



## PHYTOCHEMICAL SCREENING, ANTIOXIDANT ACTIVITY AND ANTI-BACTERIAL ACTIVITY OF HYDRO-ETHANOLIC EXTRACT OF *CAMELLIA SINENSIS* LEAVES (HECsL)

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### ABSTRACT

Recent evolution to find out drugs from natural sources has resulted in compounds that are being developed to treat cancer, resistant bacteria & viruses and immunosuppressive disorders. There is growing interest in relationship of the phytochemical constituents of a medicinal plant with pharmacological activity. In this study, phytochemical, antioxidant activity, total phenolic content and concentration of flavonoids of Hydro-ethanolic extract of *Camellia sinensis* (HECsL) were determined using spectrophotometric methods. HECsL was found to have alkaloids, phenols, flavonoids, tannin, glycosides, saponin, terpenoides, steroids and phytosteroids. The antioxidant activity of extract was expressed as percentage of DPPH radicals inhibition and IC<sub>50</sub> values (µg/ml). Values in percentage ranged to 86.37%. The total phenolic content ranged to 3.22 mg/g of HECsL extract, expressed as gallic acid equivalents. The total flavonoid concentration was 16.45 mg/g, expressed as gallic acid equivalents. The HECsL extract were evaluated for their antibacterial activity against four Gram-negative and Gram-positive bacteria using ampicillin as positive control. The study revealed that HECsL respective of their types in different concentrations inhibited growth of the test organism to varying degrees and showed maximum activity against four the bacterial strains. Expression to these results it may be concluded that HECsL extract, regarded as promising candidates for natural plant sources of antioxidants with high value and curing of various infectious diseases caused by the resistant microbes.

**Keywords:** Antioxidant, Antibacterial, Flavonoid, Phenol, HECsL, *Camellia sinensis*.

### 1. INTRODUCTION

Medicinal plants and plant-derived drugs are commonly used in different traditions all over the world and they are becoming increasingly popular in modern scientific communities as natural alternatives to synthetic chemicals. To prevent and cure different human diseases, recently considerable attention has been paid to eco-friendly and bio-friendly plants [1]. India is well known for its rich traditional systems of medicine. In Indian system of medicine, generally medicine of plant origin is preferred over the medicine of animal origin due to the abundance of natural flora. Phytochemicals are naturally occurring biologically active plant chemicals that possess protective or disease preventive properties [2].

Secondary metabolites from plants have important biological and pharmacological activities, such as anti-oxidative, anti-allergic, antibiotic, hypoglycemic and anti-carcinogenic. Many disorders in human beings such as atherosclerosis, arthritis, Alzheimer disease, cancer

etc., may be the result of increased concentrations of free radicals in an organism. Reactive oxygen species (ROS) and nitrogen species (RNS), as the most frequent pro-oxidants, either originate from normal metabolism or are induced by UV radiation and different pollutants. Harmful effects of disturbed antioxidant-prooxidant balance can be largely prevented by intake of antioxidant substances [3]. Antioxidants offer resistance against oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation and by many other mechanisms and thus prevent the disease progression [4].

Infectious disease caused by bacteria is a significant burden on public health and threaten the economic stability of societies worldwide. In particular, the widespread incorrect use of conventional antibiotics has led to the adaptation of microorganisms to these therapies and the appearance of antibiotic resistant bacteria is a serious problem [5].

The increasing antibiotic resistant pathogens and failure

of many chemotherapeutic has led the screening of medicinal plants for their antimicrobial activity [6]. Thus recently, natural products with antimicrobial activity have gained more attention due to safety concerns and increasing resistance to available antibiotics [7]. The present work was aimed to determine the phyto-chemical constituents and DPPH radical scavenging activity determination of total phenol and flavonoid content of HECsL and to evaluate their antimicrobial efficiency against some pathogenic bacteria.

## 2. MATERIAL AND METHODS

### 2.1. Plant material

*Camellia sinensis* leaves were collected from Aruvankadu in the Distinct of The Niligris, and the taxonomic identification of the plant was confirmed by Botanical Survey of India, Coimbatore (Authentication No: BSI/SRC/5/23/2019/Tech./18). The collected plant leaves were dried in the shade at room temperature for complete drying. The plant material was pulverized and used for further investigation.

### 2.2. Preparation of Plant extract

In the preliminary screening, the direct hydro-ethanolic extract of *Camellia sinensis* showed a characteristic orange to magenta colour in Shinoda test (powdered magnesium + conc. HCl) which indicated the presence of flavonoids. The color is due to the reductive conversion of flavone into the corresponding anthocyanin pigment [8].

Knowing the presence of flavonoid in hydro-ethanolic extract, the extraction was done with 10g of powdered plant material and 100 ml of light petroleum ether (B.P 40°-60°C) in a soxhlet apparatus for 18 hours to remove chlorophyll, non-flavonoid compounds and lipids dewaxing [9]. The treated material was dried and extracted with hydro-ethanolic extract using Soxhlet apparatus [10]. This fraction is referred as Flavonoid Fraction of *Camellia sinensis* (FFC).

#### 2.2.1. Phytochemical Screening assays

Preliminary phytochemical analysis of the crude sample powders was carried out. Phenols, flavonoids, alkaloids and glycosides were estimated by the methods as described [11], Terpenoids, and steroids were estimated by described method [12] and tannins by the procedure [13].

#### 2.2.2. Determination of Total Phenol content

The amount of phenol in the HECsL extract was determined by using the previously described method

[14]. To 0.5 ml of the HECsL extract (1mg/ml), 2.5 ml of 10% Folin-Ciocalteu reagent and 2 ml of Na<sub>2</sub>CO<sub>3</sub> (2% w/v) were added. The resulting mixture was incubated at 45°C for 15 minutes with intermittent shaking. The absorbance was measured at 765nm. Results were expressed as milligrams of gallic acid (0.1-0.5 mg/ml) dissolved in methanol.

#### 2.2.3. Determination of Total flavonoid content

Total flavonoid estimation was performed by using Aluminium chloride Colorimetric method [15]. To 1ml of the HECsL extract (1mg/ml), 3 ml methanol, 0.2 ml of aluminum chloride, 0.2 ml of potassium acetate and 5.6 ml of distilled water were added. The mixture was incubated at room temperature for 30 minutes and absorbance was measured at 420 nm. Results were expressed as milligram of gallic acid (0.1-0.8 mg/ml) dissolved in Methanol.

#### 2.2.4. Antioxidant-Scavenging activity of DPPH radicals

The determination of DPPH scavenging activity of the plant extract was done by using the method described earlier [16]. The sample extracts (25µl) and 0.48ml of methanol were added to 0.5ml of methanolic solution of DPPH. The mixture was allowed to react at room temperature for 30 minutes. Methonal alone served as blank and DPPH in methanol, without the plant extracts, served as positive control. After 30minutes of incubation, the discolouration of the purple colour was measured at 518nm. Ascorbic acid was used as a positive control.

The radical scavenging activity of the plant extract was calculated using the equation:

$$\text{Scavenging activity (\%)} = \frac{A_{518}(\text{sample}) - A_{518}(\text{control})}{A_{518}(\text{control})} \times 100$$

#### 2.2.5. Antibacterial activity

Four bacterial strains Gram-negative bacteria: *Escherichia coli* and *Staphylococcus aureus*, Gram-positive bacteria: *Klebsiella oxytoca* and *Bacillus subtilis* were taken. All the tested strains are reference strains, and were collected from Microbiological Laboratory. The bacterial cultures were grown on nutrient agar medium (Hi Media, pH 7.4) at 37°C respectively. The cultures were maintained at 4°C.

In the present study, the antibacterial activities of HECsL extract were screened by agar well diffusion method [17]. The antimicrobial compounds present in the plant extract were allowed to diffuse out into the

medium and allowed to interact in a plate seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimetres.

#### 2.2.5.1. Broth microdilution method

The broth microdilution method was carried out in a 96-well microtiter plate to determine the minimum inhibitory concentration (MIC). The different concentrations of compounds (1, 0.5, 0.25 and 0.125mg/ml) were diluted in Mueller Hinton broth and the final volume was maintained at 100 µl. The final concentration of DMSO was less than 1%. Five µl of an overnight grown bacterial culture was added to the test medium to bring the final inoculum size to  $1 \times 10^5$  cfu/ml. The agar plates were incubated at 37°C for 16 h and the absorbance was read at 600 nm. The lowest concentration of the compound that inhibits the complete growth of the bacterium was determined as the MIC [18]. The percent growth inhibition was calculated by comparison with a control using the formula indicated below.

$$\% \text{ of growth inhibition} = \left\{ \frac{(\text{Control-Test})}{\text{Control}} \right\} \times 100$$

### 3. RESULTS AND DISCUSSION

#### 3.1. Phytochemical screening of HECsL extract

The results of the phytochemical screening of secondary metabolites in HECsL extract are shown in Table 1. The phytochemical analysis conducted on HECsL extract revealed that alkaloids, phenols, flavonoids, tannin, glycosides, saponin, terpenoides, steroids and phyto-steroids were present in considerable amounts.

**Table 1: Phytochemicals Assay**

Phytochemicals	Presence
Alkaloids	+
Phenols	+
Flavonoids	+++
Tannin	+++
Glycosides	+++
Saponin	+
Terpenoides	+++
Steroids	+
Phytosteroids	++

+++>++>+ Represents intensity of color formation (Presence of Specific Compound)

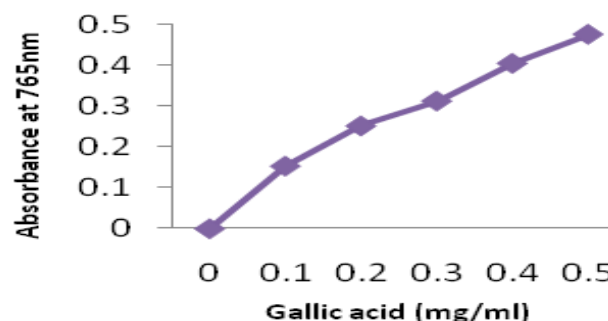
- Represents no color formation (Absence of Specific compounds)

Rukshana et al., 2017 [19] observed the presence of different phytochemical constituents in the ethanolic extract of *Pergularia daemia* leaf that includes: alkaloids, glycosides, terpenoids, steroids, carbohydrate, tannins and flavonoids.

A study done by scientists [20] showed that qualitative phytochemical analysis of the leaves of *Camellia sinensis* revealed the presence of steroids, terpenoids, tannins, flavonoids, saponins, cardiac glycoside and trace amount of alkaloids the aqueous extract.

#### 3.2. Determination of total Phenol content

The estimation of percentage yield, total phenolic content (TPCs) of HECsL extract is presented in Fig. 1. The total phenol content of the HECsL extract was found to be 3.22 mg gallic acid equivalent/g of extract power (fig. 1) respectively with reference to standard. Thoo et al., 2010 [20] also stated that the excess extraction time lead to reduction of phenolic and antioxidant yields. This is because antioxidants are potentially prone to degradation if exposed to ambient condition for long duration. Phenolic compounds such as, flavonoids are considered to be the major contributors to the antioxidant capacity of plants [21].

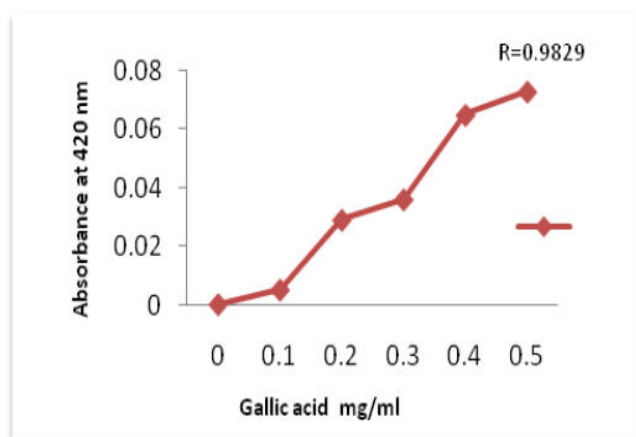


**Fig. 1: Determination of total Phenol content of HECsL extract**

#### 3.3. Determination of Total flavonoid content

The estimation of percentage yield, total flavonoid content of HECsL extract is presented in Fig. 2. The flavonoid contents of the HECsL extract were found to be 16.45 mg gallic acid equivalent/g of extract powder respectively with reference to standard.

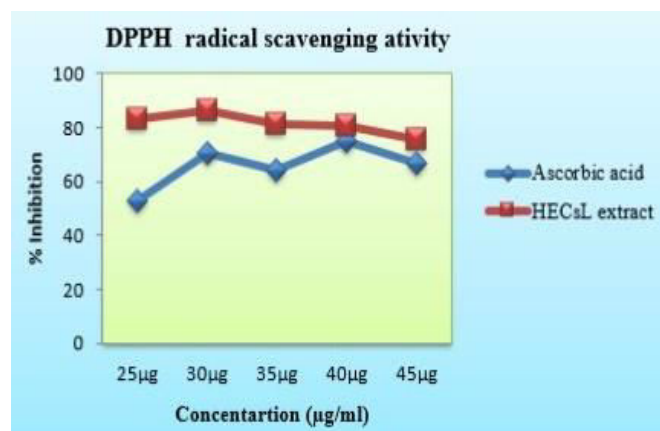
The total flavonoid content was calculated using a calibration curve for quercetin ( $R_2 = 0.9829$ ). All samples were analyzed in triplicates. The compounds such as flavonoids, which contain hydroxyls, are responsible for the radical scavenging effect [22].



**Fig. 2: Determination of total flavonoid content of HECsL extract**

### 3.4. Antioxidant - Scavenging activity of DPPH radicals

The percentage inhibitions of scavenging activities of HECsL extract for DPPH are shown in Fig. 3. The percentage inhibition of scavenging activities of the HECsL extract was increasing in dose-dependent manner which was comparable to that of standard at the same concentration.  $IC_{50}$  value of the HECsL extract was determined to be between 25-45  $\mu$ g/ml the percentage inhibition was 86.37%.



**Fig. 3: DPPH Activity- Percentage Inhibition at various HECsL extract and Standard (Ascorbic acid)**

Inhibition of DPPH was estimated to indicate the possible antioxidant compound from plant resources [23]. Similar, studies Moattar et al., 2017 [24] studied the methanolic extract to possess a significant inhibitory activity of DPPH radical (43.99%). Similarly, the inhibition percentage (IP) and inhibition concentration

( $IC_{50}$ ) values are considered to be a good measure of the antioxidant efficiency of pure compounds and extracts results showed that methanolic extracts of all the *Terminalia alata*, *Terminalia arjuna*, *Terminalia bellarica* and *Terminalia catappa* species bark and fruit pulp is preferable sources for extracting pure antioxidant compounds as they showed high IP values (93.95-95.42 IP) [25].

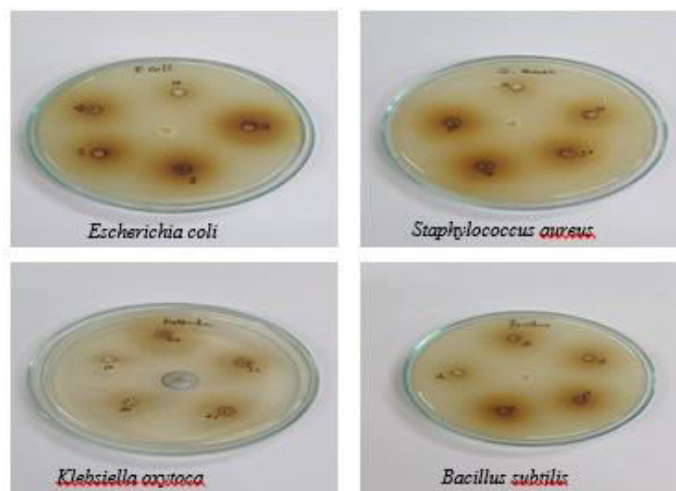
### 3.5. Antibacterial activity

The antibacterial activity against HECsL extract showed zones of inhibition of bacterial growth which varied against test organisms with different concentration 10-50  $\mu$ l (Table 2 and Fig. 4). The inhibition zones produced by the sample extract compared with zones produced by ampicillin were used as a control. Here obtained results revealed that the plant extracts were able to resist against some of the bacterial species. The HECsL extract showed moderate activity in 50  $\mu$ l concentration against *Escherichia coli* (1.2mm), *Staphylococcus aureus* (0.8mm), *Klebsiella oxytoca* (0.8mm) and *Bacillus subtilis* (0.9mm).

Abdullah et al., 2011 [26] reported that the antibacterial activity are in agreement with the findings where Methanol, Ethyl acetate and hexane extracts inhibited the growth of *Bacillus subtilis* to 12.75, 8.75 and 11.50 mm, respectively.

Extracts did not show any activity with distilled water. Additionally, all the extracts were not active against the growth of *Escherichia coli* [27].

From the studies of Salem et al., 2013 [28] the results revealed that different leaf extracts of *Callistemon viminalis* had a promising antibacterial activity.



**Fig. 4: Antibacterial activity HECsL extract**

**Table 2: Antibacterial activity of HECsL extract**

Name of the organism	Control	HECsL extract (mm)				
		10 µl	20 µl	30 µl	40 µl	50 µl
<i>Escherichia coli</i>	0.3	0.3	0.4	0.6	0.8	1.2
<i>Staphylococcus aureus</i>	0.5	0.3	0.4	0.3	0.6	0.8
<i>Klebsiella oxytoca</i>	0.2	0.1	0.2	0.4	0.3	0.8
<i>Bacillus subtilis</i>	0.1	0.2	0.6	0.5	0.7	0.9

Zone of inhibition in mm

### 3.5.1. Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration was done for selected organism using petroleum ether, chloroform, ethanol and aqueous extracts which gave maximum zone in well diffusion method the results are presented in Table 3.

The HECsL extract (10-50µl) were inoculated against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella oxytoca* and *Bacillus subtilis*. Bacterial strains of *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella oxytoca* and *Bacillus subtilis* were found to be sensitive in 30µl HECsL extract.

Strains exhibiting the highest inhibitory zones were not always those which are most sensitive. These results are consistent with those reported with previous studies [29]. Indeed, the zones of inhibition do not reflect the antibacterial effectiveness of a product; it can be affected by the solubility, diffusion and evaporation of the extract [30].

**Table 3: Antibacterial Activity of HECsL extract against Organisms by Minimum Inhibitory Concentration (MIC) method**

Name of the organism	HECsL extract (mm)				
	10 µl	20 µl	30 µl	40 µl	50 µl
<i>Escherichia coli</i>	-	+	+	-	-
<i>Klebsiella oxytoca</i>	+	-	+	+	+
<i>Staphylococcus aureus</i>	+	-	-	+	-
<i>Bacillus subtilis</i>	-	+	+	-	-

## 4. CONCLUSION

Now a day's antioxidative properties of the plants have become a great interest due to their possible uses as natural additives to replace synthetic ones. In this present study, hydro-ethanolic extract of *Camellia sinensis* leaves (HECsL) shows antioxidant property which might be helpful in preventing the progress of various oxidative stress related diseases followed by the DPPH free radical scavenging assay. The scavenging activity of HECsL extract shows similar trend with the result of total phenolic content and total flavanoid

indicating that the free radical scavenging activity of HECsL extract and also it can have great potential as antimicrobial components against microorganisms. Thus the present data suggests that HECsL extract can be used as a good source of natural antioxidants for health benefits and further isolation of bioactive compounds are required for identifying the unknown compounds to establish their pharmacological properties.

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