

## Antioxidant activity, polyphenolic composition and *in vitro* antibacterial and antifungal activities of tea seed oil

H.K. Ruto<sup>a,b,✉</sup>, J.O. Yugi<sup>a</sup>, S.O. Ochanda<sup>b</sup> and C. Bii<sup>c</sup>

<sup>a</sup>University of Kabianga – Biological sciences

<sup>b</sup>Kenya Agricultural and Livestock Research organization – Tea Research Institute

<sup>c</sup>Kenya Medical Research Institute – Centre for Respiratory Diseases Research

✉Corresponding author: henrikuto2017@gmail.com

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**SUMMARY:** The polyphenolic composition and antioxidant activity of tea seed oil from *C. sinensis* TRFK 301/5 (green colored) and TRFK 306 (purple colored) and *C. oleifera* were evaluated. The total polyphenolic content, total catechins and catechin fractions were significantly different in the oils. *C. oleifera* contained significantly ( $p \leq 0.05$ ) higher amounts of catechins and polyphenols than *C. sinensis*. *C. oleifera* also exhibited a higher DPPH radical scavenging activity ( $18.81 \pm 0.46\%$ ) compared to *C. sinensis* (TRFK 306;  $15.98 \pm 0.13$  and TRFK 301/5;  $14.73 \pm 0.47\%$ ). The antimicrobial activities of tea seed oil and two selected oils (olive and eucalyptus oil), were also evaluated against *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, *Cryptococcus neoformans* and *Trichophyton mentagrophytes*. *S. aureus* was significantly inhibited by the oils compared to *E. coli*. The oils inhibited the growth of *T. mentagrophytes* and *C. albicans*, although they had no effect on *C. neoformans*. Tea seed oil is a potential source of beneficial phytochemicals and potent antimicrobial agents.

**KEYWORDS:** Anti-microbial activity; Antioxidant activity; Catechins; Polyphenol; Tea seed oil

**RESUMEN:** *Actividad antioxidante, composición polifenólica y actividades antibacterianas y antifúngicas in vitro del aceite de semilla de té.* Se evaluó la composición polifenólica y la actividad antioxidante del aceite de semilla de té de *C. sinensis* TRFK 301/5 (color verde) y TRFK 306 (color púrpura) y *C. oleifera*. El contenido polifenólico total, catequinas totales y fracciones de catequinas fueron significativamente diferentes en los aceites. *C. oleifera* contenía cantidades significativamente mayores ( $p \leq 0.05$ ) de catequinas y polifenoles que *C. sinensis*. *C. oleifera* también exhibió una mayor actividad de eliminación de radicales DPPH ( $18,81 \pm 0,46\%$ ) en comparación con *C. sinensis* (TRFK 306;  $15,98 \pm 0,13$  y TRFK 301/5;  $14,73 \pm 0,47\%$ ). También se evaluó la actividad antimicrobiana del aceite de semilla de té y dos aceites seleccionados (aceite de oliva y de eucalipto) frente a *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, *Cryptococcus neoformans* y *Trichophyton mentagrophytes*. *S. aureus* fue inhibido significativamente por los aceites en comparación con *E. coli*. Los aceites inhibieron el crecimiento de *T. mentagrophytes* y *C. albicans*, sin embargo, no tuvieron ningún efecto sobre *C. neoformans*. El aceite de semilla de té es una fuente potencial de fitoquímicos beneficiosos y potentes agentes antimicrobianos.

**PALABRAS CLAVE:** Aceite de semilla de té; Actividad antimicrobiana; Actividad antioxidante; Catequinas; Polifenol

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## 1. INTRODUCTION

Tea is used to manufacture tea products that are used in beverages all over the world. The products come mainly from the leaf. The tea plant has been given a lot of attention by researchers due to its immense health benefits (Schneider and Segre, 2009). In China for example *C. oleifera*, is the most important and widely distributed of the *camellia* species and is used in seed oil production which is important in traditional therapy for burns and stomach aches, ringworms and dandruff in China. The oil is used as a crop food oil, and additives for cosmetic products among other uses, while tea seed cake, a by-product, is utilized as feed additives in aquaculture and livestock production (George *et al.*, 2013). Iodine value, saponification value, peroxide value, free fatty acids, total polyphenols and antioxidant activity were determined. The oil yields ranged between 16 to 25% w/w. Iodine value was in the range of 86 to 91 g I<sub>2</sub>/100 g, peroxide value < 3.5 meq O<sub>2</sub>/kg, saponification value between 182 to 187 mg KOH/g, free fatty acid < 1.5% oleic acid, total polyphenols 0.036 to 0.043 mg/L gallic acid and antioxidant activity of between 14 to 21% 2,2-diphenyl-1-picrylhydrazyl (DPPH). Whole tea seed powder is mainly used in aquaculture as feed additives to help prevent bacterial infections in fish (Rico *et al.*, 2012).

*C. sinensis* is majorly grown for its leaves used in the manufacture of black tea, the most consumed beverage second only to water. However, *C. sinensis* has been seen to produce a lot of seeds especially when under stressful environmental conditions. The tea seed oil from *C. sinensis* is rich in phytochemicals such as polyphenols, catechins, saponin and squalene. In other studies, these phytochemicals have been demonstrated as antimicrobial agents and also have been shown to exhibit antioxidant properties.

Infectious diseases caused by various microorganisms are a burden globally. To combat these diseases, it is essential that novel antimicrobial agents are developed. Doctors utilize synthetic antibiotics all over the world to manage and treat microbial related disease. However, most antibiotics are synthetic and may have adverse side effects on many occasions (Cunha, 2001).

The antimicrobial properties of various plant extracts are due to the various phytochemicals available in them which are used to carry out life functions as well as defense mechanisms against predators (Tayel

*et al.*, 2011). Most of these phytochemicals have been found useful by humans especially for treating various diseases. Phytochemicals are usually found in the barks, leaves, roots and other parts of plants. The presence of phytochemicals in plants is a major contributor of the use of plants as natural medicine worldwide (Ahmad and Beg, 2001).

The *in vitro* antimicrobial activity of extracted tea seed oil has not been researched extensively. The eradication of multidrug resistant microbes is currently a challenging task in the fight against both existing and emerging infectious diseases and as such there is need for continued search for new antimicrobial products for use in the fight against microorganisms. Therefore, the main aim of this study was to determine the antioxidant activity, polyphenolic composition and establish *in vitro* antimicrobial activities of tea seed oil against *E. coli*, *S. aureus*, *C. albicans*, *C. neoformans* and *T. mentagrophytes*.

### 1.1. Uses of tea seeds

Tea seeds have been perceived to be a rich source of saponin. Five saponins have been distinguished in tea seeds, particularly Theasapogenal A, B, C, D and E. These saponins have been observed to be pharmacologically dynamic with articulated antiexudative and disease prevention properties. Saponin from tea has a wide array of biological activities that include but are not limited to foam-stabilization and emulsification. Tea saponin has been used in aquaculture to kill unwanted fish as well as insects in prawn ponds and in the control of pests and mites (Yamauchi *et al.*, 2001). Other physiological functions displayed by tea saponin include anti-inflammatory and expectorant properties (Morikawa *et al.*, 2006).

The phytochemicals in tea have been extensively studied and are well known for their myriad biological activities which include but are not limited to antibacterial activity, antifungal activity anticancer properties, anti-inflammation properties, antiviral activity, prevention of cardiovascular disorders and also anti-obesity and hypo-lipidemic effects (Lin and Lin-Shiau, 2006). The polyphenols in tea are mainly catechins which include epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), (+) catechin (C), gallic acid (GA) and epigallocatechin gallate (EGCG). The main mechanisms of these polyphenols are that they have anti-oxidative activity and can scavenge reactive nitrogen and oxygen

species by chelating redox-active transition metal ions. The tea seed oil from *C. sinensis* also has been shown to contain these phytochemicals (Njuguna *et al.*, 2014).

Tea seed component determination has shown that tea seed oil is composed of linolenic, palmitic, linoleic and oleic acids. According to (Guynot *et al.*, 2003), the fatty acid chains, just like the polyphenols, have exhibited antibacterial and antifungal activities.

## 2. MATERIALS AND METHODS

### 2.1. Sample collection and preparation

The seeds of *Camellia sinensis* (L. O Kuntze), were collected in September 2019 at the Tea Research Institute seed Barrie located at the KALRO-TRI, (0.3722 °S, 35.3483 °E: Elevation 2180 m above sea level). Samples were collected in triplicate. Only healthy and mature seeds were picked. The seeds were de-husked, cleaned and rinsed using distilled water and then dried.

### 2.2. Chemical and reagents

The standards used for chromatography were purchased from Sigma Aldrich and included Gallic acid (GA), Epigallocatechin (EGC), caffeine, (+) catechin (C), epigallocatechin gallate (EGCG) and Epicatechin gallate (ECG). The reagents used for the study included Folin-ciocalteu reagent, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), Methanol, *n*-hexane, Acetonitrile, Acetic acid, Ethylenediaminetetraacetic acid (EDTA), Dimethyl sulfoxide (DMSO), Ascorbic acid, and culture media (Milton Hueller (MH) and the Sabour and dextrose agar (SDA) used were analytical grade and were obtained from Sigma-Aldrich Chemical Company, Germany through Kobian Suppliers.

### 2.3. Methodology

#### 2.3.1. Extraction of tea seed oil

Dried tea seed samples were de-husked and ground using a pestle and mortar and dried at low temperatures for the subsequent extraction of seed oil. Extraction was done according to the soxhlet method with hexane as solvent. Extraction was done for 8 hours with a sample-to-solvent ratio of 1:20. The oil extract was then put into a rotary evapora-

tor to concentrate and expel the hexane solvent at 60 °C. The concentrated sample was then placed in an oven at 80 °C to remove residual solvent. The samples were weighed and then transferred to sterilized bottles for further analysis.

#### 2.3.2. Extraction for catechin, polyphenols and antioxidant assay

Sample extraction for catechins, total polyphenols and antioxidant activity determination were done simultaneously using a similar method. The extraction method was liquid-liquid extraction (LLE), which involved the use of *n*-hexane, methanol and water to extract the catechins and total polyphenols from the seed oil. Two grams of seed oil were dissolved in 2.0 mL of *n*-hexane in a graduated 10-mL tube. The mixture was mixed in a vortex machine for 1 minute. This was followed by liquid-to-liquid extraction. 4 mL of 80:20 methanol to water were added to the graduated tube and vortexed for 10 seconds. The mixture was then centrifuged at 3500 RPM for 10 minutes. The supernatant was then decanted and poured into a graduated extraction tube. The supernatant was further subjected to a second and third extraction with 4 mL with an 80:20 methanol-to-water ratio. After the third extraction the sample was centrifuged and the supernatant put in sterilized sample tubes and stored at -15 °C for further analysis.

### 2.4. Empirical studies

#### 2.4.1. Determination of the polyphenolic composition and profiles of camellia oil of *Camellia sinensis* and *Camellia oleifera*

Total catechin and individual catechin levels of *C. sinensis* and *C. oleifera* seed oil profile were determined using the HPLC method and the polyphenol levels were measured using Folin Ciocalteu reagent method developed in 1999 with slight modifications (Singleton *et al.*, 1999). In this study all the standards and reagents used were pure analytical grade with 99.9 % purity and were obtained from Sigma-Aldrich chemical company.

#### 2.4.2. Determination of polyphenol from tea seed oil

The total phenolic content from tea seed oil was determined according to a method described by (Singleton *et al.*, 1999). The method utilizes Folin-Cio-

calteau reagent. 1 mL of sample was pipetted into a 100 mL volumetric flask, with 5 mL of Folin-Ciocalteu reagent added and a further 10 mL of 20% w/v sodium carbonate solution. The mixture was then topped up with distilled water to the 100-mL mark. The mixture was mixed thoroughly and left to stand for 1 hour. The absorbance was then determined at a wavelength of 725 nm using UV-Vis spectrophotometer (UV 1800, Shimadzu, Japan). The result was expressed as a gallic acid equivalent.

#### 2.4.3. Determination of total catechin and individual catechin fractions from tea seed oil

THE Catechin fraction detection and quantification employed a Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method. Catechin standards were obtained from Sigma and Aldrich i.e. (+) - Catechin (C), (-) - epicatechin (EC), epigallocatechin (EGC), (-) - epicatechingallate (ECG) and (-) - epigallocatechingallate (EGCG). Double distilled water was used for dilutions. All other reagents used were HPLC grade i.e. n-hexane, methanol, ethylenediaminetetraacetic acid (EDTA) and ascorbic acid. The catechin contents in the methanolic extracts of the crude oils were quantitatively estimated by RP-HPLC (Shimadzu LC 20, Japan). 1.0 mL of sample was pipetted into a graduated tube, 10% v/v stabilizing solution was added to make up 5 mL. The mixture was then passed through a 0.45 µm nylon membrane filter and transferred to sample vials. A Shimadzu LC 20 AT HPLC system fitted with a SIL 20A auto-sampler, an SPD-20 UV-Visible detector, a class LC10 chromatography work station and a Gemini 5 µm C6 Phenyl 110Å, 250 mm × 4.6 mm i.d (Phenomenex, Torrance, CA, USA) separation column was used. Catechin quantification was done using the caffeine calibration curve with individual catechin relative response factors. Total catechin (TC) was calculated by adding up individual catechin fraction i.e. ECG + EGC + EC + EGCG + C = % TC.

#### 2.4.4. Determination of the antioxidant activity of *Camellia sinensis* and *Camellia oleifera* seed oil

The antioxidant activity was measured using the DPPH radical scavenging method developed in 1958 (Blois, 1958) and later modified in 2001 (Morales and Jiménez-Pérez, 2001). The procedure was slightly modified to fit this particular study.

#### 2.4.5. Antioxidant activity assay

The extracted sample was brought to room temperature and a 400 µl aliquot of the oil sample was mixed with 3 mL of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH). The DPPH was 74 mg/L in 80% Methanol. The resulting mixture was vortexed for 1 minute and put in a dark room for 1 hour. The absorbance of the blank solution i.e. DPPH and sample was then read at 520 nm in a UV-Vis spectrophotometer. The percentage antioxidant activity was calculated using the formula:

$$100(\text{CC}-\text{CD}/\text{CC});$$

\*where CC is the absorbance of the blank sample, CD the absorbance of the test sample.

#### 2.4.6. In-vitro antibacterial and antifungal activities of *C. sinensis*, *C. oleifera* seed oil, eucalyptus oil and olive oil

The microbes used for the study were American Type Culture Collection (ATCC), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and fungi *Candida albicans* (ATCC 90028), and the other two were *Cryptococcus neoformans* and *Trichophyton mentagrophytes*.

The antimicrobial activities of the oils were determined according to protocols from the National Committee of Clinical and Laboratory Standards (NCCLS). Bacteria cultures were kept in Milton-Hueller (MH) while those of the fungi were kept in a Sabouraud Dextrose Agar (SDA) medium supplemented with Chloramphenicol (50 mg/mL) and Streptomycin sulfate (500 µg/mL) and stored at +4 °C. The Barium sulphate standard equivalent to McFarland No. 0.25 was used to give a cell density of 1.5 (x) 10<sup>8</sup>/mL. The inoculum of approximately 1.5 (x) 10<sup>8</sup> cells was spread uniformly onto MH agar containing 0.2% glucose and 0.5 g/mL of methylene blue dye. Excess inoculum was drained and allowed to rest for approximately 1 hour. The medium was then left to stand for 30 minutes to allow the solution to diffuse into the agar medium. The discs impregnated with the respective concentrations of oil were then put on the plates and incubated for 24 hrs at 35 °C and later the zones of inhibition were measured in millimeters. Every assay was done in triplicate.

#### 2.5. Statistical Analysis

ANOVA was used to determine the mean differences in the biochemical properties of the camellia

TABLE 1. Total catechin levels and catechin fractions of tea seed oil

	Tea varieties		
	<i>C. sinensis</i> - TRFK 306	<i>C. sinensis</i> -TRFK 301/5	<i>C. oleifera</i>
Gallic Acid	0.54±0.01 <sup>b</sup>	0.54±0.03 <sup>b</sup>	0.62±0.04 <sup>a</sup>
Epigallocatechin	1.06±0.25 <sup>a</sup>	0.87±0.18 <sup>a</sup>	0.99±0.00 <sup>a</sup>
+Catechin	0.14±0.02 <sup>a</sup>	0.14±0.01 <sup>a</sup>	0.16±0.01 <sup>a</sup>
Epicatechin	0.71±0.08 <sup>a</sup>	0.62±0.05 <sup>b</sup>	0.70±0.05 <sup>a</sup>
Epigallocatechin-3-Gallate	0.96±0.12 <sup>ab</sup>	0.90±0.06 <sup>b</sup>	1.07±0.02 <sup>a</sup>
Epicatechingallate	1.22±0.11 <sup>b</sup>	1.26±0.03 <sup>b</sup>	1.67±0.02 <sup>b</sup>
Total Catechin	4.62±0.27 <sup>b</sup>	4.33±0.21 <sup>b</sup>	5.22±0.09 <sup>a</sup>

Means with the same letter in the same row are not significantly different at  $P \leq 0.05$  according to the Tukey test  $p < 0.05$ . Number of replicates = 3. *C. sinensis* = *Camellia sinensis*, *C. oleifera* = *Camellia oleifera*, TRFK 306– Tea research foundation of Kenya tea variety 306/1, TRFK 301/5– Tea research foundation of Kenya tea variety 301/5

oil and to compare the differences in the antibacterial and antifungal activity of the oils studied among the test microbes. Graphs were plotted and LSDs used to separate the means.

Probability was set at 95% for the significant confidence interval to determine the differences in the mean diameters of the inhibition zones.

A correlation analysis was carried out using Excel 2013 to determine the relationships among the catechin fractions, total catechins, antioxidant activity and total phenolic content of camellia oil its antimicrobial activity. All Statistical analysis was done using SAS 9.1 and Excel 2013.

### 3. RESULTS

#### 3.1. Polyphenolic composition and profiles of *Camellia sinensis* and *Camellia oleifera* seed oil. Catechin profile of tea seed oil

The catechins identified in tea oils were epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG). The two main gallated catechins present were EGCG and ECG, while the others were non-gallated. Generally, the TC levels were higher in *C. oleifera* than those of *C. sinensis*.

The various individual catechin profiled showed a significant difference ( $p \leq 0.05$ ) among the tea oils. Epicatechingallate was the most abundant in all the oils assayed while +C was the least abundant. The total catechin levels ranged from  $4.08 \pm 0.27\%$  to  $4.60 \pm 0.09\%$ . *C. oleifera* had the highest total catechin content at ( $4.60 \pm 0.09\%$ ) while the *C. sinensis* clone TRFK 301/5

TABLE 2. Catechin retention times

Catechin fractions	RT (Mins)
Gallic acid	4.61
Epigallocatechin	8.92
Epicatechin	15.58
Epigallocatechin gallate	19.82
Epicatechin gallate	24.91

Catechin retention times in minutes; RT – retention time; Mins - minutes

had the lowest at ( $3.79 \pm 0.2\%$ ). It is also notable that the *Camellia sinensis* varieties (TRFK 301/5 and TRFK 306) did not differ significantly at  $P \leq 0.05$  (Table 1).

Under the present conditions, the retention times (min) for the standard compounds were 4.61, 8.920, 15.58, 19.82, 24.91 for Gallic acid, Epigallocatechin, Epicatechin, Epigallocatechin gallate, Epicatechin gallate, respectively (Table 2). The HPLC chromatogram graphs obtained had similar chemical compositions but with different peak areas. There were statistical differences ( $p < 0.05$ ) in all catechin fractions assayed in this study. *C. sinensis* exhibited lower levels of catechins for all the analyzed catechin fractions than its counterpart *C. oleifera*. ECG (1.67%) was the most predominant catechin fraction in the oils. However, EGCG was not significantly different ( $p \leq 0.05$ ) for the three oils. Gallic acid did not show any significant difference ( $p < 0.05$ ) with levels ranging from  $0.54 \pm 0.01\%$ ,  $0.54 \pm 0.03\%$  and  $0.62 \pm 0.04\%$  for *C. sinensis* TRFK 306, *C. sinensis* 301/5 and *C. oleifera* oils, respectively. +C had the

lowest levels (0.14 to 0.16%) compared to the other catechin fractions (Table 5).

### 3.2. Total polyphenol levels in tea seed oil

The results revealed that oils from the different plants (*C. sinensis* clone TRFK 306 and TRFK 301/5 and *C. oleifera*) differed significantly ( $p = 0.001$ ) in the levels of total polyphenols. The total polyphenolic content ranged from 5.53 to 7.24%. *C. oleifera* had the highest polyphenol content at  $7.24 \pm 0.29\%$  and *C. sinensis* clone TRFK 301/5 had the lowest at  $4.783 \pm 0.16\%$ .

### 3.3. Antioxidant activity of *Camellia sinensis* and *Camellia oleifera* seed oil

The antioxidant activities ranged from 14.73 to 18.81%. The results indicated that there were

significant differences ( $p \leq 0.05$ ) in the antioxidant activity among the different types of tea oils ( $p=0.0005$ ). The antioxidant activity of *C. oleifera* was  $18.81 \pm 0.46\%$ , *C. sinensis* TRFK 306,  $15.98 \pm 0.135$  and *C. sinensis* 301/5,  $14.73 \pm 0.47\%$ .

### 3.4. Correlation between antioxidant activity, polyphenolic composition and profiles of *Camellia sinensis* and *Camellia oleifera* seed oil

Total catechin positively correlated with all the individual catechin fractions including total polyphenol ( $r = 0.998$ ). Total catechin also positively correlated with the antioxidant activity of the oils ( $r = 0.954$ ). The individual catechin C, EGCG and ECG positively and significantly correlated with antioxidant activity (Table 3).

TABLE 3. Correlation matrix of the biochemical parameters of tea seed oil

	GA	EGC	+C	EC	EGCG	ECG	TC	TP	AA
GA	1								
EGC	0.150210	1							
+C	1.000000	0.150210	1						
EC	0.409644	0.963428	0.409644	1					
EGCG	0.937509	0.484836	0.937509	0.701470	1				
ECG	0.996771	0.070336	0.996771	0.335068	0.906541	1			
TC	0.935507	0.489823	0.935507	0.705529	0.999984	0.904115	1		
TP	0.955144	0.436256	0.955144	0.661424	0.998502	0.928279	0.998173	1	
AA	0.954256	0.438937	0.954256	0.663658	0.998661	0.927165	0.998349	0.999996	1

TP- total polyphenols; AA- antioxidant activity; GA- gallic acid; EGC- epigallocatechin; +C- catechin; EC- epicatechin; EGCG- epigallocatechin gallate; ECG- epicatechin gallate, AA – antioxidant activity

TABLE 4. Zones of inhibition (mm) against two bacterial strains

Plants	Variety	<i>S. aureus</i> IZDs(mm)	<i>E. coli</i> IZDs (mm)
Tea	<i>C. sinensis</i> (TRFK 301/5)	11.00±1.00 <sup>c</sup>	8.00±0.00 <sup>b</sup>
	<i>C. sinensis</i> (TRFK 306)	12.00±1.00 <sup>c</sup>	8.00±1.00 <sup>b</sup>
	<i>C. oleifera</i>	11.67±0.58 <sup>c</sup>	8.33±0.58 <sup>b</sup>
Eucalyptus	Eucalyptus Citriodora	20.67±0.58 <sup>a</sup>	13.67±0.58 <sup>a</sup>
Olives	Olea europaea	18.33±0.58 <sup>b</sup>	8.67±0.58 <sup>b</sup>
DMSO (0.5 %)		6.00	6.00
Control – Chloramphenicol (0.6µg)		27.00	20.00

Means with the same letter in the same row are not significantly different at  $P \leq 0.05$  according to the Tukey test  $p < 0.05$ . Number of replicates = 3; IZDs – inhibition Zone diameters (mm); CONC – concentration; DMSO - Dimethyl sulfoxide; *S. aureus* - Staphylococcus aureus; *E. Coli* - *Escherichia coli*; *C. sinensis* - *Camellia sinensis*; *C. oleifera* - *Camellia oleifera*; TRFK 306/1– Tea research foundation of Kenya tea variety 306; TRFK 301/5– Tea research foundation of Kenya tea variety 301/5

### 3.5. Antibacterial activities of *Camellia sinensis* (TRFK 306, TRFK 301/5), *C. oleifera*, eucalyptus and olive oil

It was found that *E. coli* (ATCC 25922) was inhibited weakly by tea seed oil (Table 4). TRFK 301/5, TRFK 306 and *C. oleifera* at a concentration of 100% showed mean zones of inhibition of 8.00 mm, 8.00 mm and 8.33 mm respectively. *Eucalyptus citriodora* gave the highest inhibitory effects of  $13.67 \pm 0.58$  mm while olive oil gave a zone of inhibition of 8.67 mm. The trend was similar when the concentration was reduced to 50% whereupon *C. oleifera*, *C. sinensis* teas (TRFK 301/5 and TRFK 306) showed no inhibition. However, *Eucalyptus citriodora* and olive oil showed significant inhibition even at this low concentration.

*S. aureus* a gram-positive bacterium was inhibited by all the test samples at a concentration of 100%. Eucalyptus exhibited the highest bio-activity with IZD of 20.67 mm followed by Olive oil (18.33mm) (Table 4). The tea samples had low activity especially at 50% concentration.

Eucalyptus oil showed the highest antibacterial activity with zones of inhibition of 20.67 mm and 13.67 mm for *S. aureus* and *E. coli*, respectively (Table 4). Tea seed oil exhibited the lowest antibacterial activity. *C. oleifera* exhibited antimicrobial activity compared to *C. sinensis*, although IZDs values did not differ significantly ( $p \leq 0.05$ ) for *S. aureus* or *E. coli*. Olive oil inhibited the growth of *S. aureus* with an IZD of 18.33 mm, which was significantly ( $p \leq 0.05$ ) higher than those of the camellia oils.

### 3.6. Antifungal activity of *Camellia sinensis* (TRFK 306, TRFK 301/5), *C. oleifera*, eucalyptus and olive oil

There was minimal antifungal activity of tea seed oil against *C. albicans*, *C. neoformans* and *T. mentagrophytes*. *Eucalyptus Citriodora* oil (Essential oil), and Olive oil (seed oil) exhibited antimicrobial activity. *Eucalyptus citriodora* showed activity against *C. albicans* and *T. mentagrophytes*. TRFK 301/5 seed oil showed activity against *C. albicans* at a concentration of 100%. The antifungal activity of tea seed oil was low. *Cryptococcus neoformans* was not inhibited by any of the oils. TRFK 301/5 oil exhibited bioactive activity against *C. albicans*, and other tea oils had no activity, although Eucalyptus and olive oil exhibited antimicrobial activity at a 100% concentration. At 50% concentration no activity was noted. *T. mentagrophytes* was inhibited by all tea seed oil except seed oil from TRFK 301/5 seeds (Table 5).

### 3.7. Correlation between antioxidant activity, polyphenolic composition, catechin fractions and inhibition zone diameters

The inhibition zone diameters positively correlated with total polyphenols ( $r = 0.975$ ), antioxidant activity ( $r = 0.976$ ), gallic acid ( $r = 0.866$ ); epigallocatechin ( $r = 0.624$ ); catechin ( $r = 0.866$ ); epicatechin ( $r = 0.811$ ); epigallocatechin gallate ( $r = 0.986$ ); epicatechin gallate ( $r = 0.823$ ) (Table 13). Correlation analyses between the inhibition zone diameters of *S. aureus* with biochemical parameters of tea seed oil

TABLE 5. Zones of inhibition of three fungal strains

Plants	Variety	<i>C. albicans</i> IZDs (mm)	<i>T. mentagrophytes</i> IZDs (mm)	<i>C. neoformans</i> IZDs (mm)
Tea	<i>C. sinensis</i> (TRFK 301/5)	6.00±0.00 <sup>d</sup>	6.00±0.00 <sup>d</sup>	6.00±0.00 <sup>a</sup>
	<i>C. sinensis</i> (TRFK 306)	6.67±0.58 <sup>bc</sup>	7.00±0.58 <sup>c</sup>	6.00±0.00 <sup>a</sup>
	<i>C. oleifera</i>	6.00±0.00 <sup>cd</sup>	6.67±0.58 <sup>d</sup>	6.00±0.00 <sup>a</sup>
Eucalyptus	<i>Eucalyptus Citriodora</i>	10.33±0.58 <sup>a</sup>	10.33±0.58 <sup>a</sup>	6.00±0.00 <sup>a</sup>
Olives	<i>Olea europaea</i>	8.00±0.00 <sup>b</sup>	8.00±0.00 <sup>b</sup>	6.00±0.00 <sup>a</sup>
DMSO (0.5 %)		6.00	6.00	6.00
Control – Nystatin (30µg)		26.00	27.00	25.00

Means with the same letter in the same row are not significantly different at  $P \leq 0.05$  according to the Tukey test  $p < 0.05$ . Number of replicates = 3. IZDs – inhibition Zone diameters (mm); CONC – concentration; DMSO - Dimethyl sulfoxide; *C. albicans* = *Candida albicans*; *T. Mentagrophytes* – *Trichophyton mentagrophytes*; *C. Neoformans* – *Cryptococcus neoformans*; *C. sinensis* = *camellia sinensis*; *C. oleifera* = *Camellia oleifera*, TRFK 306/1– Tea research foundation of Kenya tea variety 306, TRFK 301/5– Tea research foundation of Kenya tea variety 301/5

TABLE 6. Correlation matrix of inhibition zone diameters to total polyphenolic content, total catechin and catechin fractions and antioxidant activity.

	GA	EGC	+C	EC	EGCG	ECG	TC	TP	AA
<i>S. aureus</i>	0.866	0.624	0.866	0.811	0.986	0.823	0.987	0.975	0.976
<i>E. coli</i>	1.000	0.150	1.000	0.410	0.938	0.997	0.936	0.955	0.954
<i>C. albicans</i>	0.500	0.931	0.500	0.995	0.770	0.429	0.774	0.734	0.736
<i>T. mentagrophytes</i>	0.632	0.901	0.515	0.981	0.986	0.429	0.936	0.736	0.780

TP- total polyphenols; AA- antioxidant activity; GA- gallic acid; EGC- epigallocatechin; +C- catechin; EC- epicatechin; EGCG- epigallocatechin gallate; ECG- epicatechin gallate; *C. albicans* = *Candida albicans*; *T. Mentagrophytes* – *Trichophyton mentagrophytes*; *S. aureus* -*Staphylococcus aureus*; *E. Coli* -*Escherichia coli*.

(Table 6) revealed that the inhibition zone diameters significantly correlated with following biochemical properties of tea seed oil: epigallocatechin gallate ( $r = 0.938$ ), epicatechin gallate ( $r = 0.997$ ), total catechin ( $r = 0.936$ ), total polyphenol ( $r = 0.955$ ) and antioxidant activity ( $r = 0.954$ ).

Correlation analyses between inhibition zone diameters of *E. coli* ATCC 25922 with biochemical parameters of tea revealed that the inhibition zone diameters significantly correlated with following biochemical properties of tea: EGCG ( $r = 0.938$ ), ECG ( $r = 0.997$ ), TP ( $r = 0.955$ ). Antioxidant activity also showed significant positive correlation ( $r = 0.954^*$ ) influence on the inhibition zone diameter.

The inhibition zone diameter of a *C. albicans* with biochemical parameters of the assayed tea samples positively correlated with total polyphenols GA ( $r = 0.500$ ), EGC ( $r = 0.931$ ), +C ( $r = 0.500$ ), EC ( $r = 0.995$ ), EGCG ( $r = 0.770$ ), TP ( $r = 0.734$ ) and AA ( $r = 0.736$ ) (Table 3).

The correlation between the inhibition zone diameters of *T. mentagrophytes* with total polyphenols and individual catechins and antioxidant activity is presented in Table 6. The TP ( $r = 0.734$ ), GA ( $r = 0.500$ ), EGCG ( $r = 0.770$ ), EGC ( $r = 0.931$ ) and antioxidant activity ( $r = 0.736$ ) significantly correlated with the inhibition zone diameters.

#### 4. DISCUSSION

In this study the total polyphenol contents of oils were determined. The results confirmed the presence of polyphenols in tea seed oil. However, the levels varied among the oils and *C. oleifera* exhibited the highest levels. Earlier, it had been demonstrated that the extent of variation in total polyphenol contents between different tea oils is as a result of the gen-

otype of the tea plant under study and other factors such as geographical origin. In the current study it was evident that genotype played an important role. *C. sinensis* tea varieties TRFK 306 and TRFK 301/5 with similar genotype had lower levels of polyphenols than *C. oleifera* oil.

The results of this study showed that the tea cultivars (*Camellia sinensis*) had significantly lower levels of polyphenols in their seed oil compared to those of its leaves. The polyphenol levels of the leaves of the tea cultivars studied ranged from 21.90% (TRFK 306) to 22.07% (TRFK 301/5) as seen in a study done by (Karori *et al.*, 2014) on the polyphenol composition of tea leaves. The polyphenol content of the tea seed oil was in agreement with work reported by George (2013). The low levels of polyphenols in the seed oil could have been due to the fact that most of the polyphenols in the seed of plants were not easily extractable using aqueous or aqueous-organic solvents as opposed to the polyphenols found in the leaves and skin of most plants.

This study showed that the catechins in the oils were similar to those found in the leaves of *Camellia* plants i.e. (-)-epigallocatechin (EGC), (+)-catechin (C), (-)-epicatechin (EC), (-)-epigallocatechin gallate (EGCG) and (-)-epicatechin gallate (ECG). From the chromatograms it was seen that the gallated catechins were the last to be eluted. The difference in retention times between the gallated and non gallated catechins was because of their polarity to the C6 column used in the HPLC system. Gallated catechins bind tightly to the C6 column unlike non gallated catechins that bind loosely and are not eluted first. In a review by Stalikas, (2007) on extraction, separation, and detection methods for phenolic acids and flavonoids, he indicated that phenolic acids are eluted from RP columns according to decreasing po-



larities. This was the case in this study whereby the gallated catechins have less hydroxy groups which exhibit less polarity, while the non gallated catechins have more of the hydroxyl groups exhibiting increased polarity, and thus the decreased polarity increased their retention time.

This study also investigated the radical scavenging activity of DPPH by tea seed oil to determine its antioxidant activity. The results obtained indicated that oils have the ability to scavenge for free radicals. This phenomenon is attributed to the polyphenolic composition in the oils. In a study done at the Tea Research Institute Kenya, by George (213), the antioxidant capacities of tea seed oil and the whole tea seed meal expressed as gallic acid equivalent showed that the seed cake had significantly higher antioxidant activity than the oil. In addition, the metal chelating capacity of tea seed cake was as high as 90% at 25 mg sample equivalent/mL, demonstrating that tea seed products can provide lasting health benefits. In an article written by (Bernatoniene and Kopustinskiene, 2018) on ‘the Role of Catechin in Cellular Responses to Oxidative Stress’, it was noted that catechin had the ability to donate hydrogen ions to stabilize free radicals. This was considered the first mechanism in the antioxidant efficacy of catechin. Other mechanisms included indirect ways such as the induction of antioxidant enzymes that inhibit the pro-oxidant enzymes and also the production of detoxification enzymes and antioxidant enzymes in a stage referred to as PHASE II (Bernatoniene and Kopustinskiene, 2018). However, the antioxidant activity of the tea seed oil in this study was lower than that observed for the leaf as seen in other studies (Karori *et al.*, 2007) twelve different types of commercial tea samples were assayed to determine their phenolic composition and antioxidant activity. Reverse phase high performance liquid chromatography using a binary gradient system was used for the identification and quantification of individual catechins. Subsequently, total phenolic content was determined spectrophotometrically according to the Folin-ciocalteus method. Total theaflavins and thearubigins were also determined. The radical scavenging behavior of the polyphenols on 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). Despite the low antioxidant activity of the tea seed oil, it can be seen as a potential source of naturally high value edible oil. Various studies have outlined the beneficial

effects of tea seed oil such as hepatoprotection (Lee *et al.*, 2007), and reduction in weight gain (Kim *et al.*, 2008). In a study done by Zhang *et al.* (2014), an ability to clear lipid peroxidation in rat livers was detected, hence alleviating liver disease.

The tea plant in other countries such as China is not only grown for the leaf but also for the seed. The common tea plant planted for seeds in China is *C. oleifera*. The seeds of *C. oleifera* are used to produce tea seed oil which is an edible oil and can also be used in the cosmetic and pharmaceutical industries (Wang *et al.*, 2017). *C. sinensis* planted mainly in Kenya has seeds that can be exploited to produce oil for consumption and commercial use (George *et al.*, 2016). In this study the oils from two tea varieties, *Camellia oleifera* and two *C. sinensis* cultivars, were evaluated for their antimicrobial activity against five microbes viz. *E. coli*, *S. aureus*, *C. albicans*, *C. neoformans* and *T. mentagrophytes*. The results obtained confirmed that tea seed oil indeed has antimicrobial properties. The tea plants were able to inhibit the growth of *E. coli* and *S. aureus*. At an oil concentration of 100%, *S. aureus*, a gram-positive bacteria, was the most susceptible microbe to the oils. This was due to the much greater binding of negatively charged EGCG to the positively charged lipid bilayer constituent of the cell membrane of gram-positive bacteria. The bactericidal effect of EGCG was attributed to membrane perturbation (Koech *et al.*, 2013). Eucalyptus oil and olive oil had better activity than the tea oils. *C. oleifera* exhibited the most activity among the teas. The low activity of tea seed oil against the microbes under study might have been due to the low levels of catechin and polyphenols. Catechin (flavonoids) contains two benzene rings A and B, which have been identified as the main bactericidal components. EGCG is the main catechin component among the catechin which highly inhibits bacterial growth (Yamada, 2013). EGCG is known to even inhibit the activity of HIV and *S. aureus* in various study models (Yamaguchi *et al.*, 2002). Another catechin, EGC, has been demonstrated to disrupt the activity of the gyrase enzyme on the DNA gyrase b subunit of bacteria after binding to the ATP site (Hoshino *et al.*, 1999). Apart from catechins, the other tea flavanols like Quercetin, Kaempferol and Myricetin were also found to have antibacterial activity against gram-positive bacteria at varying concentrations (Yoda *et al.*, 2004). Quercetin exhibited

remarkable activity against *S. aureus*, even higher than EGCG (Yoda *et al.*, 2004). Over four thousand compounds have been detected in tea. Amongst the 4000 compounds about a hundred of them have been shown to exhibit remarkable antimicrobial activity against various microorganisms (Yoda *et al.*, 2004).

## 5. CONCLUSIONS

From this study it is evident that tea seed oil is a potential source of beneficial phytochemicals that can be utilized for their health promoting effects. It was also seen that the oils have antioxidant capacity and thus can be used as a good source of food that can help prevent pathophysiological diseases associated with oxidative stress. The correlation of the antimicrobial activity of oils to its biochemical/phytochemical constituents was positively significant, suggesting that phytochemicals in oils are the main bioactive constituents that give them potent antimicrobial activity. From this study TP, EGC, EGCG, ECG, and GA were identified as the most potent antimicrobial biochemical in the assayed teas. Therefore, antimicrobial activity was higher in tea extracts containing high levels of TPP, EGCG, ECG and EGC. The *C. sinensis* and *C. oleifera* tea seed oil possessed both antifungal and antibacterial activities.

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## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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