Chemical and microbiological studies on Kenyan and Gabali Egyptian tea

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ABSTRACT

The present study was designed to compare the quality of Gabali Egyptian tea and Kenyan tea locally cultivated in Egypt. Gabali, black and green Kenyan teas were laboratory processed, then analyzed chemically and microbiologically for their quality evaluation. Representative results show that all samples of the 3 types were contaminated with aerobic mesophilic plate counts of the tea types varied from $6.1x10^3$ to $2.2x10^8$ cfu/g, spore-forming bacteria $1.1x10^2$ to $1.6x10^4$ cfu/g, coliforms (MPN) 0.36- 1.5 $x10^1$ cell/g, molds $2.3x10^2$ to $3.0x10^4$ and yeasts $1.2x10^2$ to $5.8x10^3$ cfu/g, in which gabali tea was the highest and black Kenyan tea was the lowest microbial count. Pathogenic bacteria were not in detectable level. The black Kenyan tea had the highest antibacterial activity followed by the green Kenyan tea with all solvents, but extraction of Gabali tea had antibacterial activity only by distilled water and water reflux. Total aflatoxin in concentration range of 6.1, 9.8 and >50 µg/kg in black and green Kenyan tea and Gabali tea, respectively. Phytochemical analysis of the tea samples revealed the presence of tannin in high level in black Kenyan tea (14.33%). Considering the results obtained, only black and green Kenyan tea had acceptable levels for all microbial factors in comparison with the Egyptian standards. It was concluded that Gabali tea may be high risk product and therefore, more studies are necessary to find methods of decontamination.

Keywords: *Kenyan tea, Gabali tea, microbial analysis, total aflatoxin, tannin.*

INTRODUCTION

The beverage known as tea is an infusion of variously processed leaves of an evergreen shrub Kenyan tea (*Camellia sinensis* L.), which have been reported, while gabali tea (*Pulicaria incisa*) have been less widely used.

Camellia sinensis (L.) Kuntze (Theaceae) is probably one of the most investigated plants for its medicinal and food applications, and its main use is to prepare tea, a very popular beverage made by brewing leaves and buds. Global trends in tea consumption are evolving; FAO predicts world black tea and green tea production growth respectively at 1.87 and 7.2 % annually over the next 10 years **(Piovan *et al*., 2014)**.

Green tea can be prepared as a drink, which can have many systemic health effects or an "extract" can be made from the leaves to use as medicine. It is reported to contain thousands of bioactive ingredients which are almost contributed by polyphenols which plays a key role in prevention and treatment of many diseases **(Namita *et al*., 2012)**.

Black tea has many more components than green tea, partly because of the oxidation processes; the phenolic compounds found with high concentration in back tea extract **(Passat, 2012)**.

Pulicaria incisa belongs to family Asteraceae is a woolly tomentose annual or short lived perennial with erect stems that are branched mainly from the base. It is used by the natives of some upper Egyptian areas in the form of a decoction, sweetened with sugar as substitute for tea. The plant also used as a traditional medicine for treating heart diseases by Bedouins **(Ewais *et al*., 2014)**.

Microbial population increased 8-9 times in samples stored under accelerated storages over normal storage (**Debnath *et al*., 2012)**. All tea products shall comply with microbiological regulations by the Egyptian Standards **(ES: 559-1/2005)** in which pathogenic bacteria should be absent, molds and yeasts $\leq 10^3$ and coliform group $\leq 10^1$ cfu/g. According to

Hossain *et al*. (2013), made tea liquor has shown to have antimicrobial property. Boiling in tea preparation and its' liquor antimicrobial property considerably reduced the level of microbial load to safe level for public consumption.

The presence of fungal strains in tea leaves and the presence of aflatoxin have been reported by **Viswanath *et al*. (2012)** from all stages of tea processing and manufacturing. Results presented by **Ma *et al*. (2013)**, indicated that 5.3% of tea samples contaminated with aflatoxins. There is no reported aflatoxiginic food safety hazards related to tea, but **UNBS (2013)** suggested that the maximum content of total aflatoxin in herbal tea products shall not exceed 10µg/kg.

Phytochemical screening of the herbal tea was revealed the presence of tannin **(Omogbai and Ikenebomeh, 2013)**. **Khasnabis *et al*., (2015)** found that samples of black tea had highest tannin content while green tea had lowest tannin content. Tannin content in black tea ranged from 11.76 to 15.14% with an average of 13.36%. Highest tannin content in the studied samples of green tea was 3.11% with an average of 2.65%.

The objective of this investigation was to determine the quality of the Gabali tea in a comparison with the Kenyan (black and green) tea.

MATERIALS &METHODS

- ***Samples:***

Gabali tea (*Pulicaria incise*) was collected from Gabal El-Tore, in April 2013 after rain season. Fresh harvest young tea shoots were spread in a wooden try, and dried in shade for three days at room temperature (28℃).

Kenyan fresh tea (*Camellia sinensis*) was cultivated at March 2015, in a green house at Desert Research Center, by giving nodes from a private farm in Diarb Negm, Sharkeya Governorate. The buds and the three adjacent leaves were pruned and subjected to the following steps to prepare the black and the green Kenyan tea: the black Kenyan tea **(Sato *et al*., 2007)**: The pruned leaves

were withered, rolled, fermented and dried.

The green Kenyan tea **(Singh *et al*., 2014)**: The leaves were steam blanched, withered, rolled and dried.

- ***Microbial analysis:***

Twenty-five grams of the sample were aseptically weighed in sterile stomacher bags, diluted with 225 ml peptone water, homogenized in a stomacher (Seward Stomacher 3500, Lab system, England) for 2 min (10^{-1} dilution) and serially diluted in 9 ml of peptone water **(Soriano *et al*., 2002)**. Microbial profile, i.e., Total viable bacteria, Spore-forming bacteria, Fungal (mold and yeast) count, Coliform group, *E. coli*, *B. cereus*, *Staph. aureus*, *Cl. perferingens* and *Salmonella sp*., were performed according to the procedures recommended by the International Commission of Microbiological Specification for Foods **(ICMSF, 1978 and 1996; and Harrigan, 1998)**. Each value represents the average of three replicates.

- ***Preparation of aqueous and alcoholic extracts:***

According to **Mukhtar and Ghori (2012)**, sampled were soaked in 200 ml of distilled water, ethanol (95%) and methanol (95%) and were kept at room temperature for 24 hours, then were filtered using Whatman no. 1 filter paper. The filtrate was heated at 40-50 ℃ using water bath, until thick paste is formed. The thick paste was considered as 100% concentration of extract.

- ***Antimicrobial activity:***

It was determined by the well diffusion method according to **Abu-Shanab *et al*. (2004)**. Petri plates containing 20 ml of Mueller Hinton agar medium were seeded with a 24 h culture of *E. coli* strain (ATCC 10536). Wells (6 mm diameter) were cut in the agar and 50 µl of the plant extracts were tested in a concentration of 60, 70 and 80%. Inoculation was performed at 37℃ for 24 h. The assessment of antibacterial activity was based on measurement of the diameter of the inhibition zone formed around the well. A

standard tetracycline was used as a positive control.

- ***Total aflatoxin analysis:***

Twenty five grams of ground samples were extracted and subjected to total aflatoxin Neogen Veratox® testing kits following its instructions **(AOAC, 2003 and Harrigan, 1998)**. It was determined by Competitive Direct-Enzyme Linked Immunoscorbent Assay (CD-ELISA) technique using MRX microwell reader (Dynatech Laboratories) with software version 1.2 to values in ppb or µg/kg.

- ***Phytochemical analysis:***

Tannin was determined by the protein precipitation method as described by **Egyptian standards (ES: 559-2/2005)**.

RESULTS &DISCUSSION

The results of microbial analysis including total viable bacteria, sporeforming bacteria, coliform group, *E. coli, Staph. aureus, B. cereus, Salmonella spp*., Molds and yeasts, from the 3 types of tea are described in **Fig. (1)**. these results show that all collected samples were contaminated.

On the whole, the highest bacterial count was found in Gabali tea with a total count of $2.2x10^8$ cfu/g.

Pathogens, i.e., *E. coli*, *Staph. aureus, B. cereus* and *Salmonella spp* were not found in any of the samples.

In case of black and green Kenyan tea, microbial profile, i.e., molds, yeasts, coliform group and pathogenic bacteria were of satisfactory quality according to the Egyptian standards **(ES: 5559-1/2005)**, whereas Gabali tea exceeds these parameters.

The occurrence of microorganisms in tea samples is common but unfavorable phenomenon. The results of the present study demonstrated the presence of both microbiological profile and pathogenic microbial flora.

The presence and numbers of bacteria could be explained by the fact that some of these organisms like *Bacillus sp* produce spores which are resistant to harsh processing elevated heat and dry conditions.

Therefore, they can survive for a long time in the product in a dormant state **(Omogbai and Ikenebomeh, 2013)**.

Staphylococcus aureus is organism which can be transferred from humans to teas during processing **(Omogbai and Ikenebomeh, 2013)**.

Handling and packing after drying may result in contamination of processed tea leaves with microorganisms. Moreover, environmental dust settling on different parts of the plant can potentially carry bacterial and mould spores **(Abd El-Aty *et al*., 2014).**

The use of hot water for preparing a tea infusion may reduce microbial risks. However, water at sub-boiling temperature may not eliminate all microorganisms, including bacterial and mould spores **(Mishra *et al*. 2006)**.

Plant polyphenols, tannins, have been suggested to exert their growth inhibitory effects through auto-oxidation and hydrogen peroxide production, but in certain circumstances some bacterial genes may be induced (like Oxy R in *Escherichia coli*) so that strengthening bacterial antioxidant defense mechanisms may overcome tannin inhibitory effects **(Neyestani *et al*., 2007)**.

Fig. (2), Showed an average value of tannin in black Kenyan tea of 14.33%. This coincided with those of **Khasnabis *et al*. (2015)**; it was found that samples of black Kenyan tea had highest tannin content while green Kenyan tea had lowest tannin content. Tannin content in black Kenyan tea ranged from 11.76 to 15.14% with an average of 13.36%. Highest tannin content in the studied samples of green Kenyan tea was 3.11% with an average of 2.65%.

Camellia sinensis contains many phytochemical compounds with antimicrobial properties. Tannins component (are polyphenol group) present in tea 15.20 % damage bacterial membrane, precipitate protein and having chelating properties contributed to antibacterial activity of tea.

These antimicrobial activity of tea may refer to several chemical components

found in tea like polyphenolic (these make up some 30% of dry weight) and generally known as "tannin" which are chemically different from other plant tannin, the simplest compound in this class are the catechin (found in green and black tea), inhibits the growth of many bacterial species **(Passat, 2012)**.

It was reported that the percentage of element leached to the infusion was strongly related to tannin contents in the beverage, i.e. the lower the tannins the better the leaching **(Wróbel *et al*., 2000)**.

The antibacterial activity results in **Table (1)** indicated that methanol 60, 70, 80 % gave an extraction of tea cultivated in Egypt with the highest antibacterial potency zone (2 cm). Methanol 60% gave extraction of black Kenyan tea that had high antibacterial potency zone (1.7 cm) in comparison with the other solvents. However, Gabali tea had an antibacterial potency zone with water reflux (1.3 cm). This might indicate that tea cultivated in Egypt had the highest antibacterial activity followed by black Kenyan tea with all solvents, but extraction of Gabali tea had antibacterial activity only by distilled water and water reflux.

In the same context, **Hossain *et al*. (2013)** found that boiling in tea preparation and its' liquor antimicrobial property considerably reduced the level of microbial load to safe level for public consumption. Phenols have antibacterial and anti-inflammatory activities **(Vijayvergia and Kumar, 2007)**.

Results shown in **Fig (3)**, point to an extremely high aflatoxin content in gabali tea (>50 µg/kg) followed by green Kenyan tea (9.8 µg/kg) and black Kenyan tea (6.1 µg/kg), in descending order. Whereas the reference range of the aflatoxin content in tea (10µg/kg) is mentioned only in **UNBS (2013)**, the aflatoxin in Gabali tea was above the maximum standard limit, while in black and green Kenyan tea was within the acceptable limit. Very little information is available on the mycotoxin of tea and the associated quality problems. **Prado *et al*. (2012)** reported that temperature and water activity

are the main factors that influence fungal invasion and the production of aflatoxins in stored green tea. Also, black tea is a good substrate for the production of aflatoxins by the fungus *A. flavus* **(Viswanath *et al*., 2012)**. It is from the dried leaves of the tea plant *Camellia sinensis*, processing involves a series of steps before the final product is ready. It involves withering, rolling, fermentation and firing. Fungi have been isolated from all stages of tea processing and manufacturing and recontamination of the final product after firing may also occur during sorting and packaging. Microbiological contamination of tea during processing and manufacturing may facilitate their contamination with mycotoxins **(Al-Sohaibani *et al*., 2011)**.

With respect to spoilage, the concern is mainly the possible growth of molds, when tea becomes sufficiently wet to reach a water activity of 0.7. This may occur during transportation and storage and is normally visible as a recognized defect **(Lund and Baird-Parker, 2000)**.

CONCLUSION

The results of this study have shown that microorganisms associated with Kenyan and Gabali teas. The Gabali tea is heavily contaminated with microbial and aflatoxic content above the acceptable levels contrary to Kenyan tea. These results suggest sanitary conditions at different stages in production must be improved to reduce the potential hazard to human health.

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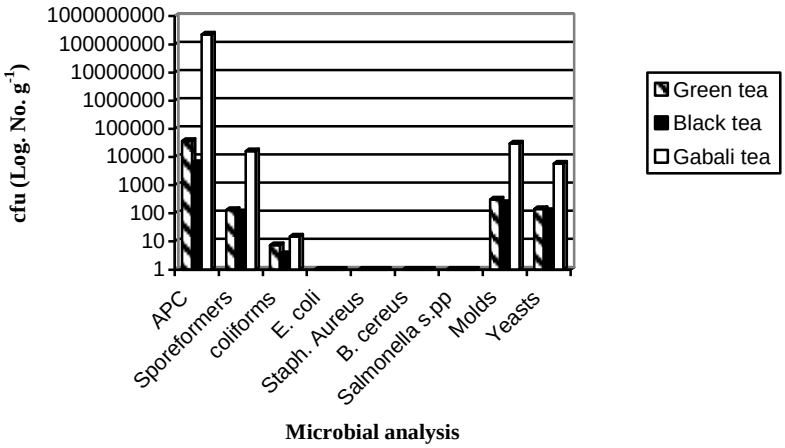

Figure 1. Occurrence of the most frequent microbial contaminants in Kenyan and Gabali Egyptian teas (in logarithmic scale).

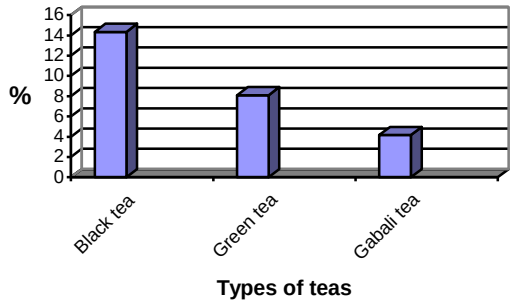

Figure 2. Occurrence of tannin in Kenyan and Gabali teas.

Table 1: Antibacterial activity of different tea extracts against *E. coli* strain.

Solvents		**Antibacterial potency zone, cm**		
		Black Kenyan tea	Green Kenyan tea	**Gabali 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 reflux		**1.5**	**1.3**	**1.5**

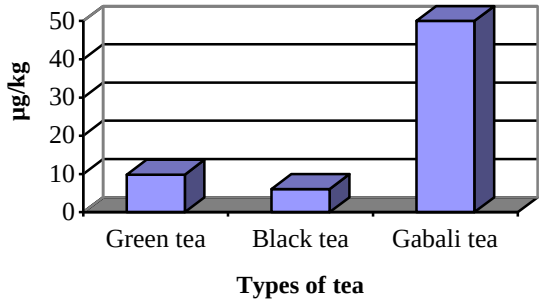

Figure 3. Occurrence of total aflatoxin in Kenyan and Gabali teas.

دراسه كيميائيه و ميكروبيه على الشاى الكينى والجبلى المصرى

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الملخص العربي

أجريت هذه الدراسه بغرض المقارنه بين جودة الشاى الجبلى المصرى والشاى الكينى المزروع محليا فى جمهورية مصر العربيه. تم تصنيع الشاى الجبلى و الشاى الكينى الأسود و الأخضر معمليا ثم تحليلهم كيميائيا و ميكروبيا لتقييم جودتهم. أظهرت النتائج تلوث الأصناف الثلاثه بالبكتريا الهوائيه و تتراوح ما بين ٦،١ x ١٠ [٣] إلى ٢،٢ x ١٠ [٨] خليه/جرام، البكتريا المكونه للجراثيم ١،١ x ١٠ [٢] إلى ١،٦ x ١٠ [٤] خليه/جرام، بكتريا مجموعة القولون (بطريقة العد الأكثر إحتمالا) ٠،٣٦ إلى ١،٥ x ١٠ [١] خليه/جرام، الفطريات ٢،٣ x ١٠ [٢] إلى ٣،٠ x١٠ [٤] والخمائر ١،٢ x ١٠ [٢] إلى ٥،٨ x ١٠ [٣] خليه/جرام، بحيث كان الشاى الجبلى الأعلى و كان الشاى الأسود الأقل فى الحمل الميكروبى و لم تظهر ميكروبات ممرضه فى أى من العينات. كان مستخلص الشاى الكينى الأسود له أعلى تأثير مضاد للبكتريا يليه الشاى الكينى الأخضر مع كل المذيبات، بينما الشاى الجبلى كان له تأثير مضاد للبكتريا فقط عند إستخلاصه بالماء المقطر والماء المعاد تكثيفه. كان تركيز الأفلاتوكسين الكلى فى حدود ٦،١ و ٩،٨ و أكثر من ٥٠ ميكروجرام/كجم فى الشاى الكينى الأسود و الأخضر و الجبلى على الترتيب. أظهر التحليل الفيتوكيميائى وجود التانين بتركيز عالى فى الشاى الكينى الأسود (١٤،٣٣٪). بالنظر إلى النتائج المتحصل عليها، وجد أن الشاى الكينى الأسود و الأخضر فقط يطابقا المواصفات القياسيه المصريه. و قد خلصت نتائج الدراسه إلى أن الشاى الجبلى يحتوى على نسبه عاليه من المخاطر مما يستوجب إجراء مزيد من الدراسات لإيجاد وسائل لتقليل الملوثات الموجوده به.

الكلمات الداله: *شاى كينى، شاى جبلى، تحليل ميكروبى، أفلاتوكسينات كليه، تانين.*