Study of the Antioxidant Effect of its Coconut Oil as Functional Food on Some Microbes and Anti-cancer Activity

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Abstract :

Virgin coconut oil (VCO) can be used as a functional food because it provides many health benefits in addition to its nutritients agent. It also contains antioxidants as well as antimicrobial and anticancer activity. The chemical composition of VCO was identified by gas chromatography–mass spectrometry GC-MS analysis. The antioxidants present in VCO were identified and polyphenols as well as flavonoids were identified using (GC-MS) and HPLC analysis. The well diffusion assay method was used to investigate the antimicrobial activity of VCO. The SRB assay assay method was used to investigate the anticancer activity of VCO. . Nine fatty acids were quantitively identified in VCO and it richs in saturated fatty acids, with high Percentge of lauric and myristic acids . GC-MS analysis and HPLC analysis of compounds contained in VCO resulted in of 17 antioxidant compounds .(VCO)has no effect on the microbes used in the sense that it has no microbial effect , VCOhas anti-cancer activity, So, this study concluded that VCO is a functional food with many health benefits in addition to its nutritive value

Key words: Virgin Coconut Oil -Functional Food – Antioxidant - antimicrobial Activity - Anti-cancer Activity

Introduction :

coconut oil (CNO) is colorless to brown-yellow edible oil produced from a coconut palm (Cocos. *nucifer*). VCO, oil can be obtained from fresh coconut flesh or from copra. The process of making VCO can be carried out in 2 ways, namely, Virgin Coconut oil (VCO) from fresh coconut flesh, known as the wet process because in this process, water is added to extract oil, while the manufacture of VCO with raw copra is known as the dry process **(Rahamat, et al ,2019).**

(VCO) is extracted from the fresh kernel without high heat or chemical treatment**. (Narayanankutty ,et al,2018).**

VCO contains 92% of saturated fatty acids(SFAs), which is significantly greater than in other commonly consumed vegetable oils. A portion size of 100 g of VCO is found to contain 890 kcal and 82.5 g of saturated fat**.(Lima'' and Block'',2019).**

VCO contain 47.5% lauric acid, 18.1% myistric acid , 8.8% palmitic acid , 7.8% caprylic acid , 6.7% capric acid , 6.2% oleic acid, 2.6% stearic acid, 1.6% linoleic acid and 0.5% caproic acid. **(Dayrit,2015)**

VCO contains saturated fatty acids including medium-chain fatty acids (mcfas) and medium-chain triglycerides (mcts). mcfa is a lauric acid which has antiprotozoal, antiviral, and antibacterial properties. mct in vco can increase immunity against disease and accelerate healing from illness and can prevents obesity (**Maromon, et al ,2020 &Ayob , et al ,2020).**

Antioxidants are a group of elements and compounds that have the ability to prevent or slow down the oxidation process in order to protect other compounds from oxygen. Antioxidants are found in the body of '" organism in the form of enzymes, glutathione" or compounds containing reduced sulfur" such as (Co-enzymes), as enzyme accompaniments. Antioxidants are also found naturally in vegetables, fruits, grains and most medicinal herbs. Interesting of in antioxidants has been increased in recent years due to their ability to immunize the body against invading germs and eliminate them. It is also protect'" the body from common age diseases. **(Chowdhury , et al;2016).**

VCO is rich in mainly α-tocopherol and lauric acid responsible for biological action **(Tangwatcharin and Khopaibool 2012; Arlee et al. 2013; Arunima and Rajamohan 2014) .**

VCO polyphenols improve the sulfhydryl form glutathione (GSH) and superoxide dismutase (SOD) activity which retains the integrity of cellular membranes. Thus, the VCO polyphenols have a potential for

being used as an anti-arthritic agent. The beneficial antioxidative effects of VCO have been studied and include effects on several antioxidant pathways in the body. **(Shahidi F, Zhong Y 2015**)

Lauric acid when metabolized converts to the compound monolaurin which has antimicrobial properties. which focused on virucidal effects of monolaurin RNA and DNA viruses. Recently, experimental outcomes from many studies discovered that monolaurin had not only antimicrobial activity against various gram-positive and gram-negative bacterial cells **(Taheri ,et al,2010 :Thaweboon, et al,2011 & Peedikayil , et al,2015) .**

Cancer is a collection of disorders characterised by the invasion and multiplication of cells (which is unlimited cell growth and division). These dividing cells have the ability to invade and kill nearby tissues, as well as travel to distant tissues, resulting in secondary cancer in the new organ. One of the classic cancer treatments, such as chemotherapy, radiation, or surgery, is used to control the metastases**(Druesne,et al ,2010)**.

(VCO) contain bioactive compounds known as natural antioxidants that may mitigate oxidative damage induced by methotrexate chemotherapy. **(Al-Dabagh,et al,2013).**

Methotrexate (MTX), loaded in layered double hydroxide nanoparticle, had exhibited a great antitumor activity against human osteosarcoma-bearing mice with reduced adverse side effects . **(Choi et al., 2013**)

Material and Methods

Materials:

Virgin coconut oil were purchased from Imtenan Company, Egypt.

Staphylococcus aureus (ATCC 25923) , *Escherichia coli* o:157(93111), *Listeria monocytogenes* (ATCC7644),*Bacillus cereus*(ATCC25923), *Pseudomonas aeroginosa* (ATCC35032)and *candida albicans* (ATCC10231) were obtained from the collection of microorganisms from the Reference Collection of Microorganisms on Research Laboratories Complex "" Faculty of Agriculture - Cairo University . Both bacteria were maintained in nutrient agar at 4 ˚C until using.

Culture media Mueller- Hinton agar and Sabouraud dextrose agar were found from the Reference Collection of Microorganisms on Research Laboratories Complex "" Faculty of Agriculture '" Cairo University.

Human tumor cell lines in this study the potential cytotoxicity of the tested compound VCO was tested one cell lines were used MCF7 lung carcinoma cell line and HCT116 colon"" carcinoma cell line , was obtained from VACSERA-Cell Culture Unit, Cairo, Egypt.

Methods:

Determination of Fatty Acids Composition :

Chemical composition of VCO by (GC-MS) analysis :

Gas-chromatography coupled with Mass spectrometry (GC-MS) was used to identify and measure the composition of fatty acids present in walnut and coconut oils.

Gas chromatography–mass spectrometryanalysis (GC-MS) for FAME :

The GC-MS system (Agilent Technologies) was equipped with gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, Cairo, Egypt.

Determination of polyphenols and flavonoids :

Antioxidants, polyphenols, and flavonoids will be determined using Gas chromatography–mass spectrometryanalysis (GC-MS) and HPLC

Determination of Antibacterial activity:

The method used to test antimicrobial effect of the samples is the well diffusion assay ,it is widely used to evaluate the anti-Timicrobial activity of plants or microbial extracts ,similarly to the procedure used in disk-diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial in- oculum over the entire agar surface. **(Valgas , et al;2007).**

Determination of Anticancer activity:

The potential cytotoxicity of VCO was determined using lung and colon human cell lines : cytotoxicity of tested VCO extract using SRB assay, The assay depends on the ability of SRB to bind to protein components of cells that have bean fixed to tissue-cultures plates by tri chloro acetic acid (TCA).SRB is a bright-pink aminoxanthere dye with two sulfonic groups that bind to basic amino-acid residues under mild acidic condititions as the binding of SRB is stoichiometric the amount of dye extracted from stained cells is directly proportional to the cell mass. **(Suresh *et al,*2006).**

Statistical analysis

Statistical analysis were done using the Statistical Package for the Social Sciences (SPSS for WINDOWS, version 11.0; SPSS Inc, Chicago). Comparative analyses were conducted using the general linear models procedure (SPSS Inc). Values of $P<0.05$ were considered statistically significant. **AOAC (2012)**

RESULTS :

Chemical composition of VCO by (GC-MS) analysis

Gas-chromatography coupled with mass spectrometry (GC-MS) was used to identify and measure the composition of fatty acids present in walnut and coconut oils. A total of 9 fatty acids were qualitatively identified in VCO. The constituents of VCO with their retention time and percentage composition are given in Table 1 :

Table 1: Fatty acids composition of virgin coconut oil''

number	Name	Fatty acids%
1	Hexanoic acid (Caproic Acid)	0.03
2	Octanoic acid (caprylic acid)	0.96
3	Decanoic acid (Capric acid)	0.81
4	Dodecanoic acid (Lauric acid)	79.44
5	Tetradecanoic acid(Myristic acid)	15.66
6	Hexadecanoic acid (Palmitic acid)	1.68
7	Methyl stearate (Stearic acid)	0.46
8	9-Octadecenoic acid (Z) (Oleic acid)	0.84
9	9,12-Octadecadienoic acid (E,E) (linoleic acid 9E,12Z)	0.11

This was observed in our study, but the percentages vary. Thus, with 79.44% the Lauric acid had the highest quantity followed by Myristic acid with 15.66%, Palmitic acid with 1.68%, caprylic acid with 0.96% , Oleic acid with .84%, Capric acid with 0.81% , Methyl stearate with 0.46% , linoleic acid 9E,12Z with 0.11% and Caproic Acid with 0.03% .

Table 2. Fatty acids composition of virgin coconut oil obtained by GS-MS method:

Number	**Name**	Fatty acids%
1	Hexanoic acid (Caproic Acid)	0.04
2	Octanoic acid (caprylic acid)	1.21
3	Decanoic acid (Capric acid)	1.03
4	Dodecanoic acid (Lauric acid)	100
5	Myristic acid)(Tetradecanoic acid	19.71
6	Hexadecanoic acid (Palmitic acid)	2.11
7	Methyl stearate (Stearic acid)	0.58
8	9-Octadecenoic acid (Z) (Oleic acid)	1.06
9	9,12-Octadecadienoic acid (E,E) (linoleic acid 9E,12Z)	0.14

The chromatographic profile of these fatty acids showed that the virgin coconut oil is rich in saturated fatty acids, with high proportions of lauric and myristic acids .

Figure 1:Fractionation of polyphenols and flavonoids components by (GC-MS) (µg/g) for virgin coconut oil :

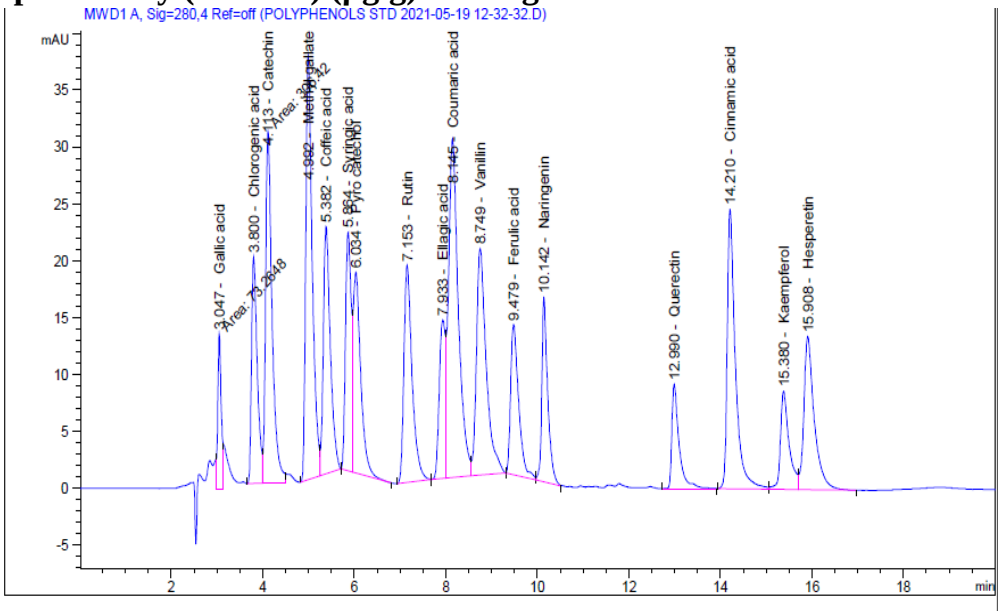


Table 3:Fractionation of polyphenols and flavonoids components by (GC-MS) (µg/g) for Virgin coconut oil :

No	RetTime [min]	Area [mAU*s]	Fatty acids %	Name
1	3.047	73.26477	1.9865	Gallic acid
2	3.800	173.34332	4.7000	Chlorogenic acid
3	4.113	306.42044	8.3082	Catechin
4	4.992	371.12375	10.0625	Methyl gallate
5	5.382	231.56790	6.2787	Coffeic acid
6	5.864	180.56229	4.8957	Syringic acid
7	6.034	205.11473	5.5614	Pyro catechol
8	7.153	237.39149	6.4366	Rutin
9	7.933	126.81020	3.4383	Ellagic acid
10	8.145	448.34357	12.1562	Coumaric acid
11	8.749	297.69907	8.0717	Vanillin
12	9.479	161.13330	4.3689	Ferulic acid
13	10.142	143.67262	3.8955	Naringenin
14	12.990	100.13819	2.7151	Querectin
15	14.210	305.11835	8.2729	Cinnamic acid
16	15.380	113.22188	3.0699	Kaempferol
17	15.908	213.24916	5.7820	Hesperetin
Total	3688.17503			

Figure 2: Fractionation of polyphenols and flavonoids components by HPLC (µg/g) for Virgin coconut oil :

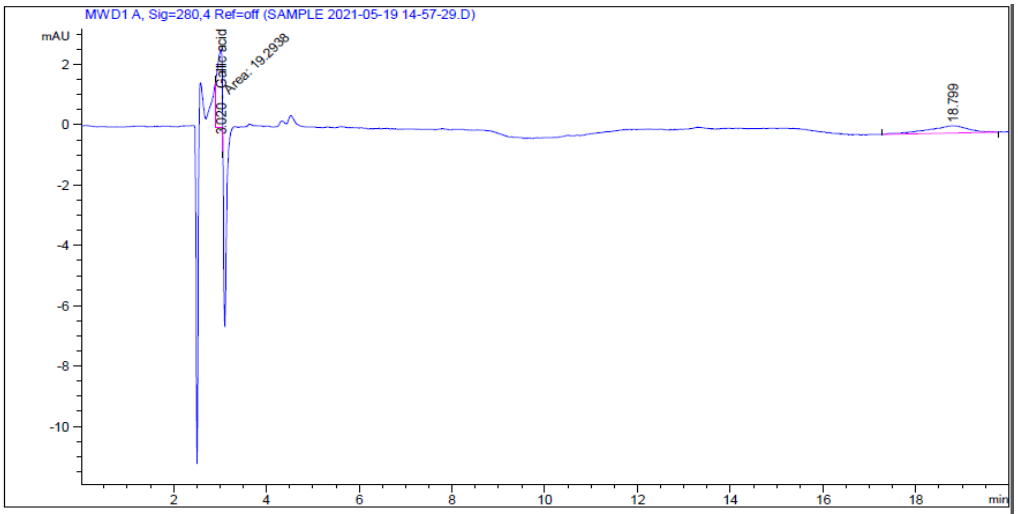


Table 4: Fractionation of polyphenols and flavonoids components by :HPLC (µg/g) for Virgin coconut oil

No	RetTime [min]	Area [mAU*s]	**Fatty acids %**	Name
1	3.020	19.29381	56.3494	Gallic acid
2	3.800	0.0000	0.0000	Chlorogenic acid
3	4.113	0.0000	0.0000	Catechin
4	4.992	0.0000	0.0000	Methyl gallate
5	5.382	0.0000	0.0000	Coffeic acid
6	5.864	0.0000	0.0000	Syringic acid
7	6.034	0.0000	0.0000	Pyro catechol
8	7.153	0.0000	0.0000	Rutin
9	7.933	0.0000	0.0000	Ellagic acid
10	8.145	0.0000	0.0000	Coumaric acid
11	8.749	0.0000	0.0000	Vanillin
12	9.479	0.0000	0.0000	Ferulic acid
13	10.142	0.0000	0.0000	Naringenin
14	12.990	0.0000	0.0000	Querectin
15	14.210	0.0000	0.0000	Cinnamic acid
16	15.380	0.0000	0.0000	Kaempferol
17	15.908	0.0000	0.0000	Hesperetin

GC-MS analysis and HPLC analysis of compounds contained in Virgin coconut oil resulted in of 17 antioxidant compounds such as Gallic acid ,Chlorogenic acid , Catechin , Methyl gallate, Coffeic acid, Syringic acid , Pyro catechol, Rutin, Ellagic acid, Coumaric acid ,

Vanillin, Ferulic acid , Naringenin , Querectin , Cinnamic acid , Kaempferol and Hesperetin shown in Table 3,4.

Table 5 : Antibacterial activity The analysis of the above mentioned sample (s) show (s) the following :

Microbial type	Diameter of the inhibition zone (mm)			
	Sample	Positive		
Escherichia coli o:157(93111)	No inhibition	15	15	15
Listeria monocytogenes (ATCC7644)	No inhibition	40	40	40
Staphylococcus aureus (ATCC 25923)	No inhibition	15	15	15
Bacillus cereus(ATCC25923)	No inhibition	30	30	30
Pseudomonas aeroginosa (ATCC35032)	No inhibition	20	20	20
Candida albicans (ATCC10231)	No inhibition	15	15	15

The results reaveled in Table 5 confirm that virgin coconut oil has no effect on the microbes used in the sense that it has no microbial effect.

Measurements of potential cytotoxicity of chemical compound by SRB assay.:

In this study using natural compounds of virgin coconut oil these plant have anti cancer activity.The potential cytotoxicity of natural virgin coconut oil was determined on human carcinoma cell lines which is (MCF7 and HCT116 carcinoma cell lines). The potential cytotoxicity of each sample for different concentrations (2.5, 5,10 and 20 mg/ml) was determined using the ELISA RADER at wave length 570 nm and 96-multwll plates .that repeted three times and in each time it was done twice and the main values was calculated. Then the relation between the concentrations of sample in (mg) against surviving fraction % of cells was curved.Also the IC50 values of thes samples were determined from potential cytotoxicity curves. Cytoxicity of Virgin coconut oil on carcinoma cell lines . This result confirms that Virgin coconut oil has anti-cancer activity.

Fig (1) The potential cytotoxicity effect of natural plants extractcts on HCT116 carcinoma cell line :

the IC50 values of thes samples were determined from potential cytotoxicity curves. Cytoxicity of Virgin coconut oil on carcinoma cell lines and found 11.7 % that while the control group 100% , The first group 100.6%, The second group 99.85% ,The third group 56.05%, And

the fourth group23.78% . This result confirms that Virgin coconut oil has anti-cancer activity.

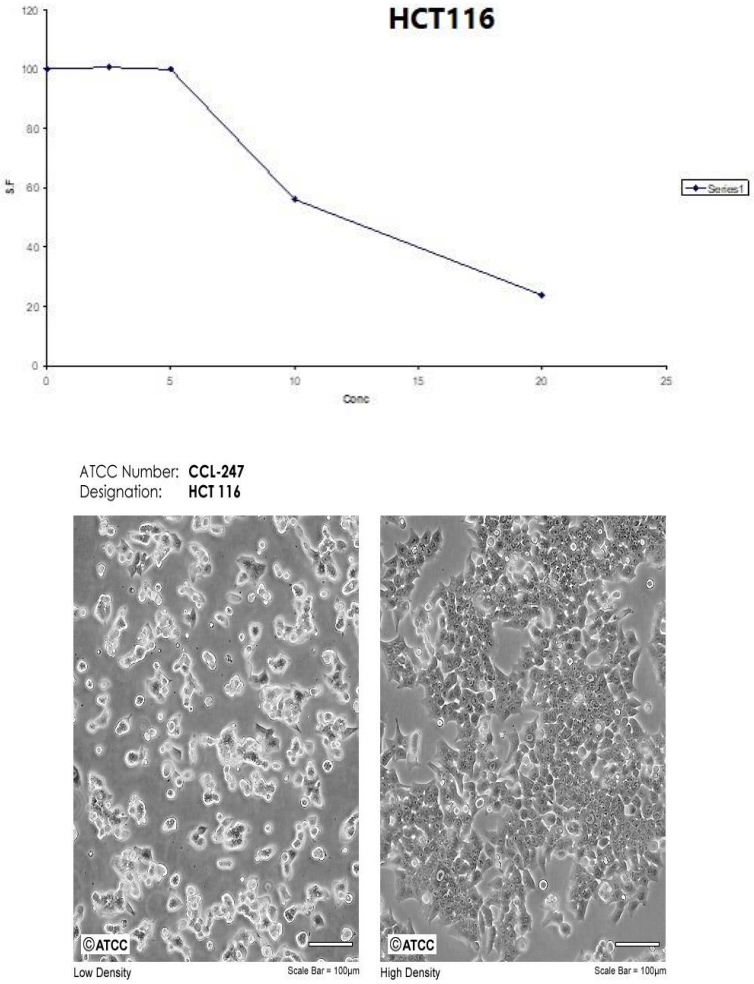
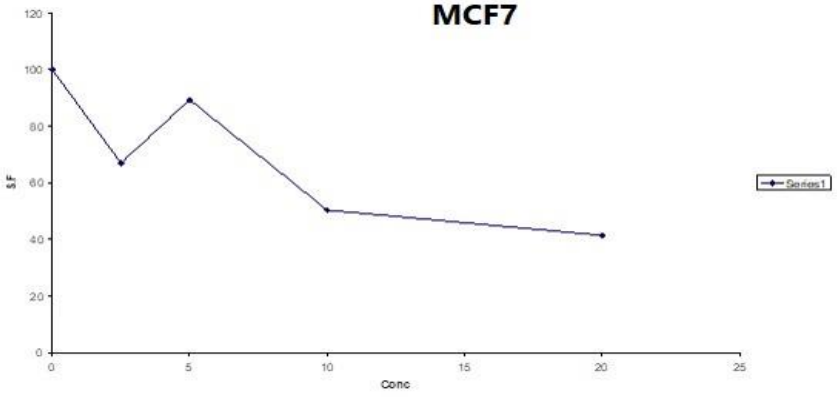
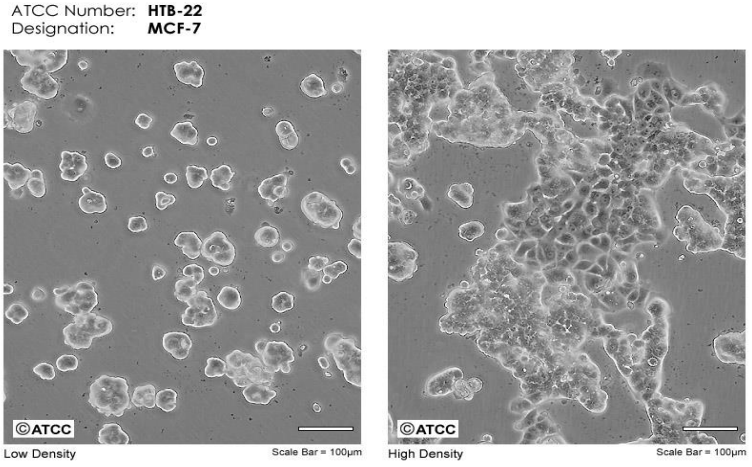


Fig (2) the potential cytotoxicity effect of natural plants extractcts on MCF7 carcinoma cell line .

the IC50 values of thes samples were determined from potential cytotoxicity curves. Cytoxicity of Virgin coconut oil on carcinoma cell lines and found 13 % that while the control group 100% , The first group 67.01%, The second group 89.33% ,The third group 50.39%, And the fourth group 41.45% . This result confirms that Virgin coconut oil has anti-cancer activity.





Discussion:

From Table 1, it can be seen that Virgin coconut oil has 99.04% saturated fatty acid and only .95% unsaturated fatty acid, mostly composed of oleic acid and linoleic acid . The presence of lauric acid (79.44%) was found in coconut oil.

This results is in line with**(Gregorio ,2005)** and **(Gopala et al,2010),** they reported that Virgin coconut oil is major source of lauric acid, with significant food and non-food uses **(Dayrit,et al,2007).**

The chromatographic profile of these fatty acid showed that the Virgin coconut oil is rich in saturated fatty acids, with high proportions of lauric and myristic acids .

(Dayrit, 2015) reported that the only disadvantage with Virgin coconut oil is the poor level of essential fatty acids. Almost 50% of fatty acids in Virgin coconut oil is lauric acid (C12H24O2) – the saturated medium-chain fatty acid which possesses the distinct properties unlike long-chain fatty acids. GC-MS analysis and HPLC analysis of compounds contained in Virgin coconut oil resulted in of 17 antioxidant compounds such as Gallic acid ,Chlorogenic acid , Catechin , Methyl gallate, Coffeic acid, Syringic acid , Pyro catechol, Rutin, Ellagic acid, Coumaric acid , Vanillin, Ferulic acid , Naringenin , Querectin , Cinnamic acid , Kaempferol and Hesperetin shown in Table 3,4.

This results is in line with Virgin coconut oil has a high percentage of phenolic acids, and these are phytochemicals, sometimes also referred to as a polyphenols. Phenolic acids are recognised for their antioxidant properties. *p*-Coumaric acid, ferulic acid, caffeic acid and catechin acid are the major phenolic acids found in coconut oil**(Marina, et al,2009).**

The result of the research is close to this conclusion reached by the researcher , **Arivalagan, et al. (2018)** identified 28 phenolic compounds including 12 flavonoids and 16 phenolic acids. Among these, the p-coumaric, ferulic, and protocatechuic acids were major phenolic acids, and apigenin, catechin, and kaempferol were the main flavonoids found in coconut testa. These antioxidant activities, which correlated with the phenolic contents were lower in RBD coconut oil.

The results in table 5 reaveled in table confirm that Virgin coconut oil has no effect on the microbes used in the sense that it has no microbial effect, and this result is consistent with the following results :

(Khoramnia A,et al,2013) reported that In this study, the oil was modified into a value-added product using direct modification of substrate through fermentation (DIMOSFER) method. The modified

coconut oils MCOs demonstrated improved antibacterial activity mostly due to the presence of free lauric acid. The highest MCFAs-rich Virgin coconut oil revealed as much as 90% and 80% antibacterial activities against *Staphylococcus aureus* and *Escherichia coli*, respectively. The results of the study showed that DIMOSFER by a local *lipolytic G. candidum* can be used to produce MCFAs as natural, effective, and safe antimicrobial agent. The produced MCOs and MCFAs could be further applied in food and pharmaceutical industries.

Using natural compound Virgin coconut oil these plant have anti cancer activity.The potential cytoxicity of natural Virgin coconut oil was determined on human carcinoma cell lines which is MCF7 and HCT116 carcinoma cell lines. The potential cytotoxicity of each sample for different concentration (2.5, 5,10 and 20 mg/ml) was determined using the ELISA RADER at wave length 570 nm and 96-multwll plates .that repated three times and in each time it was done twice and the main values was calculated. Then the relation between the concentrations of sample in (mg) and surviving fraction % of cells was curved.Also the IC50 values of thes samples were determined from potential cytoxicity curves. Cytoxicity of Virgin coconut oil on carcinoma cell lines .

Conclusions : Virgin Coconut Oil (VCO) can be used as a functional food because it provides many health benefits in addition to its nutritional content. It also contains antioxidants as well as anticancer activity. Virgin coconut oil has no effect on the microbes used .

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دراسة علي التاثير المضاد للاكسدة لزيت جوز الهند كغذاء وظيفي على بعض الميكروبات ونشاطة المضاد للسرطان

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استاذ السموم وملوثات الغذاء(المتفرغ) _قسم السموم الغذائية_المركز القومى للبحوث

الاء حمدي حلوة
مدرس مساعد بقسم الاقتصاد المنزلي كلية التربية النوعية جامعة المنوفية

الملخص العربي :

يمكن استخدام زيت جوز الهند البكر كغذاء وظيفي لأنه يوفر العديد من الفوائد الصحية بالإضافة إلى محتواه الغذائي. كما أنه يحتوي على مضادات اكسدة بالإضافة إلى نشاط مضاد للميكروبات ومضاد للسرطان. تم التعرف علي التركيب الكيميائي لزيت جوز الهند البكر من خلال تقنية تحليل كروماتوغرافيا الغاز ومضادات الأكسدة الموجودة في الزيت والبوليفينولات والفلافونويدات باستخدام تحليل كروماتوجرافيا الغاز وقياس الطيف الكتلة. تم استخدام طريقة اختبار الانتشار الجيد للتحقيق من نشاط مضادات الميكروبات لزيت جوز الهند البكر واستخدام طريقة مقايسة SRB للتحقيق من النشاط المضاد للسرطان للزيت .أظهرت النتائج أن إجمالي أحماض دهنية تم تحديدها نوعياً في زيت جوز الهند البكر وهي غنية بالأحماض الدهنية المشبعة، مع نسب عالية من أحماض اللوريك والميريستيك. أسفر تحليل كروماتوجرافيا الغاز المزود بمطياف الكتلة. للمركبات الموجودة في زيت جوز الهند البكر عن وجود سبعة عشر مركبًا مضادًا للأكسدة مثل حمض الجاليك وحمض الكلوروجينيك والكاتشين وميثيل جالات وحمض الكافيك واتضح ان زيت جوز الهند البكر ليس له أي تأثير على الميكروبات المستخدمة بمعنى أنه لا يوجد لديه التأثير الميكروبي، زيت جوز الهند البكر له نشاط مضاد للسرطان، لذلك خلصت هذه الدراسة إلى أن زيت جوز الهند البكر غذاء وظيفي له العديد من الفوائد الصحية بالإضافة إلى محتواه الغذائي.

الكلمات المفتاحية: زيت جوز الهند البكر– الغذاء وظيفي– مضاد الاكسدة– نشاط المضاد للميكروبات – نشاط المضاد للسرطان