

Research article

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Phytochemical screening, anti-inflammatory activity and anti-oxidant activity assessment of black tea, green tea and rooibos tea

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Abstract

Tea is one of the supreme consuming beverages all over the world, which possesses several health benefits as a result of the presence of different phytochemicals including polyphenols which contain important medicinal activities. This current research study is about comparing the varying efficacies of polyphenols of green, black and rooibos tea in relevance to therapeutical efficacies including anti-oxidant and anti-inflammatory. The initial phase of the study involves the extraction of phytochemical compounds from three different types of tea and their phytochemical screening using standard methodologies. The later phase involves the extensions with fractionation and estimation of phenolic compounds from the extracts. The crude and high phenolic fractions from each extract were assessed for anti-inflammatory and total anti-oxidant potency. Results authenticate that, the polyphenols produce anti-inflammatory and anti-oxidant efficacies and as a mean of comparison, green tea with its high polyphenol contents possess high efficacy in producing the values under *in vitro* conditions.

Keywords

Anti-oxidants; Anti-inflammatory; Green tea; Black tea; Rooibos tea; Polyphenols



Introduction

Tea is an infusion made from the plant leaves.<sup>[1]</sup> Tea is a refreshing beverage that is calorie-free containing no sodium, fat, carbonation or sugar and helps in maintaining a proper fluid balance in the body.<sup>[2]</sup> It is usually prepared from the leaves of *Camelia sinensis*. White, yellow, green, oolong, black and pu-erh tea are six different types of tea prepared from the leaves of *C. sinensis* using different processing methods.

Many research studies carried out in the leaves of *C. sinensis* show that, they possess vital phytochemicals especially polyphenols (such as flavonoids, tannins and less common stilbenes and lignans) which can exhibit activities, such as antibacterial, anti-oxidative, antimicrobial, anti-promotion and antitumour.<sup>[3]</sup>

Among polyphenolic compounds, flavonoids are the most abundant in our diet and tea possesses the highest content of them with catechins (epigallocatechin-3-gallate, epicatechin, epicatechin-3-gallate, epigallocatechin, catechin and galocatechin, etc.) being the largest type in growing tea leaves.<sup>[4]</sup> The aflavins found in black tea and catechins in green tea are equally effective anti-oxidants.<sup>[5]</sup> Apart from the tea of *C. sinensis*, there are few other important teas, such as rooibos tea. Rooibos tea is considered healthy as a result of the absence of alkaloids and low tannins content unlike tea from *C. sinensis*. This study was aimed to comparatively investigate the phytochemical profile of black, green and rooibos tea, and also to assess and compare their anti-inflammatory and anti-oxidant efficacies.

Materials

Black tea, green tea and rooibos tea powders were purchased from the local market in Chennai. All the chemicals used in the research work were of analytical grade and were purchased from Hi-media and Merck Chemicals, India.

Methodologies

Extraction

5 g of three different types of tea was heated with 100 ml of distilled water over a thermostatic water bath for 30 min at 60-70°C. The extract was filtered using Whatmann No. 1 filter paper. The extracts were labelled and refrigerated for further use.

Qualitative analysis for phytochemicals:

Test for alkaloids:

3 ml of extract was treated with 1 ml of Drangendroff's reagent and was observed for the change in turbidity.<sup>[6]</sup>

Test for flavonoids:

3 ml of extract was treated with few drops of sodium hydroxide solution and was observed for the formation of yellow colour.<sup>[6]</sup>

Test for phenols and tannins:

2 ml of extract was treated with 2% solution of FeCl<sub>3</sub> and observed for the formation of bluish black colour.<sup>[6]</sup>

Test for polyphenols:

10 ml of extract was heated over a steam bath for 30 min. Then it was treated with 1% FeCl<sub>3</sub> and observed for the bluish black precipitate.<sup>[7]</sup>

Test for steroids:

Aqueous extract was treated with 2 ml of chloroform, and concentrated sulphuric acid was added and observed for dark pink colour formation.<sup>[8]</sup>

Test for terpenoids:

Crude extract was treated with 2 ml of chloroform and evaporated; following that, 2 ml of concentrated sulphuric acid was added and observed for reddish brown colour change.<sup>[9]</sup>

**Test for anthraquinones:**

The extract was treated with dilute sulphuric acid; then 1 ml of diluted ammonia was added and observed for colour change to red.<sup>[9]</sup>

**Test for saponins:**

The extract was shaken with water (1:1 ratio) in a test tube and was warmed in a water bath and observed for the stable froth.<sup>[9]</sup>

**Test for cardiac glycosides:**

The extract was dissolved in 5 ml of methanol and then followed by 10 ml of 50% HCl. It was then heated over a steam bath for 30 min. 5 ml of Fehling's solution was added; the mixture was boiled for 5 min and then observed for the formation of brown ring.<sup>[10]</sup>

**Separation of phenolic compounds through column chromatography**

Column chromatography was performed on the classic 20 cm long × 2 cm diameter glass column packed with silica gel of mesh size 230–400. 5 ml of crude extract was allowed to move through the stationary phase; ethanol was used as mobile phase for elution in 1:1 ratio. Ten fractions were collected and the purified extract was used for further studies.<sup>[11]</sup>

**Quantification of phenolic compounds using Folin-Ciocalteu's assay**

In this experiment, the crude extract of black, rooibos and green tea, and their corresponding column fractions were quantified for phenolic compounds. The assay mixture was made with 400 µl of sample, 1.5 ml of Folin-Ciocalteu's reagent and 1.5 ml of 7% sodium bicarbonate solution. Gallic acid was used as standard in the concentrations of 0.25, 50, 75, 100, 125, 150 and 175 µg/ml. The assay mixture was incubated at room temperature for 90 min. After 90 min, the absorbance was measured against a blank at 725 nm using a Spectrophotometer.<sup>[12,13]</sup>

**Anti-inflammatory assay**

In the anti-inflammatory assay, 0.5 ml of purified fraction of each type of tea and the crude extract was tested. Human red blood cell suspension for the *in vitro* anti-inflammatory analysis was prepared using the methodology as mentioned by Manohari et al.<sup>[14]</sup> The sample was made up to 1 ml with distilled water. Following this, 1 ml of phosphate buffer, 2 ml of hyposaline and 0.5 ml of HRBC suspension were added. Distilled water was used as control. The assay mixture was incubated at 37°C for 30 min and estimated at 560 nm. The percentage of stabilisation was calculated using the following equation<sup>[14]</sup>:

$$\% \text{Stabilisation} = 100 - \frac{\text{optical density of sample} - \text{optical density of control}}{100}$$

**Total anti-oxidant efficiency analysis using phosphomolybdenum assay**

The purified fractions of each type of tea extract were tested for the total anti-oxidant efficacy using the phosphomolybdenum assay. Two test samples (100 and 200 µl) were taken and made up to 300 µl with distilled water. Following this, phosphomolybdenum reagent (made by mixing 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added and assayed in Spectrophotometer at 695 nm. Ascorbic acid was used as standard in the concentration of 375, 560 and 920 µg/ml.<sup>[15]</sup>

**Results and Discussion**

The study was aimed at performing a comparative investigation about the phytochemicals present in black, green and rooibos tea, and their efficacy in exhibiting anti-inflammatory and anti-oxidant activities. Among these different types of teas tested, black and green teas were prepared from the plant *C. sinensis*, and rooibos from the leaves of rooibos plant. However, the production process varies for each tea and hence the active molecule concentration also varies.<sup>[16]</sup>

Green tea is produced from the fresh leaves by steaming to inactivate the enzymes; whereas, the black tea is prepared through curing and maceration of leaves, and the rooibos tea is prepared through partial oxidation.<sup>[17]</sup> Based on the variation in the making process, even the tea leaves from the same plant constitute different compounds.<sup>[18]</sup>

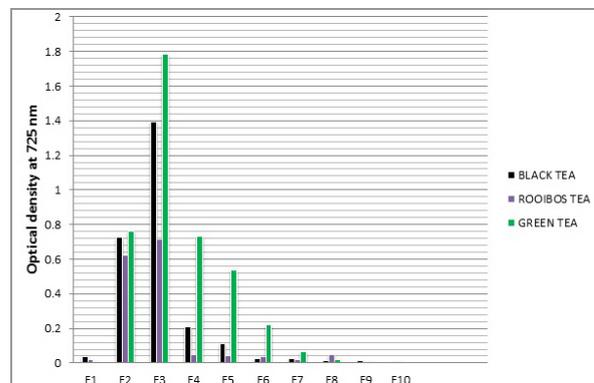
All the three tea extracts were tested for basic phytochemical screening and the results based on the observation are tabulated (Table 1).

Table 1. Phytochemical screening

S. No.	Phytochemical	Black tea	Green tea	Rooibos tea
1.	Alkaloids	-	-	-
2.	Polyphenols	+	+	+
3.	Flavonoids	+	+	+
4.	Tannins	+	+	+
5.	Phenols	+	+	+
6.	Saponins	+	+	-
7.	Terpenoids	-	-	-
8.	Steroids	+	+	+
9.	Anthraquinones	-	-	-
10.	Cardiac glycosides	+	+	+

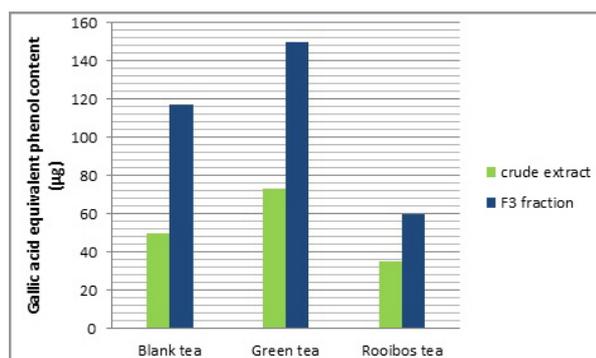
The screening tests on black, green and rooibos tea extracts revealed the presence of phenols especially flavonoids and tannins. Phenol test produced remarkable results which effectively confirmed the presence of good quantity of phenolics in the tea leaves of interest.<sup>[19,20]</sup>

Fig 1. Total phenolic content of column fractions in µg/ml

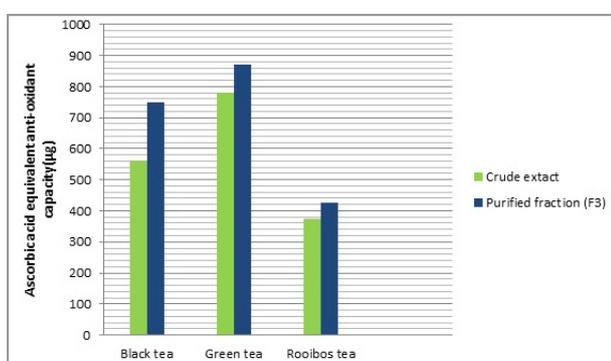


The purified extracts were subjected to quantification using Folin-Ciocalteu's phenol assay. Ten fractions were collected using ethanol as eluent.<sup>[21]</sup> Among different fractions, F2 and F3 contain more amount of phenol in all the tea extracts (Fig. 1). However, the phenolic content of green tea<sup>[22]</sup> was high in more number of fractions F2, F3, F4 and F5. Relatively, black tea extracts produced higher phenolic fractions than the rooibos.

The Folin-Ciocalteu's phenol assay was also performed in a comparative way to assess the phenol content in the crude and column fraction. The results (Fig. 2) have clearly shown that, the fractions from column chromatography contain more concentration of phenol content.

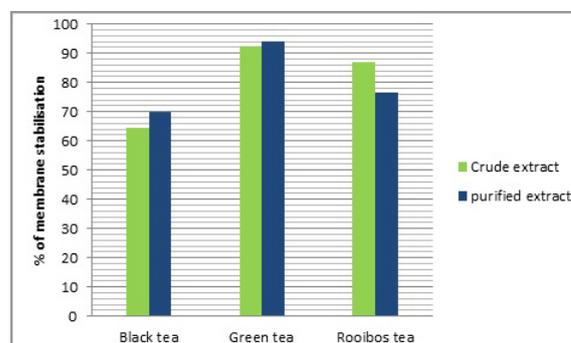
**Fig. 2. Total phenolic content of different types of tea**

Tea polyphenols are known for the anti-oxidant activity. The anti-oxidant efficacy of all the three tea extracts and the purified fractions were tested using the standard total anti-oxidant measurement assay. Among the tested tea extracts, green tea has shown increased activity in comparison to black and rooibos tea<sup>[23]</sup> as a result of variation in the polyphenol content (Fig. 3).<sup>[24,25]</sup>

**Fig. 3. Anti-oxidant activity of different types of tea**

The anti-inflammatory effect of three different teas was tested for anti-inflammatory potential. Among the results obtained, green tea was found to produce more membrane stabilisation followed by black

tea and then the rooibos tea. However, the anti-inflammatory efficacy of the purified fractions of black and green tea was slightly higher than the crude extracted, and in the case of rooibos, the potency was little lesser than that of the crude (Fig. 4).<sup>[14]</sup> This may be due to the presence of other phytomolecules in the crude extract, such as alkaloids.<sup>[25]</sup>

**Fig. 4. Anti-inflammatory activity of different types of tea**

### Conclusion

Tea is one among the best refreshment beverages used widely around the countries of the world. Apart from the refreshment, tea also adds valuable inputs to our healthy life. This *in vitro* study on different tea extracts had strengthened the above mentioned fact. All the three tested tea leaves had shown positive result for the presence of phenolic compounds; also, their purified phenolic fractions had shown therapeutic efficacy that was slightly higher than the crude extract under *in vitro* conditions. Though all the tested tea leaves had shown medicinal benefits, the green tea had remarkably exhibited some higher values which are a considerable interest.

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