

**Antioxidant and antibacterial studies on tea
cultivated in Kenya, Shaay Gabali and
Kenyan tea cultivated in Egypt**

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Abstract :

Tea has excellent flavour and enjoyable effect on the human body. A recent trial was successfully carried out to plant Kenyan tea in a private farm in Sharkia Governorate, Egypt. A wild plant, *Pulicaria incisa*, grow in Southern Sinai, Halayeb and Shalatin, is used as a tea substitute. The extraction by different solvents, the antioxidant and the antibacterial activities for the three types of tea were assessed. The extraction by water reflux gave the highest percentage in the three types of tea, and chloroform was the solvent that had the least extraction from all samples. This signified that most compounds found in the Kenyan black tea, Shaay Gabali and Kenyan tea cultivated in Egypt were polar. Kenyan tea cultivated in Egypt gave the highest antioxidant and antibacterial activities.

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Introduction :

Tea is one of the popular beverages in the world because of its excellent flavour and effect on the human body. These qualities distinguish tea from other products of plant origin, which impart good taste and aroma to various foods (Bokuchava and Skobeleva, 1969). The usefulness of the tea plant is due to the special composition of its young leaves, which provides the specific material for producing tea. Such materials include polyphenols and caffeine (Werkhoven, 1988).

Sen Gupta *et al.* (1958) indicated that water soluble extract in black tea ranged from 26.2 to 50.3%. Pure compounds were assayed for antioxidative activity and lipoxygenase inhibition activity. Flavanol showed the strongest antioxidative and lipoxygenase inhibition activity (Bijun-Xie *et al.*, 1993). Catechins and theaflavins contributed to antioxidant characteristics of tea. Black and green teas were not different in phenol content, in antioxidant strength or in antioxidant potential (Vinson and Dabbagh, 1998). The antioxidant effect of tea extracts was well correlated to their antimutagenicity in some cases but varied with the mutagen and antioxidative properties (Yen and Chen, 1995).

Shabana *et al.* (1988) studied the potential medicinal activity of wild Egyptian plants. They found that when the extracts of 60 desert plant species were tested on 5 species of bacteria and 3 species of fungi, *Pulicaria incisa* was from the most potent types. El-Kamali *et al.* (1998) collected the aerial parts of *Pulicaria undulata* that were screened for antibacterial properties. They found that the essential oils exhibited activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*.

Some observations indicated that those who continuously drink a large amount of green tea had less tooth decay. After a year of continuous surveillance at elementary schools, this was proven (Onishi et al., 1981a, b). They also reported that the green tea extract contained many active substances for caries prevention. The active principles have not yet been thoroughly defined, although several polar polyphenolic compounds in the green tea had already been reported as moderate antibacterial principles (Sakanaka et al., 1989) against *Streptococcus mutans*, which is one of the bacteria responsible for causing dental caries (Hamada and Slade, 1980).

Therefore, the safety of these products is the first consideration. It is possible that edible plants, beverages, and food spices may be a superior source of new antimicrobial agents. With this in mind, the same green tea flavour compounds were also tested against 12 additional selected microorganisms (Himejima and Kubo, 1991). They found that the antimicrobial activity of the 10 most abundant volatile components of green tea flavour was examined. The activity of each volatile substance was moderate but broad in spectrum.

The aim of the present work is to evaluate the antioxidant and antibacterial characteristics of each of Kenyan black tea, Shaay Gabali and the Kenyan tea cultivated in Egypt.

Materials and Methods

Materials:

Kenyan black tea:

Camellia sinensis (**Theaceae**).

One kg of processed tea was imported from Kenya.

Shaay Gabali:

Pulicaria incisa (Boulos, 1995) (**Compositae**).

Collected from Southern Sinai, Halayed and Shalatin.

Kenyan green tea:

Camellia sinensis (**Theaceae**)

Experimentally cultivated in Egypt.

Methods:

Preparation of sample:

- The Kenyan black tea samples were ground using a high speed to pass in a 0.56 mm. sieve.
- Shaay Gabali fresh plants were dried at room temperature. Leaves were separated and well ground to pass in a 0.56 mm sieve.
- The fresh plants of Kenyan green tea cultivated in Egypt were dried at room temperature. Leaves were separated and well ground to pass in a 0.56 mm sieve.

Determination of antioxidant activity of tea:

a) Preparation of extractions:

- * 50 ml chloroform were added to 5 gms tea and allowed for 24 hours, then the solution was filtered by filter paper Wattman No. 1 using another 50 ml chloroform.
- * Different solvents were used i.e. methanol (60%, 70%, 80%) ethanol (60%, 70%, 80%), distilled water, and tea was refluxed with distilled water.
- * The filtered solutions were evaporated under vacuum, the dried extractions were weighed and percentages were calculated according to A.O.A.C. (1984).

b) Determination of antioxidant activity:

- * 10 ml methanol 90% dissolved 0.02 gm of the different dried extracts.
- * Different solvent extracts were added to 100 ml sunflower oil at concentration of 0.02%.
- * Oxidation at 60.C (oven test) and measurement of peroxides were carried out according to A.O.A.C. (1984).
- * Stability curves were plotted as milliequivalent peroxide/kg sample, the time (hours) to reach a peroxide value and the induction period were calculated according to A.O.A.C. (1990).

Determination of antibacterial activity:

The agar medium was composed of (gm/l) = peptone 6.0, yeast extract 3.0, beef extract 1.5, dextrose 1.0, agar 15 and distilled water up 1000 ml. Methylene blue 0.5 ml was added after the digestion of the agar medium which turned the colour of the medium from pale yellow to blue. The pH was 6.5 after sterilization. The agar medium was seeded with the standard inoculum of the test organism [*E. coli* (0157)] before solidification. About fourteen-ml agar medium was poured into sterile petri dishes of diameters 150 x 20 mm under aseptic conditions, mixed and allowed to set on a leveled plate form to obtain a homogenous seeded layer of the same thickness. Pores of 9 mm diameter were introduced in the solid agar medium by a sterile cork borer 0.1 ml of the aqueous extract to the pores under aseptic condition.

The petri dishes were stored at 5°C in a refrigerator for one hour to permit a good diffusion of the extracts. The plates were then in-

cubated at 37°C for 48 hours to obtain clear transparent inhibition zones. Diameters of inhibition zone were recorded (Abou-Zeid and Shehata, 1969).

Results and Discussion

Extraction by different solvents

From Table (1) it could be observed that chloroform was the solvent that had the least extraction from all samples. This signified that most compounds found in the Kenyan black tea, Shaay Gabali and tea cultivated in Egypt were polar compounds. It might be also noticed that extraction percentage decreased with the increase in concentration of both methanol and ethanol solutions. This might emphasize the previous conclusion that most compounds found in all teas were polar compounds. In addition, extraction with water increased under reflux.

Table (1) Tea extraction by different solvents.

Solvents		Percent Extract, %		
		Kenyan Black Tea	Shaay Gabali	Locally Cultivated Kenyan Tea
Chloroform		3.44	4.78	4.26
Methanol	60%	33.85	12.61	21.94
	70%	31.48	9.67	20.66
	80%	22.99	9.46	19.81
Ethanol	70%	34.06	8.59	17.15
	80%	30.73	8.15	15.44
Distilled Water		20.84	13.91	15.12
Water Refluxed		41.79	26.63	48.39

Extraction with methanol and ethanol of Kenyan black tea and tea cultivated in Egypt were relatively higher than that with water. This might be attributable to the presence of organic compounds in these teas which were insoluble in water but soluble in organic solvents such as methanol and ethanol. Generally, it was noticed that extraction of Kenyan black tea with methanol, ethanol and water had the highest values followed by extraction of the tea cultivated in Egypt while it was found to be the least in Shaay Gabali.

Antioxidant activity

The antioxidant activity of tea extracts was listed in Table (2). It could be observed that tea cultivated in Egypt gave higher antioxidant activities when chloroform, methanol 60%, methanol 70% and ethanol 70% were used for extraction. Shaay Gabali gave higher antioxidant activities when methanol 70% and ethanol 80% were used for extraction, as the induction periods were 19.6 and 10 hours, respectively.

When methanol concentration diluted from 70% to 60%, it extracted components with antioxidant activity that decreased 2.5 times. This was compatible with the findings of Bassiouny et al. (1988), which showed that the antioxidant activity of spearmint extract decreased when added to soybean oil at a concentration of 0.02% instead of that of 0.01%. The present work indicated that, the extraction with chloroform yielded components with pro-oxidant activity as the induction period was 2.5 hours. It was also noted that the Kenyan black tea under the extraction conditions of ethanol had no antioxidant activity. Addition of 0.02% of chloroform extraction from Kenyan black tea exhibited pro-oxidant activity to sunflower oil.

Table (2) Antioxidant activity.

Solvents		Induction Period		
		Kenyan Black Tea	Shaay Gabali	Locally Cultivated Kenyan Tea
Chloroform		3.5 hours	2.5 hours	19 hours
Methanol	60%	--	7 hours	21 hours
	70%	--	19.6 hours	19 hours
Ethanol	70%	5 hours	--	19 hours
	80%	5 hours	10 hours	4 hours

Blank (IP) → 5 hours.

Tea cultivated in Egypt might be considered the best source of antioxidant activity compared with the other two teas, when all solvents were used in extraction with the exception of ethanol 80%. Serafini et al. (1996) and Gerhard et al. (1989) reported that, in vitro, the antioxidant capacity of green tea was much higher than that of black tea, since polyphenols are higher in green tea.

The highest induction period was found when methanol 60% was used for the extraction process from tea cultivated in Egypt. Addition of such extraction in concentration of 0.02% increased stability period of sun flower oil by four times compared to the oil without any addition (control sample). Considering tea cultivated in Egypt as a raw material, components soluble in ethanol 80% exhibited pro-oxidant activity since the induction period was (4 hours) compared with the induction period of blank (5 hours).

Antibacterial activity

Table (3) showed no difference in antibacterial effectiveness, when different concentration of methanol was used in the extraction procedure from tea cultivated in Egypt. Ethanol 70% extract exhibited higher antibacterial activity. Extraction by reflux gave extracts with high antibacterial activity than that using water extraction under room temperature, this phenomena was found in all raw materials investigated.

It could also be noticed that Kenyan black tea had a higher antibacterial potency zone when methanol 60%, methanol 70% and refluxed water were used for extraction following a descending order. When ethanol 80% and distilled water were used in the extraction process from tea cultivated in Egypt, components were extracted with less antibacterial potency zone (1.2 cm). Components extracted by ethanol 80% and distilled water from Kenyan black tea had the least antibacterial potency zone (1.1 cm).

Kenyan black tea was lower than the tea cultivated in Egypt as an antibacterial agent when methanol and ethanol were used for extraction. Results presented in Table (3) recommended that chloroform should not be used in the extraction process from the material under investigation. Chloroform extracted components had no antibacterial effect. This might be attributed to the non-polar nature of such components, as the non-polar components are soluble in the non-polar solvents (Chloroform). Such non-polar components are water-insoluble and do not diffuse into the media because these compounds are partially or even entirely evaporated from the paper disk when the solvent was removed. Several polar polyphenolic compounds in the green tea

had already been reported as moderate antibacterial principles (Sakanaka et al., 1989) against *Streptococcus mutans*.

Table (3) Antibacterial activity.

Solvents		Antibacterial Potency Zone, cm		
		Kenyan Black Tea	Shaay Gabali	Locally Cultivated Kenyan Tea
Chloroform		0	0	0
Methanol	60%	1.7	0	2.0
	70%	1.6	0	2.0
	80%	1.3	0	2.0
Ethanol	70%	1.3	0	1.7
	80%	1.1	0	1.2
Distilled Water		1.1	1.2	1.2
Water Refluxed		1.5	1.3	1.5

Results (Table 3) showed that, in Kenyan black tea, antibacterial activity increased as methanol concentration decreased. Methanol 60, 70 and 80% extracted components with different antibacterial potency zone of 1.7, 1.6 and 1.3 cm respectively. The antibacterial potency zone was 2.0 cm when different concentrations of methanol were applied on tea cultivated in Egypt. Different concentrations of ethanol extracted components with different antibacterial activity were found either in tea cultivated in Egypt or in Kenyan black tea. From the previous results, it could be observed that antibacterial activity of different extracts from teas under investigation, might be attributed to their polarity. The relationship between compounds activity and their

structure were described by Farag et al. (1989) and Kubo et al. (1992).

Considering the foregoing results and discussions and conclusions reached by Abd El-Razik Ali et al. (1992); Gad et al. (1989) and Shelef et al. (1984), it could be concluded that hydrophilic/lipophilic balance, relative basicities and the structure of the compounds were factors likely to be involved in their antimicrobial activity. Shaay Gabali exhibited no antibacterial effect when methanol and ethanol were used for extraction. Only water that extracted components with low antibacterial activity (1.2-1.3cm).

Yoshino et al. (1996). reported that extracts of green and black tea leaves showed strong antimicrobial activities against *Streptococcus mutans*. Results encountered (Table 3) showed that edible beverages might be a superior source of new antibacterial agents, as the safety of these materials would have the first consideration. From Tables (2 and 3), it could be concluded that methanol 60% had the extract that had the highest antioxidant and antibacterial activities from tea cultivated in Egypt.

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دراسات على المواد المضادة للأكسدة والمضادة للبكتريا للشاي المنزرع فى كينيا والشاي الجبلى والشاي الكينى المنزرع فى مصر

يتميز الشاي بطعمه وتأثيره الجيد على جسم الإنسان - ترجع هذه الفائدة إلى مكونات أوراقه الخاصة التى تعطى أجود إنتاج من الشاي . وقد نجحت محاولات زراعة الشاي الكينى فى مزرعة خاصة فى محافظة الشرقية بمصر. يوجد نبات برى يسمى الشاي الجبلى *Pulicaria incisa* ينمو فى مناطق مختلفة من جنوب سيناء . وقد اكتشف العلماء أن المستخلص لهذا النبات يعمل على خفض الكوليسترول ، كما أنه يستخدم كمنشط وكبديل للشاي لاحتوائه على الكافيين. وقد أجرى هذا البحث على المواد المستخلصة من الشاي الكينى الأسود والشاي الجبلى والشاي الكينى المنزرع فى مصر. فقد تم الحصول على المستخلص بمذيبات مختلفة للثلاث أنواع من الشاي . ودرست الأنشطة المضادة للأكسدة ، والمضادة للبكتريا .

وقد أشارت نتائج النشاط المضاد للأكسدة إلى أن الشاي المنزرع فى مصر أعطى أعلى فترة ثبات *induction period* حيث كانت القيم ٢١ ، ٦ ، ١٩ ، ٥ ساعات للشاي المنزرع فى مصر ، الشاي الجبلى والشاي الكينى الأسود ، على التوالى ، مما يشير إلى أن الأخير كان أقل نشاط مضاد للأكسدة مقارنة بالنوعين الآخرين. كما أوضحت نتائج النشاط المضاد للبكتريا إلى أن الميثانول ٦٠ ، ٧٠ ، ٨٠٪ أعطى مستخلص للشاي المنزرع فى مصر ذو تأثير أعلى مضاد للبكتريا (٢ سم) ، وأعطى الميثانول ٦٠٪ مستخلص للشاي الكينى الأسود ذو تأثير مضاد للبكتريا عال (٧ ، ١ سم) مقارنة بالمذيبات الأخرى. بينما كان للشاي الجبلى تأثير مضاد للبكتريا مع الماء باستخدام المكثف (٣ ، ١ سم) ويمكن أن يستنتج من ذلك إلى أن الشاي المنزرع فى مصر له أعلى نشاط مضاد للبكتريا يليه الشاي الكينى الأسود مع جميع المذيبات ، لكن مستخلص الشاي الجبلى كان له نشاط مضاد للبكتريا فقط مع الماء المقطر والماء باستخدام مكثف.