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Antioxidant, Antimicrobial and Anticancer Activities of Egyptian *Conocarpus erectus* L. leaves Extracts

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Conocarpus erectus L. (Family Combretaceae) is a low branching evergreen shrub or tree with a typical height of up to 6 meters, growing spread in Egypt. It contains phenols such as flavonoids and tannins as major constituents. Folk remedy has used parts of this species in its native countries ,showed high antioxidant, antimicrobial and anticancer activity due to the presence of phenolic compounds. . In this study, the antioxidants, antimicrobial and anticancer activity of three different extracts (ethanolic , cold aqueous and hot aqueous) from leaves of this plant were assessed. The antioxidant properties of all extracts were found to be associated with the total content of phenolic compounds. Also showed high free radical scavenging activity toward DPPH radical , it could be used the hot and cold water extracts as an effective natural antioxidant. The different extracts were subjected to chromatographic fractionation with high-performance liquid chromatography. The major and sharp peaks in each sample were identified or tentatively identified based on matching with some standard compounds. Pyrogallol , caffeine and e- vanillic were the major phenolic compounds in ethanolic extract 3500, 730 and 1020 ppm ,respectively but the major phenolic compounds in cold aqueous extract were pyrogallol, salicylic, epi- catachin, gallic and protocatchouic 870, 320, 210, 163 and 110 ppm ,respectively. The extracts were also assessed against two gram-positive bacteria (*Bacillus ceries* , *Staphylococcus aureus*) and two gram-negative bacteria (*Escherichia coli, Salmonella* typhimurium). Nevertheless results showed all the extracts possess antibacterial activity, while the extracts of crude leaf were more active in cold aqueous of hot aqueous in case of Staph. aureus. The cytotoxic activity of these were measured, the most efficient cytotoxic activity against (breast cancer) MCF7 in ethanolic extract with IC50 = 99 followed by hot aqueous extract (HAE) with IC50 = 100 then cold aqueous extract (CAE) with IC50 = 102 , while the most efficient cytotoxic activity against (liver cancer) HEPG2 in hot aqueous extract with IC50= 37.9 followed by ethanolic extract with IC50 = 39.3 then cold aqueous extract with IC50= 50.9 . From previous results can be utilization from leaves extract as a natural food additives.

Introduction

Natural products from some of these natural resources continue to be used in pharmaceutical preparations either as crude extracts, fractions, pure compounds or analogous compounds from highly active isolated compounds (Abdel-Hameed et al., 2012).

The World Health Organization (WHO) indicated that medicinal plants are every plant which, in one or more of its structures, contains elements that may be utilized for helpful purposes, or which are forerunners for chemo-pharmaceutical semi synthesis (Saroya et al., 2011). The therapeutic plants may either be the wild or the developed plant particularly for restorative purposes. As there are broad home grown pharmaceuticals accessible in the present time, the alike material may be characterized in various structures, for example the powdered plant material may be measured as both a natural material and/or herbal preparation and when it's accessible in a form of the packing material, it's considered as home grown restorative product (Daniela et al., 2016). In every an herbal preparations, it's easy to systematize in terms of a defined amount and concentration of an active components, if the medicinally active constituents have been recognized and are notable. The herbal drugs are the more extensive term which embraces herbs, herbal preparations, and materials counting an ended herbal product (WHO ,2007).

Medicinal plants are the richest bio-resource of drugs of traditional medicines, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Nirmala et al., 2011). Due to the multitargeting effect, inexpensive and safety of plant-based products compared to synthetic agents, there is a need for more and more searching and discovering of new drugs from plants.

Conocarpus erectus L. is one of two species in the genus Conocarpus, in the family Combretaceae growing on shorelines in tropical and subtropical regions around the world. *Conocarpus erectus;* known in English as buttonwood or button mangrove; is an evergreen tree 6 m tall with spreading crown, grey or brown bark, glaucous medium-green leaves and greenish flowers in dense cone-like heads in terminal panicles (Bailey, 1976). It is used in some countries as folk remedy for anemia, catarrh, conjunctivitis, diabetes, diarrhea, headache, gonorrhea, bleeding, tumors, syphilis and fever (Irvine, 1961). The leaves were eaten or boil water and drunk to treat fever (Duke and Wain, 1981). This plant may have great relevance in the prevention and therapies of diseases in which oxidants or free radicals are implicated after more in vivo studies (Abdel-Hameed et al., 2013). The extract of *C. erectus* from different parts (leaves, stems, fruits, and flowers) showed high antioxidant, hepatoprotective and anticancer activity due to the presence of phenolic compounds. It is an attempt to review the pharmacognostic characteristics, traditional uses, phytochemistry and biological activities of the plant. (Maryam et al., 2015). Extracts of different parts of *C. erectus* investigated antimicrobial activity against gram-positive, gram-negative, acid-fast bacteria and fungi. The broad-spectrum antibacterial activity of the plant extracts, confirms its use as a health remedy in folklore medicine (Shohayeb et al., 2013).

In this work, a phytochemical study of leaves of *C. erectus* and their biological activities as antioxidant, antibacterial and anticancer were done. as is the main aim in this research.

Materials and Methods

Materials

- *Plant material:* Conocarpus erectus L. leaves were collected from Horticultural Research Institute, Agricultural Research Center, El-Giza Governorate, Egypt. They were carefully washed with tap water. Stems were removed and the green tissue was dried at room temperature and pulverized into powder with an electric blender .
- **Standards**: All authentic standards were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The stock standard solutions were prepared by dissolving the standard phenolic compounds and flavonoids in the appropriate volume of 50% aqueous methanol to produce a final concentration of 1 mg/ml. Stock/working solutions of the standards were stored in the dark at -18º C.

Bacterial strains

- Cultures were prepared for in vitro antibacterial assay of the four bacteria strains, two Gram positive: *Bacillus ceries* (ATCC33018); and *Staph. Aureus* (DSM 20231) and two Gram negative *Escherichia coli* (ATCC69337) and *salmonella typhimurium* (ATCC14028). Those strains were provided by Microbiologics® USA. Tested organisms were sub cultured on nutrient agar (Oxoid laboratories, UK) slopes. These stock cultures were stored in the dark at 4°C until used.

Methods

Preparation methods of natural extracts

- *Alcoholic extract:* The plant powder (300 g) of each part was soaked in 1500 ml ethanol for one week at room temperature with shaking day by day followed by filtration and again extraction for four times. (Abdel-Hameed et al.,2012)
- *Cold aqueous extract:* The extract was prepared by infusion from 260 g of dried leaves of *C. erectus* L. The material was weighed, ground and the infusion was performed by adding distilled water at room temperature for 30 minutes and stored at 5°C.
- *Hot aqueous extract :* The extract was prepared by infusion from 260 g of dried leaves of *C. erectus* L. The material was weighed, ground and the infusion was performed by adding distilled water at 100°C for 30 minutes. The aqueous extract was lyophilized and stored at 5°C. (Nascimento et al., 2016).

Analysis methods

- Total phenols and flavonoids contents: Total phenols and flavonoids contents were described by Arnous et al. (2001) and Joyeux et al. (1995), respectively.

- *Determination of antioxidant activity:* Antioxidant activity of Conocarpus erectus L. leaves extracts were determined by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method according to (Brand-Williams et al., 1995).

- *Determination of Chlorophyll A, chlorophyll B and carotenoids*: Chlorophyll A, chlorophyll B and carotenoids were determined as described by Arnon (1949) and Wettstein (1957), respectively.

- *HPLC analysis of Polyphenols and flavonoids*: Polyphenols and flavonoids contents in the extracts of Conocarpus erectus L. leaves were determined using HPLC (Agilent series 1200). Column temperature was maintained at 35 . gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml/min. This method modified of (Goupy et al., 1999) and (Mattilla et al., 2000), respectively.

- *Determination of total tannins*: Tannins contents in the extracts of Conocarpus erectus L. leaves were determined by vanillin hydrochloric acid method described by (price et al., 1978).

Antimicrobial activity

Antimicrobial activity was determined using the agar well diffusion assay method as described by Holder and Boyce (1994). DMSO was used as a negative control. Bacterial cultures were incubated at 37°C for 24 hr. Antimicrobial activity was determined by measurement zone of inhibition (Agwa et al., 2000).

Determination of anticancer activity

Effect of *Conocarpus erectus* L. leaves extracts as anticancer measurement of potential cytotoxicity activity against the breast carcinoma cell line (MCF7) and liver carcinoma cell line (HEPG2) were tested by SRB assay using the method of Skehane et al. (1990).

Statistical analysis: The obtained data were exposed analysis of variance. Duncan's Multiple range tests at ($p \leq 0.05$) level was used to compare between means. The analysis was carried out using the PRO-ANOVA procedure of Statistical Analysis System (SAS, 1996).

Results and Discussion

Antioxidant activity of Conocarpus erectus L. leaves

The total amounts of phenols, flavonoid and tannin components of ethanolic,cold aqueous and hot water aqueous extracts fractions were chemically estimated. Table 1 showed that the hot water extract fraction of leaves contains high total phenolic contents equivalent to 211.7mg/g GAE whereas the Cold aqueous extract fractions of leaves has the lowest total phenolic contents (186.2mg/g GAE). On the other hand the ethanolic extract fraction of leaves has the highest total flavonoid contents equivalent to 57.2mg/g RE followed to cold aqueous extract 27.6 mg/g RE. The estimation of total tannin showed high content of the ethanolic extract than lack gradual in cold aqueous extract and hot aqueous extract. The model of scavenging of the stable DPPH radicals is awidely method to evaluate the antioxidant activity of the investigated sample in relatively short time compared with other methods. The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Gulcin et al., 2004). The addition of the investigated extracts to the DPPH solution caused a decrease in the optical density at 517 nm (the maximum absorption of a stable DPPH radical at 517 nm).The decrease in absorbance of DPPH radical caused by antioxidants is due to the reaction between antioxidant molecules and radical which results in the scavenging of the radical by hydrogen donation.This is visualized as a discoloration from purple to yellow. The total antioxidant activity was the highest in ethanolic extract 66.7% and it was not signaficant in cold and hot water extract 51% and44%, respectively. The activities of extracts were in order :ethanolic exectract >hot water extract > cold water extract leaves. This result is agreement with (Madsen et al., 1996) who reported that phenol and flavonoid compounds play an important role as antioxidants and a good correlation between the concentration of plant phenolic and the total antioxidant capacity. These data are in harmony to those of Gallegos-Infant et al. (2010) who found that temperature affects on the total phenolic content positively, higher temperatures, higher phenolic contents were observed and (Seok-Moon et al., 2004) who indicated that total phenol contents and the antioxidants activity were significantly affected by heating temperature and duration of treatment and the heating process can be used as a tool for increasing the antioxidant activity.

TABLE 1. Antioxidant activity of *Conocarpus erectus* L. leaves extracts.

Antioxidant activity	Conocarpus erectus L. leaves		
	EOH	CAE	HAE
Total phenols(mg gallic acid equivalents/g plant extract)	$206.5^{a}{}_{\pm}6.30$	$186.2^{b}{}_{\pm}3.02$	$211.7^{a}{}_{\pm}2.4$
Flavonoids (mg rutine quivalents/g plant extract)	$57.2^{a}{}_{\pm}2.10$	$27.6^{b}{}_{\pm}0.38$	$17.6^{c}{}_{\pm}1,5$
Total antioxidant activity (%)	$89.5^{a}{}_{\pm}4.00$	$66.01^{c}{}_{\pm}1.8$	$69.78^{b}{}_{\pm}5.2$
Total tannins (mg gallic acid Plant part equivalents/g plant extract)a equivalents/g plant extract)b equivalents/g plant extract)	$66.7^{a}{}_{\pm}0.13$	$51^{b}{}_{\pm}0.11$	$44^{c}{}_{\pm}0.3$

Values are means of three replicates ±SE.
Values followed by the same letters within the same row were not significantly different at 0.05 level
EOH (ethanolic extract)
CAE (cold aqueous extract)
HAE (hot aqueous extract)

Chlorophyll A, B and carotenoids (mg/100g) in Conocarpus erectus L. leaves extracts

As shown in Table 2 chlorophyll A, B and Carotenoids. It could be , noticed that chlorophyll A, B and Carotenoids (mg/100g) were highest in ethanolic extract were (0.68, 0.13 and 0.06 mg/100g) , (0.16, 0.12 and 0.03 mg/100g) and (0.087 , 0.016 and 0.011), respectively, followed by cold aqueous extract then hot aqueous extract .These results are in harmony to Hande et al., (2008) who reported that chlorophylls are easily degraded by heat.

The concentration of phenolic compounds found in Conocarpus erectus L. leaves extracts

The concentrations of identified components were determined using the obtained calibration curves and listed in Tables 3 and 4. Most of phenolic compounds selected in Table 3 were identified in each sample. In the present study, pyrogallol, caffeine and e- vanillic were the major phenolic compounds 3500, 730 and 1020 ppm , respectively in ethanolic extract but the major phenolic compounds in cold aqueous extract were pyrogallol, Salicylic, epi- catachin gallic and protocatchouic 870, 320, 210, 163 and 110 ppm, respectively. It also could be noticed that gallic, pyrogallol, protocatchouic, chlorogenic, catechol, catechin, caffeic, 4- amino benzoic acid, ferulic ,iso-ferulic, epi- catachin, ellagic , alpha-coumaric, salycillic, 3,4,5 methoxy, cinnamic, coumarin, p- coumaric and cinnamic were higher in hot aqueous extract than cold aqueous extract.

The concentration of flavonoid compounds of Conocarpus erectus L. leaves extracts

The results in Table 4 indicated that ethanolic extract was rich in flavonoids for each aqueous extracts and the highest concentration being rosmarinic (1060 ppm) forward to quercetrin and quercitin (230 and 120 ppm). While the cold aqeous extract was the highest in hesperidin rosmarinic and luteolin 140 ,30 and 30 , respectively. It could be also , noticed that the lowest amount of flavonoids in hot aqueous extract. Inderjit , 1991 revealed that hesperidin is a naturally occurring bioflavonoid, a compound in plants with antioxidant properties. Bioflavonoid also provide the color, flavor and aroma to plant. It is commonly referred to as vitamin P. it is'not naturally occur in the body ; you can only get it through foods or synthetic supplement. Rosmarinic solubility in water slightly soluble and well soluble in most organic solvents, in plants it is supposed to act as a preformed constitutively accumulated defense compound (Petersen et al., 2003). luteolin is often glycosylated in plants, and the glycoside is hydrolyzed to free luteolin during absorption (Mann, 1992).

TABLE 2. Chlorophyll A, B and Carotenoids (mg/100g) in *Conocarpus erectus L. leaves* extracts.

Item	Concentration (mg/100g)		
	EOH	CAE	HAE
Chlorophyll A	0. 68^{a}±0.006	0.16^{b}±0.005	0.087^{c}±0.005
Chlorophyll B	0.13^{a}±0.007	0.12^{b}±0.005	0.016^{c}±0.007
Carotenoids	0.06^{a}±0.007	0.03^{b}±0.007	0.011^{c}±0.006

Values are means of three replicates ±SE.
Values followed by the same letters within the same row were not significantly different at 0.05 level
EOH (ethanolic extract)
CAE (cold aqueous extract)
HAE (hot aqueous extract)

TABLE 3. The concentration of phenolic compounds found in *Conocarpus erectus* L. leaves extracts.

phenolic compounds	Concentration (ppm)		
	EOH	CAE	HAE
Gallic	170^{a}	163^{b}	170^{a}
Pyrogallol	3500^{a}	870^{c}	1414^{b}
3- hydroxytyrosol	30^{a}	30^{a}	--
Protocatchouic	380^{a}	110^{c}	160^{b}
Chlorogenic	230^{b}	160^{c}	290^{a}
Catechol	190^{b}	14^{c}	212^{a}
Catechin	80^{a}	43^{c}	65.4^{b}
Caffeine	730^{a}	10^{b}	--
Caffeic	80^{b}	60^{c}	115^{a}
P- OH benzoic	290^{a}	30^{b}	--
4- amino benzoic acid	250^{a}	140^{c}	224^{b}
Vanillic	90^{a}	4^{b}	--
Ferulic	90^{b}	70^{c}	100^{a}
Iso-ferulic	10^{b}	2^{c}	12^{a}
Epi- catachin	200^{b}	210^{c}	290^{a}
Reversetrol	20^{a}	1^{b}	--
E- vanillic	1020^{a}	20^{b}	--
Ellagic	170^{a}	60^{c}	160^{b}
Alpha- coumaric	50^{a}	6^{c}	30^{b}
Salycillic	420^{b}	320^{c}	580^{a}
3,4,5 Methoxycinnamic	40^{a}	3^{c}	20^{b}
Coumarin	6^{b}	1^{c}	40^{a}
p- Coumaric	40^{a}	12^{c}	30^{b}
Cinnamic	40^{b}	22^{c}	42^{a}
Benzoic	190^{a}	30^{b}	--

Values followed by the same letters within the same row were not significantly different at 0.05 level
EOH (ethanolic extract)
CAE(cold aqueous extract)
HAE (hot aqueous extract)

TABLE 4. The concentration of flavonoid compounds of *Conocarpus erectus* L. leaves extracts.

Flavonoids	Concentration (ppm)		
	EOH	CAE	HAE
Naringin	20[a]	4[b]	0.3[c]
Rutin	40[a]	2[b]	3.3[b]
Hesperidin	--	140[a]	101[b]
Rosmarinic	1060[a]	30[b]	0.9[c]
Quercetrin	230[a]	12[b]	2.0[c]
Quercitin	120[a]	3[c]	6.3[b]
Luteolin	--	30[a]	29.39[a]
Kaempferal	60[a]	1[b]	0.09[b]
Hespertin	100[a]	5[b]	1.5[c]
Apegenin	30[a]	1[b]	0.95[b]
7- Hydroxyl-flavone	0.016[a]	0.04[a]	--

Values followed by the same letters within the same row were not significantly different at 0.05 level
EOH (ethanolic extract)
CAE (cold aqueous extract)
HAE (hot aqueous extract)

Antibacterial activity of extracts of Conocarpus erectus L. leaves on various clinical strains

Phytochemical constituents of medicinal plants are secondary metabolites that may act as antimicrobial agents (Marjorie, 1999). As shown in Table 5, aqueous extracts were active against for the tested bacteria (*Bacillus ceries, Staph.aureus, E.coli and Salmonella typhimurium*).The results indicated that the aqueos extracts of leaf crude were active in cold and hot extracts, therefore, extracts of crude leaf were more active in cold extract of hot extract in case of *Staph.aureus*.

extract of hot extract in case of *Staph.aureus*.

TABLE 5. Antibacterial activity of the extracts of *Conocarpus erectus* L. leaves on various clinical strains.

Organisms Bacterial	stains code Control	Gram Stain	Distilled sterile water	EOH	CAE	HAE
				Inhibition zone diameter (mm)		
Bacillus ceries	(ATCC-33018)	+	0.00	1.2[b]	1.7[a]	1.6[a]
Staph.aureus	(DSM-20231)	+	0.00	1.4[b]	1.8[a]	1.3[b]
E.coli	(ATCC -69337)	-	0.00	1.6[a]	1.36[b]	1.53[a]
Salmonella typhimurium	(ATCC-14028)	-	0.00	1.0[b]	1.5[a]	1.5[a]

Values followed by the same letters within the same row were not significantly different at 0.05 level
(EOH (ethanolic extract
(CAE (cold aqueous extract

Efficiency of Conocarpus erectus L. leaves extracts as anticancer agent

In 2018, 1,735,350 new cancer cases and 609,640 cancer deaths are projected to occur in the United States (Siegel et al., 2018). 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 (Hamed et al., 2016). 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 (Nagarajan et al., 2013).

Results in Table 6 showed that cytotoxic activity of *Conocarpus erectus* L. leaves as anticancer agent. As illustrated in Table 6, the most efficient cytotoxic activity of *Conocarpus erectus* L. leaves extracts was against (liver cancer) HEPG2 and (breast cancer) MCF7. Also, data indicated that the most efficient cytotoxic activity against (breast cancer) MCF7 in ethanolic extract with IC50 = 99 followed by hot aqueous extract with IC50 = 100 then cold aqueous extract with IC50 = 102 , while the most efficient cytotoxic activity against (liver cancer) HEPG2 in hot aqueous extract with IC50= 37.9 followed by ethanolic extract with IC50 = 39.3 then cold aqueous extract with IC50= 50.9 .

The current result was confirmed by Abdel-Hameed et al. (2012) who reported that the cytotoxicity of the ethyl acetate and n-butanol fractions of the different parts of C. erectus in HepG2 and MCF-7 cell lines by using the sulforhodamine B (SRB) and also confirmed by Maryam. (2015) who showed high cytotoxic activity toward the HepG2 cell line of the stems and leaves of *Conocarpus erectus* L.

(HAE (hot aqueous extract

TABLE 6. Cytotoxic activity of *Conocarpus erectus* L. leaves extracts.

Concentration (/ml)	Inhibition percent					
	MCF7 (breast)			HEPG2 (liver)		
	EOH	CAE	HAE	EOH	CAE	HAE
0.0	0.00	0.00	0.00	0.00	0.00	0.00
12.0	4.2	3.8	5	13.2	8.5	11.9
25.0	12.6	10.1	11.4	39.9	36.8	40.2
50.0	39.8	30	40.4	57.3	50.6	53.5
100.0	40.2	40.1	40.2	72.6	68.5	70.4
IC 50	99	102	100	39.3	50.9	37.9

MCF7 (breast carcinoma cell line)
HEPG2 (liver carcinoma cell line)
EOH (ethanolic extract)
CAE (cold aqueous extract)
HAE (hot aqueous extract)

Conclusion

From the aforementioned results, the extracts of *Conocarpus erectus* leaves present antioxidant, antimicrobial and anticancer properties, it could be concluded that the leaves extract can be used as a natural food additives instead of artificial food additives because of its negative effect on human health. and these findings contribute to scientific information for the effectiveness on use of this plant in the development of a phytotherapic compound.

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النشاط المضاد للأكسدة و الميكروبات و الأورام لمستخلصات أوراق نبات الكربس المصري

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نبات الكاربس هو شجرة دائمة الخضرة يصل ارتفاعها الى ٦ متر و منتشر زراعته فى مصر. يتميز بإحتوائه على الفينولات من أمثلة الفلافونيدات و التانينات و تعتبر مركبات أساسيه فيه. و يستخدم كعلاج عشبى فى العديد من الدول . أظهر نشاط عالى كمضاد للأكسده و الميكروبات والأورام و يرجع ذلك لوجود المركبات الفينوليه مثل الفلافونيدات و التانينات ويستخدم كعلاج عشبي فى العديد من الدول حيث أظهر نشاط. فى هذه الدراسه تم تقييم ثلاثة مستخلصات من أوراق هذا النبات كمضادات للأكسده و الميكروبات و الأورام (مستخلص ايثانولى - مستخلص مائى بارد مستخلص مائى ساخن)

وجد أن الخواص المضادة للأكسده لهذه المستخلصات مرتبطه بمحتوى المركبات الفينوليه و أظهرت نشاط عالى للشقوق الحره و كان أكثرهم المستخلص الأيثانولى يتبعه المستخلص المائى الساخن ثم المستخلص المائى البارد (٥.٩٨ و ٨٧.٩٦ و ١٠.٦٦) على التوالى. مما يجعل استخدام المستخلصات المائيه الساخنه و البارده كمصدر طبيعى مؤثر كمضاد أكسده. تم تحليل المستخلصات المختلفه على جهاز التحليل الكروماتوجرافى السائل و التعرف عليها بالمقارنه ببعض المركبات القياسيه.فكان البيروجالول و الكافيين و الفانيلك المركبات الفينوليه الرئيسيه فى المستخلص الإيثانولى(٠٠٥٣ و ٠٣٧ و ٠٢٠١) مائة جزء فى المليون على التوالى و لكن والمركبات الفينوليه الرئيسيه فى المستخلص المائى البارد هى البيروجالول و السليسليك و ابيكاتشين و الجاليك و البروتوكاتشويك(٠٧٨و ٠٢٣و٠١٢و ٣٦١ و ٠١١) مائة جزءفى المليون على التوالى. و تم تقييم المستخلصات كمضادات لنوعان من الميكروبات الموجبه لجرام و لنوعان من البكتريا السالبه لجرام وأظهرت النتائج نشاط فعال مضاد للميكروبات و كان اكثرهم فاعليه المستخلص المائى البارد عن الساخن فى حالة استافيلو كوكس اريوس.

تم قياس النشاط المضاد للأورام لهذه المستخلصات لأورام الثدى والكبد فكان النشاط المضاد لأورام الثدى فى المستخلص الايثانولى بينما كان أعلى نشاط مضاد لأورام الكبد فى المستخلص الساخن. وبناء على ما سبق يمكن الأستفادة من مستخلص الأوراق وذلك بأستخدامه كأضافات طبيعه للأغذية بدلا من الأضافات الصناعية و التى لها تأثير سلبى على صحة الأنسان .