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Comparative phytochemical analysis, antimicrobial and anti oxidant activity of the methanolic extracts of the leaves of *Coffea Arabica* and *Coffea Robusta*

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ABSTRACT

The methanolic extracts of the leaves of Coffea arabica (CA) and Coffea robusta (CR) were evaluated for its anti oxidant and anti microbial activity. The total phenolic content, flavonoid content and condensed tannin content were determined by the Folin ciocalteu method, Aluminum chloride colorimetric method and vanillin assay respectively. Antimicrobial activity was determined by using cup diffusion assay while the anti oxidant activity was evaluated using DPPH. The total phenolic content was found to be 27.04 µg/g and 21.80 µg/g, total flavonoid 10.90 µg/g and 8.08 µg/g, and condensd tannins 3.1 and 2.9 respectively for Coffea arabica (CA) and Coffea robusta(CR) respectively. Coffea robusta showed better anti microbial and anti bacterial activity when compared to Coffea arabica.

Key words: Antioxidant activity, antimicrobial activity, *Coffea arabica* , *Coffea robusta*

INTRODUCTION

Coffea arabica and *Coffea robusta* are the two important classes of coffee belong to the family rubiaceae.Coffee has been used traditionally in the treatment of asthma, atropine-poisoning, fever, headache, jaundice, malaria, migraine, narcosis, sores and vertigo. Coffee enemas have been used for cancer. Caffeine is the most important constituent of coffee which is widely used as stimulant [1]. A number of beneficial health properties have been attributed to coffee, among them are diuretic, antimicrobial and antioxidant activities[2].Constituents such as such as caffiene, chlorgenic acid, caffiec acids condensed pro anthocyanidins, quinic acid, and ferulic acid have been reported to anti oxidant and posses anti bacterial activity[3]. The review of

literature has revealed that most of the work on coffee has been done on the beans of coffee [4, 5]. Therefore, the aim and objective of the present study was to evaluate the phytoconstituents, antioxidant and antimicrobial properties of methanolic extracts of the leaves of *Coffea arabica* and *Coffea robusta*

MATERIALS AND METHODS

Plant material:
The leaves of *Coffea arabica* and *Coffea robusta* were collected from rural areas of Chickmangalore District, Karnataka and were identified and authenticated by the Prof Manjula Srinivisan, HOD, Department of Botany, Krupanidhi College, Bangalore. The material was shade dried, pulverized and preserved in air tight containers until further use.

Chemicals: All chemicals for extraction, isolation, antimicrobial and anti oxidant activity were obtained from Merck and SD fine chemicals.

Preparation of the extracts:
The methanolic extracts of both the dried powder (1 kg) of the leaves was prepared by using Soxhlet apparatus. The extracts were filtered using Whatman filter paper and then concentrated using a Rotary evaporator

Phytochemical screening;
The extracts were then subjected to preliminary phytochemical analysis i.e., alkaloids, phenolic compounds, tannins, carbohydrates, proteins, amino acids and saponins using standard procedures [6].

Determination of total Phenolic content in the leaves of *Coffea arabica* and *Coffea robusta [7]*:
The percentage of total phenol was estimated by the Folin ciocalteu method for both the extracts. The extracts (1mg/ml) and different dilutions of standard gallic acid were mixed separately with 1ml of Folin ciocalteu reagent and a solution of 7% sodium carbonate was also added. The mixtures were incubated at room temperature for 90 mts. The total phenolic content for the extracts was determined by colorimetry at 750 nm. A standard curve for gallic acid in methanol was prepared using different concentrations i.e. using 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, 500µg/ml, 600 µg/ml and 700 µg/ml. The total phenolic content was expressed in terms of gallic acid equivalents.

Determination of flavonoids in Coffea arabica* and *Coffea robusta [7]:
Aluminum chloride colorimetric method was used for the determination of total flavonoids in the extracts. The extracts (1mg/ml) in methanol were mixed with 0.1ml of 1M potassium acetate, 1.5ml of methanol, 0.1ml of 10% aluminum chloride and 2.8ml of distilled water. This was maintained at room temperature for about 30mts. The absorbance of this mixture was measured at 415 nm. A calibration curve for the standard quercitin was obtained by taking 12.5ml, 25ml, 50ml, 75ml and 100ml in methanol. The total flavonoid contents were calculated as quercitin equivalent by plotting the absorbance versus concentration

Colorimetric estimation of condensed tannins by vanillin assay [8]: The amount of condensed tannins contents were calculated as catechin equivalent from the calibration curve of standard catechin by plotting the absorbance versus concentration

Different dilutions were prepared of standard catechin ranging between 50-350 mcg/ml. this were transferred to two sets of tubes and the volume in each of the tubes was made up to 1ml with methanol. The tubes were incubated at 30^0 C in a water bath and to this 5ml of working reagent i.e. one part 1%vanillin with one part 8% concentrated HCl was added at an interval of 1mt to one set of the tubes and 5ml of 4% HCl was added to the other set at intervals of 1.0mt (blank).The samples were kept in the water bath for 20 mts and the absorbance was recorded at 500nm maintaining the difference of 1mt as the color continues to develop when left for long time. The absorbance of the blank was subtracted from that of the sample containing vanillin reagent.Amount equivalent to 200mg of the extract was dissolved in 10ml of methanol 1.0 ml of this was transferred to a tube and the same procedure was followed.

Anti microbial studies of isolated compounds by cup plate method [9].
Anti microbial activity of the extracts was evaluated by using cup plate method. Sterile plates were prepared and 0.1 ml of the inoculum from standardized culture of *Staphylococcus aureus, Bacillus subtilis, Eschericia coli, Klebsiella pneumoniae* and *Candida albicans* were spread uniformly. The cups were made using a sterile borer and 100µl of the extract substance, standard antibiotic and the solvent control were added in each cup separately. The plates were placed at 4^0C for 1 h to allow the penetration of the test solution into the medium and plates were incubated at optimal temperature for a period of time sufficient for the growth .The zone of inhibition of microbial growth around the well was measured in mm.

Anti oxidant [10]:
The anti oxidant activity of the extracts were evaluated by using 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH).The stock solution of the extracts were prepared in methanol (10 mg/ml**)**. The working solutions (10, 20, 40, 80,100,120,140,180 and 200 mcg/ml) of the extracts were made from the stock solution by making appropriate dilutions. The anti oxidant activity of the plant extract was determined depending on the radical scavenging effect of the stable DPPH. The DPPH was prepared as 0.002% solution in methanol and mixed with 1ml of both the standard and the samples. The prepared solutions were placed in the dark for ½ hour and the absorbance was measured at 517nm. A blank was prepared using 2ml methanol and 2ml of DPPH. The % absorbance was calculated using the formula

$$\% \text{ Absorbance} = \frac{A - B}{A} \times 100$$

Where A is the absorbance of the blank and B is the absorbance of the sample.

RESULTS

The preliminary phytochemical analysis revealed the presence of alkaloids, glycosides, phenolic compounds, carbohydrates, sterols, tannins and proteins.

The amount of phytoconstituents was more in CR compared to CA i.e. phenolic acid was found to be 21.80 µg/g and 27.04 µg/g, total flavonoid 8.08 µg/g and 10.90 µg/g and condensed tannins was found to be 2.9 and 3.1 in CR and CA respectively as shown in table 1.

Table1: Phenolic acid, total flavonoids and condensed tannins content in the leaves of *Coffea arabica* and *Coffea robusta*

Extract	Phenolic content µg/g	Total flavonoid µg/g	Condensed tannins
Coffea Arabica	21.80	8.08	2.9
Coffea robusta	27.04	10.90	3.1

Anti microbial effect of the methanolic extract of the leaves of *Coffea arabica* and *coffea robusta* by cup plate method:

Coffea arabica and *coffea robusta* exhibited activity against *Staphylococcus aureus Bacillus subtilis,Eschericia coli,* and *Klebsiella pneumoniae* at 200 mcg/ml. while *coffea robusta* exhibited activity against *Staphylococcus aureus, Bacillus subtilis,Eschericia coli* at both 100 and 200 mcg/ml and *Klebsiella pneumoniae* at 200 mcg/ml. Both the extracts did not show any activity against *Candida albicans*

Table 2: Anti microbial effect of test drugs at 200 mcg/ml and 100 mcg/ml by cup plate method

Sl. no	Sample	Zone of inhibition in mm									
		Staphylococcus aureus		*Bacillus subtilis*		*E. coli*		*Klebsiella pneumoniae*		*Candida albicans*	
		200	100	200	100	200	100	200	100	200	100
1	*Coffea arabica*	17	NI	15	NI	19	NI	10	NI	NI	NI
2	*coffea robusta*	19	10	18	10	23	13	15	NI	NI	NI
Standard drugs	Streptomycin (100 mcg/ml)	33	17	29	15	31	14	25	17	-	-
	Flucanozole (25mcg/ml)									18	14

NI: No Inhibition.

Anti oxidant activity of the isolated compounds:

The results of the anti oxidant activity revealed that *Coffea robusta* had better anti-oxidant activity when compared to *Coffea Arabica.*

Table 3: Anti oxidant activity of the methanolic extract of *Coffea arabica* and coffea robusta (absorbance and % inhibition)

Mcg/ml	Methanolic Extract of *Coffea arabica*		Methanolic Extract of *Coffea* robusta	
	AB	% inh	AB	% inh
10	0.081	74.36	0.065	79.43
20	0.076	75.94	0.061	80.69
40	0.074	76.58	0.058	81.64
80	0.071	77.21	0.055	82.51
100	0.069	78.16	0.051	83.86
120	0.066	79.11	0.048	84.81
140	0.064	79.74	0.045	85.75
180	0.060	81.01	0.041	87.02
200	0.057	81.92	0.039	87.65

DISCUSSION AND CONCLUSION

Literature has revealed that a number of plants posses anti oxidant and antimicrobial activity due to the presence of various phyto constituents like phenolic acids,tannins,flavonoids etc[12,13].Plants contain different types of antioxidant molecules, like phenolic compounds i.e. phenolic acids, flavonoids, and tannins, tocopherols, carotenoids and ascorbic acid . These natural antioxidants are present in different parts of the plants such as wood, bark, leaves, roots etc. Phenolic compounds are an important class of compounds due to ability to function as terminators of free radical chains or chelators metal ions. They play an important role in stabilizing the lipid oxidation. The amount of phenolic content was found to be 21.80 and 27.04 in CA and CR respectively. The amount of total flavonoid was found to be 10.90 and 8.08 and condensed tannins were found to be 3.1 and 2.9 in CR and CA respectively. The phenolic compounds by virtue of their structure i.e. presence of hydroxyl groups contribute to the anti oxidant activity.

Bacteria have different degrees of sensitivity to antimicrobial compounds such as caffeine, volatile and non-volatile organic acids, and aromatic compounds like phenolic compounds, aldehydes, ketones and esters. CR variety has been reported to be more inclined to antimicrobial activity than CA. Antibacterial properties have been reported be due to the presence of caffeic acid, chlorogenic acid and protocatechnic acid, all of which are present in coffee. Coffee is a rich source of antioxidants, including caffeine, hydroxycinnamic acid derivatives i.e. caffeic, coumaric, chlorogenic, ferrulic acids, flavonoids and polyphenols [14].The results of our study indicated that the amount of phenolic acids, condensed tannins and flavonoids were found to be more in CR when compared to CA. The anti microbial and anti oxidant activity were more significant in case of C*offea robusta*. Caffeine is the most important constituent of coffee which is reported to posses both anti oxidant and antibacterial activity. Literature has also revealed that the amount of caffeine is more in CR [15]. From the results of our study it can be concluded the CR has shown better anti microbial and anti oxidant activity as the amount of phytoconstituents present in it are more when compared to CA.

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