucked onto the stub. Samples were then viewed under the VPSEM at 500x magnification. Together with the image, elemental analysis using EDX was carried out by using scanning electron microscope (LEO 1455 Variable Pressure-EDX). Numerous crystals can be seen distributed in clusters within the cell wall of *Cola nitida*. The crystals were variable in shape and size with diameter ranging from 2 to 10 µm. The crystals were densely packed with limited space in between them (FIG. 1). The elemental analysis results (Table 1) showed that the sample consisted of many important minerals like oxygen ($49.79 \pm 0.36 \%$), carbon ($47.56 \pm 0.33 \%$), potassium ($1.93 \pm 0.15 \%$), phosphorus ($0.43 \pm 0.08 \%$) and magnesium ($0.27 \pm 0.05 \%$).

Sample	Symbol	Weight (%)
Oxygen	O	49.79 $\pm$ 0.363
Carbon	C	47.56 $\pm$ 0.332
Magnesium	Mg	0.27 $\pm$ 0.054
Potassium	K	1.93 $\pm$ 0.145
Phosphorus	P	0.43 $\pm$ 0.080

Table 1. Quantitative elemental analysis by using EDX-VPSEM

Potassium is important in regulating the transfer of nutrients to the cells, phosphorus plays a role in metabolic reaction in the body, while magnesium plays a significant role in photosynthesis, carbohydrate metabolism, nucleic acids and binding agents of cell walls.

- 5 The antioxidant properties of *Cola nitida* were evaluated by using Ferric thiocyanate (FTC)-Thiobarbituric acid (TBA) method, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method, total phenolic and total flavonoid content. FTC evaluates amount of peroxides at the initial stage of lipid peroxidation while TBA shows the amount of peroxides at the final stage of lipid peroxidation. Results of ANOVA analysis indicated that
- 10 antioxidant activity of *Cola nitida* was significantly higher ($p < 0.05$) than positive control, vitamin E. From the FTC method, the total antioxidant activity was evaluated to be 31.61 ± 0.01 % at the concentration of 1 mg/ml, compared to vitamin E (28.46 ± 0.11 %). While from TBA method, *Cola nitida* exhibited 58.85 ± 0.11 % antioxidant activity compared to standard; α -tocopherol (58.10 ± 0.69 %). However, the results were not significantly
- 15 different ($p > 0.05$). The DPPH radical scavenging activity of *Cola nitida* methanolic extract was evaluated to be 78.21 ± 0.11 % at the concentration of 4.96 mg/ml (Table 2).

Antioxidant assays	Percentage of activities (%)	
	Cola nut	Vitamin E (standard)
FTC	31.61 ± 0.01	28.46 ± 0.11
TBA	58.85 ± 0.11	58.10 ± 0.69
5 DPPH	78.21 ± 0.11	91.47 ± 2.24

Table 2. Total activities of cola nut with different assays. Results were compared with standard (vitamin E). Values are expressed as mean ± standard deviation of six replicate measurements.

The total phenolic content of *Cola nitida* was expressed as gallic acid equivalents (GAE) which was 68.41 ± 0.01 mg GAE/g sample. While the total flavonoid content of *Cola nitida* methanolic extract which was expressed as rutin equivalents (RE) was detected to be 30.04 ± 0.04 mg RE/g sample (Table 3).

Sample	Total phenolic content	Total flavonoid content
	(mg GAEs/g sample)	(mg REs/g sample)
15 <i>Cola nitida</i>	68.4±0.01	30.04±0.04

Table 3. Values are expressed as mean ± standard deviation of six replicate measurements.

The approach to the discovery of new anticancer drugs has recently evolved from a reliance on empiric cell-based screening for cytotoxic testing, *in vitro* including the specific molecular lesion thought to be responsible for the development and maintenance of the malignant phenotype in various form of cancer. Current legislation demands that source of drugs such as herbal medicine; food additives or drugs go through extensive cytotoxic testing before they are released to the consumer. Cytotoxic activities of *Cola nitida* were

studied by using MTT (3, 4, 5, dimethylthiazol-2, 5 diphenyl tetrazolium bromide) assay. Micro-culture Tetrazolium Salt (MTT) assay was used in the present invention to measure the amount of cell viability. Cells, grown in a 96 wells tissue culture plate, were incubated with the yellow MTT solution for approximate 4 hours. After this incubation period, purple
5 formazan salts crystals were formed. These salt crystals are insoluble in aqueous solution, but may be solubilized by adding the solubilization solution and incubating the plates overnight in humidified atmosphere (37°C, 5% CO₂). The solubilized formazan product is spectrophotometrically quantified using an ELISA reader. An increase in number of living cells results in an increase in the total metabolic activity in *Cola nitida*
10 sample. This increase directly correlated to the amount of purple formazan crystals formed, as monitored by the absorbance. Percentage of cell viability was measured by comparing the optical density (OD) against the control. The antiproliferative activities are presented as percentage of cell viability versus concentration. Percentage of cell viability could be seen decreasing with the increasing of sample's concentrations (FIG. 2). *Cola*
15 *nitida* was found to inhibit 50% cell growth of HepG-2 (human hepatocellular carcinoma cell line) at the concentration of $8.01 \pm 0.23 \mu\text{g/ml}$ and $78.00 \pm 0.11 \mu\text{g/ml}$ on MCF-7 (human mammary carcinoma cell line. No effect was detected on Chang normal cell line. *Cola nitida* seed possessed the strongest IC₅₀ value on HepG-2 liver cancer cell lines and the mechanism of the cytotoxic effect was studied through apoptosis pathway using flow
20 cytometry analysis. The elimination of tumor cells by the induction of apoptosis has become an important and a new technique and approach in cancer therapy. From flowcytometry analysis (FIG. 3), *Cola nitida* was shown to cause apoptosis in HepG-2. The result showed that the quantity of apoptotic cells was higher in the cancer cells treated with *Cola nitida* compared to control. Thus, it can be concluded that the

antioxidant properties inclusive of the useful minerals of *Cola nitida* might be one of the mechanisms of cytotoxic properties on HepG-2 and MCF-7 cell lines. Further investigation had been carried out to study the anticancer effect of *Cola nitida* extract towards neoplastic hepatocytes *in vivo*. This study was based on the hepatocarcinogenesis model of Solt & Farber (1976) for the duration of 11 weeks. Diethylnitrosamine (DEN) and acetylaminofluorene (AAF) were used as the initiator and promoter, respectively, but without partial hepatectomy. Water extract of *Cola nitida* was used *in vivo* to create natural conditions as consumed by human. Male Sprague Dawley rats were used to investigate the anticancer properties of *Cola nitida*. As the liver was the main organ that was affected by carcinogens such as DEN and AAF, this model was appropriate as a liver cancer model. The severity of hepatocarcinogenesis by DEN/AAF with and without *Cola nitida* was confirmed by evaluating the relative body and liver weight as well as the morphological changes from the gross results. However, the severity of hepatocarcinogenesis will be further confirmed from the results of histological changes with hematoxylin and eosin (H&E) stained slides, liver enzyme activity (microsomal gamma-glutamyl transpeptidase; GGT, cytosol glutathione s-transferase; GST), blood alpha feto protein (AFP) concentration and AFP gene expression in tissue by reverse transcription-polymerase chain reaction (RT-PCR) that are still in process. Changes of body weight of rats during 8 weeks of bioassay are shown in FIG. 4. It shows the effect of *Cola nitida* on weight gains of rats during hepatocarcinogenesis. With the exception of DEN/AAF rats, body weight of the rats increased as the time of experiment increased. Body weights of carcinogenesis induced rats increased more slowly compared to non-cancer rats. The body weights of DEN/AAF induced rats decreased after the injection of DEN. After the supplementation of 0.02% AAF, the body weight gain in DEN/AAF induced

rats was very small compared to the non-cancerous rats. Rats' starvation at termination of bioassay caused a decrease in body weight in all groups. Table 3 evaluates the final body weight and liver weight as well as the relative liver weight profile of the rats.

Cancer rats (C) and cancer rats supplemented with *Cola nitida* (CCn) had significantly
5 higher ($p < 0.05$) ratio of liver weight to body weight compared to the normal (N), normal
rats supplemented with *Cola nitida* (NCn) and cancer rats supplemented with control drug;
glycyrrhizin (CG). There was no significant different of body weight profile of rats in
different groups except for the DEN/AAF or cancer (C) group. Body weight profile of rats
might also be affected by other factors such as age, environment, genetic, health level
10 and diet changes associated with food consumption. The decreased in body weight might
be due to low food consumption by the DEN/AAF treated rats at the neoplastic stage.
There were significant differences ($p < 0.05$) of relative liver weight between the groups.
However, there was no significant different ($p > 0.05$) between normal rats (n) and cancer
rats supplemented with *Cola nitida* (CCn). There was also no significant different ($p > 0.05$)
15 among the normal rats supplemented with *Cola nitida* (NCn) and cancer rats
supplemented with control drug; glycyrrhizin (CG).

Sample weight	Final body weight (g)	Liver weight (g)	Relative liver
			(g/100g body weight)
Normal	363.20±25.23	10.00±0.00	2.75±0.00 ^c
5 Normal + <i>Cola nitida</i>	365.07±21.36	10.44±0.73	2.86±0.20 ^b
Cancer	330.80±6.83	9.67±0.58	2.93±0.17 ^a
Cancer + <i>Cola nitida</i>	335.54±32.31	9.25±0.71	2.76±0.21 ^c
Cancer + Glycyrrhizin	359.00±28.85	10.22±1.30	2.85±0.36 ^b

- 10 Table 3. Final body weight, liver weight and relative weight of rats. Values shown are mean ± standard deviation. Values of different superscript letters showing significant difference at p<0.05

The significant difference of relative liver weight between the normal (N) and cancer (C) rats indicated that combination of DEN and AAF caused severity of cancer incident in a short time even without the stimulating factor that is the partial hepatectomy. Hepatocarcinogenesis inhibits the regenerating of liver cells following partial hepatectomy, and at higher dose it may lead to liver cell death. Focal, slowly proliferating cell population, called hyperplastic nodules, occur in the liver before the appearance of cancer. Proliferation of cells will increase the size of the liver. Therefore, assessment of the liver weights to body weight ratio was used to investigate the change in the liver size. It was shown that the rats induced with DEN and AAF had higher liver weight to body weight ratios than that of the normal rats. In the present invention, the ratio of liver weight to body weight of the rats induced with DEN and AAF and given *Cola nitida* was significantly lower than the rats induced with DEN and AAF without *Cola nitida*. The results of this research showed effect of DEN/AAF on liver in the process of

hepatocarcinogenesis and that *Cola nitida* was able to improve or ameliorate its effect. From the gross results, livers of the rats induced with DEN/AAF exhibited pale reddish colour compared to the normal groups. Liver nodules appeared in the form of whitish nodules in liver rat induced with DEN/AAF without *Cola nitida* or cancer rat (C).

- 5 The present invention contributes to the new finding towards anticancer agent from *Cola nitida* contributing to the effort of treatment and suppressive of the cancer diseases. *Cola nitida* had been examined for its surface morphology, as well as the element content. For antioxidant activity, total antioxidant activity of methanol crude extract from *Cola nitida* was determined by using Ferric-thiocyanate (FTC)-Thiobarbituric acid (TBA) method. The
- 10 DPPH radical scavenging activity of *Cola nitida* methanolic extract was also evaluated, besides total phenolic and total flavonoid content. The cytotoxic activity was carried out through the growth of viable cell using MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay. *Cola nitida* seed possessed the strongest IC₅₀ value on HepG-2 liver cancer cell lines and was further assayed with flowcytometry analysis.
- 15 Further investigation had been carried out to investigate more beneficial medicinal properties of *Cola nitida*. Effect of *Cola nitida* on hepatocarcinogenic rats had been studied. From the gross results, differences could be seen in normal rat liver (N), normal rat liver treated with *Cola nitida* (NCn), cancer rat liver (C), cancer rat liver treated with *Cola nitida* (CCn) and cancer rat liver treated with control drug; glycyrrhizin (CG). Besides
- 20 that, the results for the body and liver weight profile exhibited differences as well.

Cola nitida had been examined for its surface morphology and also the element content. Numerous crystals can be seen distributed in clusters within the cell wall of *Cola nitida*. The crystals were variable in shape and size with diameter ranging from 2 to 10 µm. The

crystals were densely packed with limited space in between them. The elemental analysis results showed that the sample consisted of many important minerals like oxygen ($49.79 \pm 0.36 \%$), carbon ($47.56 \pm 0.33 \%$), potassium ($1.93 \pm 0.15 \%$), phosphorus ($0.43 \pm 0.08 \%$) and magnesium ($0.27 \pm 0.05 \%$).

- 5 For antioxidant activity, total antioxidant activity of methanol crude extract from *Cola nitida* was determined by using Ferric-thiocyanate (FTC)-Thiobarbituric acid (TBA) method. Results of ANOVA analysis indicated that antioxidant activity of *Cola nitida* was significantly higher ($p < 0.05$) than positive control, vitamin E. From the FTC method, the total antioxidant activity was evaluated to be $31.61 \pm 0.01 \%$ at the concentration of 1
- 10 mg/ml, compared to vitamin E ($28.46 \pm 0.11 \%$). While from TBA method, *Cola nitida* exhibited $58.85 \pm 0.11 \%$ antioxidant activity compared to standard; α -tocopherol ($58.10 \pm 0.69 \%$). The DPPH radical scavenging activity of *Cola nitida* methanolic extract was evaluated to be $78.21 \pm 0.11 \%$ at the concentration of 4.96 mg/ml. The total phenolic content of *Cola nitida* was expressed as gallic acid equivalents (GAE) which was $68.41 \pm$
- 15 0.01 mg GAE/g sample. While the total flavonoid content of *Cola nitida* methanolic extract which was expressed as rutin equivalents (RE) was detected to be 30.04 ± 0.04 mg RE/g sample. The cytotoxic activity was evaluated through the growth of viable cell using MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay. *Cola nitida* was found to inhibit 50% cell growth of HepG-2 (human hepatocellular carcinoma cell line) at
- 20 the concentration of $8.01 \pm 0.23 \mu\text{g/ml}$ and $78.00 \pm 0.11 \mu\text{g/ml}$ on MCF-7 (human mammary carcinoma cell line). No effect was detected on Chang normal cell line. *Cola nitida* seed possessed the strongest IC_{50} value on HepG-2 liver cancer cell lines and was further assayed with flowcytometry analysis.

From flowcytometry analysis, *Cola nitida* was shown to cause apoptosis in HepG-2. The result showed that the quantity of apoptotic cells was higher in the cancer cells treated with *Cola nitida* compared to control. Thus, it can be concluded that the antioxidant properties inclusive of the useful minerals of *Cola nitida* might be one of the mechanisms of cytotoxic properties on HepG-2 and MCF-7 cell lines. Further investigation on effect of *Cola nitida* on liver cancer was carried out through the hepatocarcinogenesis study. Results from the present invention indicates that *Cola nitida* may have anticancer properties against DEN-initiated and AAF-promoted hepatocarcinogenesis in rats.

CLAIMS

1. *Cola nitida* is an anticancer agent, the anticancer properties include antioxidant activities (FTC-TBA, DPPH), antioxidant minerals, total phenolic and total flavonoid content.
- 5 2. The anticancer properties according to claim 1, wherein the antioxidant activities are in the following values: FTC ($31.61 \pm 0.01 \%$), TBA ($58.85 \pm 0.11 \%$) and DPPH ($78.21 \pm 0.11 \%$).
3. The anticancer properties according to claim 1, therein the antioxidant minerals are present in the following ratio: oxygen ($49.79 \pm 0.36 \%$), carbon ($47.56 \pm 0.33 \%$),
10 potassium ($1.93 \pm 0.15 \%$), phosphorus ($0.43 \pm 0.08 \%$) and magnesium ($0.27 \pm 0.05 \%$).
4. The anticancer properties as claimed in claim 1, whereby the total phenolic and total flavonoid content are present in the following value: total phenolic ($68.41 \pm 0.01 \text{ mg GAE/g sample}$) and total flavonoid ($30.04 \pm 0.04 \text{ mg RE/g sample}$).
5. The anticancer agent as claimed in claim 1, wherein the cancer is liver cancer.
- 15 6. A use of an effective amount of *Cola nitida* in the manufacture of a medicament for preventing or treating cancer, particularly liver cancer in a patient in need thereof.

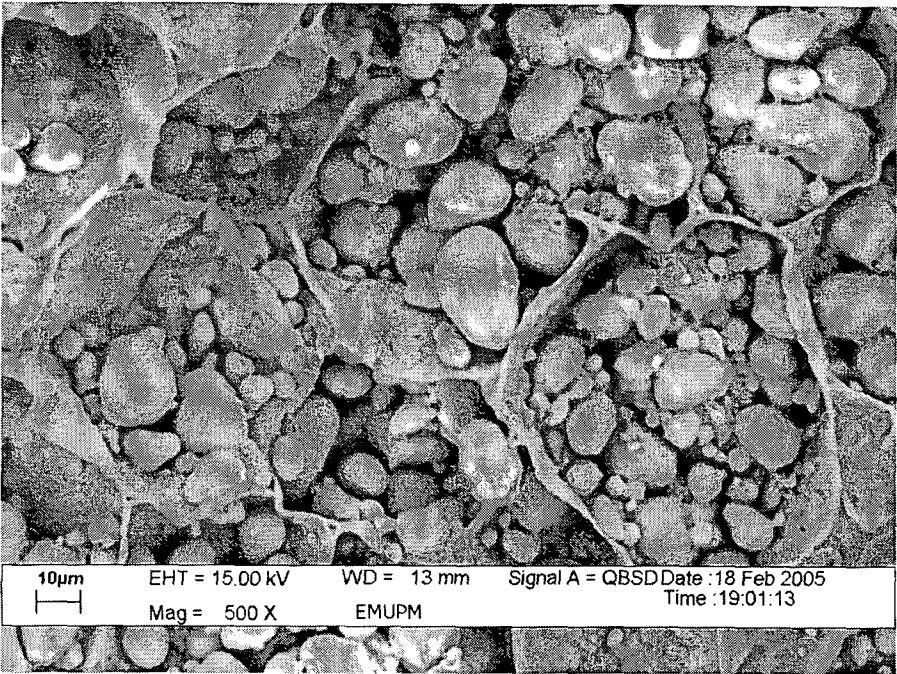


FIG. 1

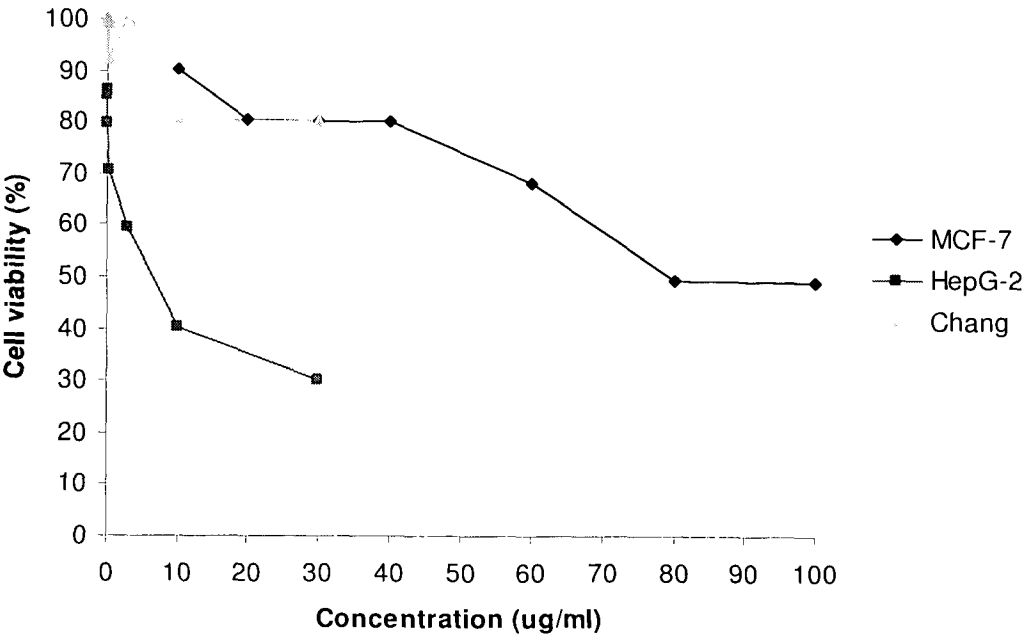


FIG. 2

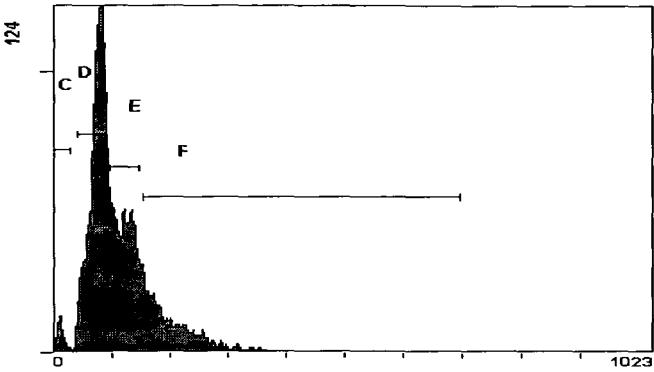


FIG. 3 (a)

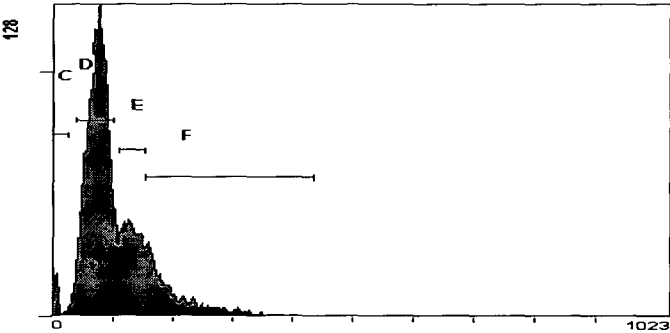


FIG. 3 (b)

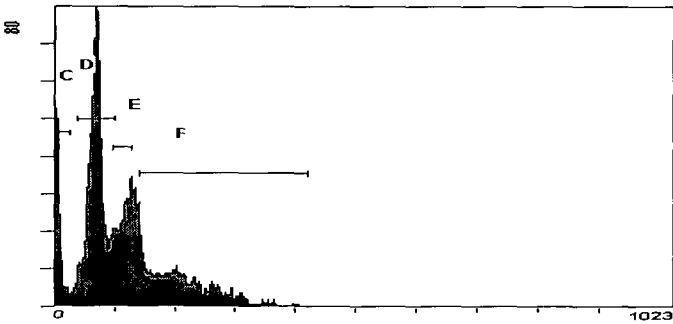


FIG. 3 (c)

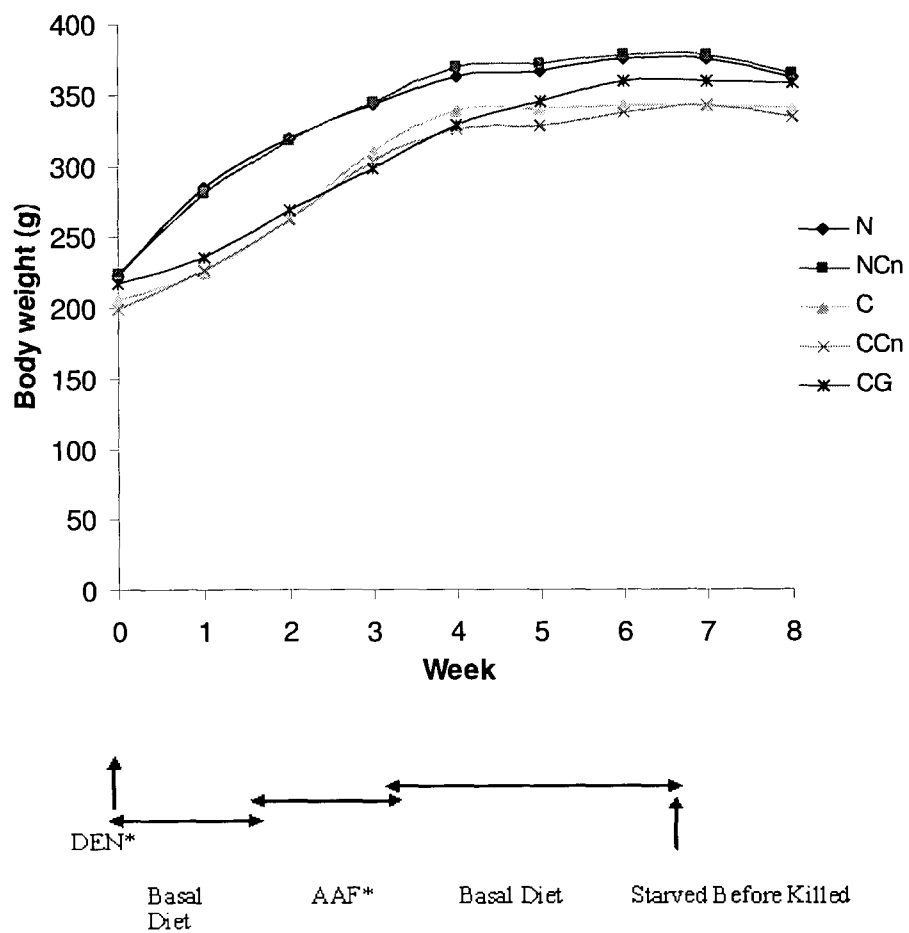


FIG. 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/MY2009/000204

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K36/185 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data, COMPENDEX, EMBASE, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2006/024385 A1 (PEDERSEN MARK A [US]) 2 February 2006 (2006-02-02) paragraphs [0033], [0040], [0077]	1-6
X	US 7 030 092 B1 (LEVINE SCOTT [US]) 18 April 2006 (2006-04-18) column 28, line 51 - line 53; claims 11,18,29	1-6
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☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

25 February 2010

Date of mailing of the international search report

11/03/2010

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Pilling, Stephen

INTERNATIONAL SEARCH REPORT

International application No
PCT/MY2009/000204

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ATAWODI SUNDAY ENE-OJO ET AL: "Content of polyphenolic compounds in the Nigerian stimulants Cola nitida ssp. alba, Cola nitida ssp. rubra A. Chev, and Cola acuminata Schott & Endl and their antioxidant capacity." JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY 28 NOV 2007, vol. 55, no. 24, 28 November 2007 (2007-11-28), pages 9824-9828, XP002570170 ISSN: 0021-8561 abstract final paragraph of "results and discussion" -----	1-6
X	Anonymous: "Cola nut" Internet Article: Herbal extracts plus 16 October 2007 (2007-10-16), XP002570171 Retrieved from the Internet: URL: http://web.archive.org/web/20071016162138/http://herbalextractsplus.com/cola-nut.cfm [retrieved on 2010-02-23] "Beneficial uses" -----	1-6
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X	FONTENOT ET AL: "Characterization of Bizzy Nut extracts in estrogen-responsive MCF-7 breast cancer cells" TOXICOLOGY AND APPLIED PHARMACOLOGY, ACADEMIC PRESS, US, vol. 220, no. 1, 20 March 2007 (2007-03-20), pages 25-32, XP005927328 ISSN: 0041-008X abstract "Discussion" -----	1-6

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/MY2009/000204

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