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DEVELOPMENT OF HERBAL TEA CONCENTRATE FROM KAHWA LEAVES

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Abstract

Kahwa or the Kashmiri Green tea is a staple beverage consumed in Kashmir in all seasons, but especially winter. Kahwa is traditionally a mix of green tea extract and other spices such as saffron, cardamom and is occasionally supplemented with pepper or cinnamon and almond pieces. Having several health benefits, Kahwa is popular in other regions of northern India as well. In the present study, the method for preparing a Kahwa concentrate is described. Double-boiling method was used for reducing the aqueous Kahwa extract, followed by testing of the concentrate for antimicrobial and antioxidant activities. The extract showed a zone of inhibition ranging from 0.75cm to 1.1 cm against *E. coli* cells and showed an antioxidant activity at 40.68%. The extract was also evaluated for suitability of taste using the 9-point Hedonic Scale and was found to be acceptable with a good score.

Keywords **:** Antibacterial, Antioxidant, Functional Properties, Kahwa Leaves, Herbal Tea.

Introduction

One of the most commonly consumed beverages is "Tea", which is originally originated from china and is one of the food products globally. India is the 2nd largest producer of the tea after china (Bassi *et al*., 2020; Mo *et al*., 2008). Nowadays, consumer has become well aware and concerned about their health, hence they are demanding for natural as well as healthy food (Hoque *et al*., 2018). Hence, tea seems to be the valuable food product because of its aroma and taste. Therefore, tea has gained the significant attention among the wellness beverages in the food market (Farooq & Sehgal; Nookabkaew *et al*., 2006).

According to tradition, tea is generally categorized as black, green, herbal and oolong tea and is generally distinguished based on their processing method (Joshi and Kumar, 2017; Kumar *et al*., 2015; 2016; Sheibani *et al*., 2016; Singha *et al*., 2013). *Camellia sinensis,* is the most common plant from which herbal tea and other types of tea have been derived (Zhang *et al*., 2019). Major difference in the herbal tea preparation from other tea is that it doesn't require fermentation and has various health promoting phenolic compounds (Khan and Mukhtar, 2013; Sunil and Kumar, 2019). Moreover, herbal tea has been comprehended in literature to contain 4000 diverse bioactive compounds, in which polyphenols hold the one-third ratio and rest is covered by flavonoids and tannins (Anand *et al*., 2015). Vitamin P, predominantly found in the green tea is accorded dur to the presence of catechin flavonoids (Chacko *et al*., 2010). Due to association of health benefit role in Alzheimer's Disease, Anti-Viral, Blood Pressure, Depression, Diabetes, Heart Diseases, Parkinson Disease, Skin-Care and Weight loss abilities in herbal tea has made it the imperative target for research (Dey *et al*., 2017; Hussain and Koul, 2018; Mak, 2012; Manvitha and Bidya, 2014; Namdev and Gupta, 2015; Singh and Singh, 2018). Therefore, antibacterial and antioxidant potential of herbal tea are being explored for developing natural as well as healthy beverage (Jassal & Thambyrajah, 2018; Kaur & Kumar, 2017; Kumar *et al*., 2017a; Malik *et al*., 2016; Nazir *et al*., 2016; Pereira *et al*., 2018). The current study focusses on the preparation of Kahwa leaves concentrate and evaluate the presence of flavonoids, antibacterial and antioxidant activity.

Materials and Methods

Sample Collection

Kahwa leaves were purchased from a local market in Jalandhar. The collected leaves were powdered by using mortar and pestle, sieved and stored in air-tight container for further use. The sample was labelled as Kahwa Leaves (KL).

Processing method for Kahwa Leaves (KL)

For this purpose, KL were subjected to three different processing conditions in order to retain the high concentration of bioactive molecules. The Tea concentrates were prepared from 1g of KL by Rotary Vacuum Evaporation as stated by Al-Farsi and Lee (2008). And, another method named Double boiling method, in which 1g leaves were added to 100 mL of water and boiled till the dark color was obtained. Later the colour and smell of sample was recorded (Banerjee and Chatterjee, 2015). In another method i.e. cold extraction method in which 1.4g of KL were blended in 200 mL of cold water and were kept in shaking incubator for 12-14 hours. Both samples i.e. different type of herbal tea concentrate obtained were kept in refrigerator for further analysis (Gião *et al*., 2009).

Antioxidant Potential of Herbal Tea Concentrate

The antioxidant potential of both the tea extracts was conducted by following the Blois (1958). The samples of varied concentration (25µL and 50µL) were prepared by diluting it with 0.1M Tris-HCl (800µL) and DPPH solution (1mL) in test tube (Mohan *et al*., 2011). After mixing, all the test tubes containing the sample were kept for 10 minutes in dark. Blank was prepared without adding the extract into it and 99.5% ethanol was used as reference. Optical Density of varied concentration was measured by taking the absorbance at 517 nm and calculated by the given formula:

$$\text{Radical Scavenging Activity (\%)} = \frac{\text{OD of the Blank} - \text{OD of the Sample}}{\text{OD of the Blank}} \times 100$$

Antimicrobial Activity of Herbal Tea Concentrate

Antimicrobial Activity of Herbal Tea Concentrate was evaluated against *E. coli.* The test strain of *E. coli* was cultured in Luria Broth. The evaluation plates were prepared using Mueller Hinton Agar and 100µL of culture was spread with sterile spreader on the plate (Gupta *et al*., 2013; Priadarshini *et al*., 2013). Then, the plates were used for assessing the antimicrobial activity of Herbal Tea Concentrate via Agar diffusion method. For which, puncturing syringe was used to make the wells in the plates. 50µL of different dilutions of v/v (%) (i.e. 20, 40, 60, 80) were added to respective wells. One well in the plate was used as negative control. The plate was incubated at 37°C for 24 hours and later were observed for inhibitory zone around the well (Digvijay and Bharadwaj, 2017; Kumar *et al*., 2017b; Suay *et al*., 2000; Thomas *et al*., 2011)

MTT Assay of Herbal Tea Concentrate

The MICs of yeast was assessed by microtiter plate assay as per the procedure of **Pierce *et al.* (2008)**. For this each well of 96-well microtiter plate was filled with 100µL of yeast culture containing 10^5 cells/well. Whereas, the herbal extract was added as follow i.e. Column 1-2 = no sample, Column 3 (cold extract) = 20µL each row, Column 4(cold extract) = 50µL each row, Column 5 (cold extract) = 100µL each row, Column 6(herbal formulation) = 20µL each row, Column 7 (herbal formulation) = 50µL each row, Column 8(herbal formulation) = 100µL each row, Column 9(diluted 10x cold extract) = 100µL each row. The volume differences were made-up using water. After adding this microtiter plate was incubated at 37°C for 24 h. After that, 20µL of MTT (5g/L) was added in each well and the microtiter plate was again incubated at 37°C for 4 h. Then finally the liquid media was removed and Dimethyl sulfoxide (DMSO) was added. The absorbance was then recorded at 570 nm.

Sensory Evaluation

Sensory evaluation was done by following the guidelines stated by Watts *et al* (1989). The attributes like Flavor, Appearance, Aroma, Texture and Taste of Herbal Tea concentrate were evaluated by fifty customers. The customers were requested to score the herbal tea concentrate on hedonic scale of 9 to 1. (Akila *et al*., 2018)

Result and Discussion

Processing method for Kahwa Leaves (KL)

The extraction of the tea extracts was done by all the three methods. The samples prepared by cold extraction and double boiling method were collected for further analysis. Whereas, the prepared by rotary vacuum evaporation was discarded as the concentrate was very less. Hence, further all the analysis was conducted on the extracts obtained by cold extraction and double boiling method

Table 1: Antioxidant Activity of Different Concentrations of Kahwa Extract

Sample	Quantity	Optical Density	Antioxidant Activity
Tea Extract	0µL	0.717	0%
	25µL	0.626	12.7%
	50µL	0.650	9.34%
Cold Extract	0µL	1.020	0%
	25µL	0.638	37.45%
	50µL	0.605	40.68%

Table 2: Antimicrobial Activity of Hot and Cold Kahwa Extracts

Sample	Dosage (v/v %)	Standard Error
Hot Tea Extract	Blank	0
	20	0.775±0.032
	40	0.775±0.043
	60	0.8125±0.074
	80	0.9625±0.062
Cold Extract	Blank	0
	20	0.45±0.064
	40	0.4±0.041
	60	0.5±0.041
	80	0.675±0.075

Table 3: Results for MTT assay of cold extract sample and herbal formulation at 570 nm. Column 1 (no sample), Column 2 (no sample), Column 3 (40% cold extract), Column 4 (60% cold extract), Column 5 (80% cold extract), Column 6 (40% Hot Extract), Column 7 (60% Hot Extract), Column 8 (80% Hot Extract), Column 9 (diluted 10x cold extract), Column 10 (diluted 10x Hot Extract)

Sample Name	Mean Absorbance	Toxicity
1	1.355±0.185	NA
2	1.210±0.140	NA
3	2.527±0.218	-
4	2.668±0.059	-
5	3.136±0.125	-
6	2.377±0.539	-
7	2.829±0.409	-
8	3.115±0.095	-
9	1.787±0.279	-
10	2.425±0.106	-

Antioxidant Potential of Herbal Tea Concentrate

The antioxidant potential of varied volume was determined by measuring the optical density at 517 nm and result are depicted in Table 1. The result obtained revealed that antioxidant potential of the herbal tea concentrate prepared by cold extraction method was high in comparison to the herbal tea concentrate prepared by double boiling method. This suggests that double boiling method degrade the chemical which reacts with the free radicals to antioxidant activity. The results obtained were similar with antioxidant potential of *Cinnamon* bark and *Tinospora cordifolia* stems result obtained by Namdev and Gupta (2015).

Antimicrobial Activity of Herbal Tea Concentrate

The antimicrobial activity of both tea extract and cold extract are shown in Table 2. Both the extract with varied concentration showed the inhibitory effect on *E. coli.* The inhibitory effect of Tea extract was more in contrast to the cold extract. The result obtained in our study was similar to the result of Archana and Abraham (2011).

MTT Assay of Herbal Tea Concentrate

The MTT Assay of both tea extract and cold extract are shown in Table 3. The result obtained showed the increase in the reading on increasing of concentration of sample from 20µL to 100µL for both samples. On comparing the result, cold extract sample reduced the cell toxicity in comparison to the double boiled sample.

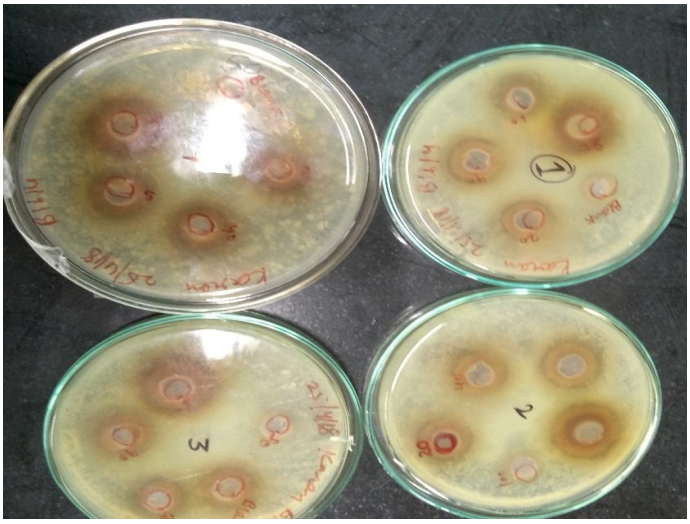

Fig. 1: MHA plates showing zone of inhibition upon treatment with dilutions of Kahwa Extracts

Sensory Evaluation

The Herbal Tea extract was presented to fifty customers for evaluating the acceptability of the product. The result of sensory evaluation of tea extract for overall acceptance by fifty customers on the hedonic scale of 9 to 1 is illustrated in Figure 3.

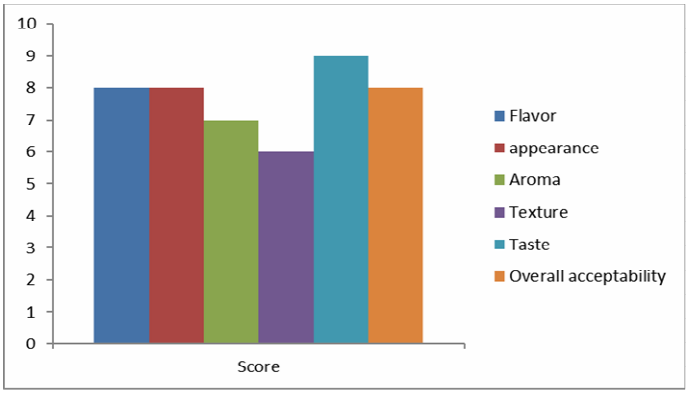

Fig. 3: Overall Acceptability of the Tea Preparation on a 9-point Hedonic Scale.

Conclusion

The result of antibacterial potential, antioxidant potential and phytochemical of Kahwa leaves revealed that it can serve as the valuable source of flavoring and nutraceutical agent. Various health benefits associated with these herbal tea makes it the ideal and psychological health rejuvenator. Even, Kahwa leaves tea holds various health benefits, but it lacks the distinct flavor which could make it appealing for consumption. Therefore, it would be effective to blend the Kahwa leaves with other herbs like *Cinnamon* bark and *Tinospora cordifolia* stems to enhance its flavor, appeal and palatability without compromising its health benefits. As taste appeals more to the costumer than the nutritional and health benefits, therefore infusion of this Kahwa leaves with other herbs in future research will serve as better alternative to flavored teas as it will impart health benefits also.

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Conflict of Interest

The authors declare no conflict of interest

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