



PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF ROASTED BEANS OF *COFFEA ROBUSTA*

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ABSTRACT

Coffee consumption has been growing globally due to its pleasant aroma and health benefits. The present study investigated the presence of phytochemicals along with its antioxidant activity. Three different extracts were prepared from the roasted beans of *Coffea robusta* and the preliminary phytochemical screening for the crude extracts showed results for phenolics, flavonoids, steroids, flavonoids, saponins and terpenoids compounds in each of the extracts. High antioxidant activity was observed in methanolic extracts using DPPH and FRAP assay. Regarding the antimicrobial activity, the highest zone of inhibition was observed in methanol extract against *Alcalygens denitrificans*. Thus, roasted beans of *Coffea robusta* could be good source of natural antioxidants and antimicrobial agents.

KEY WORDS

Coffea robusta; Phytochemicals; Antifungal; Antibacterial; Antioxidants

INTRODUCTION

Human beings have been using bioactive compounds produced by plants as remedies to improve their health and cure illness. Coffee is a popular beverage that is widely consumed around the globe due to its physiological effects as well as its pleasant taste and aroma. *Coffea robusta* is an important class of coffee belonging to the family rubiaceae. Caffeine is the most important constituent of coffee which is widely used as stimulant. Various studies have revealed a number of beneficial health properties of coffee diuretic, antimicrobial and antioxidant activities [1, 2, 3].

Phytochemicals are natural bioactive compounds present in plants that have defense role. The plants produce these chemicals to protect themselves, but recent research exhibits their importance in human health [4, 5]. Antioxidant acts as radical scavengers and reduction of oxidative damage to macromolecules thus protecting the human body from various diseases [6]. Coffee is also rich source of the alkaloids, especially caffeine which stimulates the central nervous system

and has role in diuretic and peripheral vasoconstriction. Caffeine also contributes to the brew bitterness [7].

Therefore, the present study focuses on the evaluation of phytochemicals, antioxidant and antimicrobial properties of three different extracts of the roasted beans of *Coffea robusta*.

MATERIALS AND METHODS

Sample Collection

Roasted beans of *Coffea robusta* were collected in a sterilized bag from Coorg district of Karnataka. The sample was brought to laboratory. The coffee beans were ground into fine powder and three extracts viz. methanol, aqueous and chloroform were prepared. 15 g of powdered coffee sample was mixed in 75 ml of each solvent and the mixture was stirred for 24 hours. The suspended mixture was filtered through whatman's filter paper and filtrate was collected. This procedure was repeated thrice to get three filtrates and residue. The filtrates were then dried at room temperature. Each of the solvents was evaporated and

the gummy solid thus obtained was labeled and stored for further use.

Phytochemical Qualitative Analysis

Methanol, aqueous and chloroform plant extracts were assessed for the existence of the phytochemicals by using the following standard methods [8, 9].

Test for carbohydrates

To 1 ml of extract, added few drops of Molisch's reagent and then added 1 ml of concentrated sulphuric acid at the side of the tubes. The mixture was then allowed to stand for 2 to 3 minutes. Formation of violet coloured ring indicated the presence of carbohydrates in the sample extract

Test for proteins

About 1.0 ml of each crude extract was taken and two drops of freshly prepared 0.2% Ninhydrin reagent was added and heated. The appearance of pink or purple colour indicated that the presence of proteins, peptides or amino acids.

Test for phenolics

To 1.0 ml of crude extract, few drops of 5% aqueous ferric chloride solution was added. Formation of bluish black colour indicated that the presence of phenolics.

Test for Saponins

5.0 ml of distilled water was mixed with 1.0ml aqueous crude plant extract in a test tube and it was mixed vigorously. The frothing was mixed with few drops of olive oil and mixed vigorously, and the foam appearance showed the presence of saponins.

Tests for Flavonoids

2 ml of each extract was added to few drops of 20% sodium hydroxide. Formation of intense yellow colour was observed. To this, few drops of 70% dilute hydrochloric acid were added and yellow colour disappeared. Formation and disappearance of yellow colour indicated the presence of flavonoids in the sample extract.

Test for Terpenoids

2.0 ml of chloroform was added with the 5 ml aqueous plant extract and evaporated on the water bath and then boiled with 3 ml of concentrated H₂SO₄. Formation of grey color showed the presence of terpenoids.

Test for Steroids

2 ml of chloroform and concentrated H₂SO₄ were added with the 5 ml aqueous crude plant extract. In the lower chloroform layer red color appeared that indicated the presence of steroids.

Quantitative estimation of total phenolics and flavonoids

Total phenols were estimated using Folin-Ciocalteu method [10]. To 0.5mL of each extract solution 0.5ml of 1N Folin-Ciocalteu reagent was added. The mixture was kept at room temperature for 5 min, followed by the addition of 1ml of 20% Na₂CO₃. After incubating for 10 min at room temperature, the absorbance was measured at 750nm. Gallic acid was used as standard. The concentration of phenolics was calculated as per following equation.

$$\text{Absorbance} = 0.0364 \text{ gallic acid } (\mu\text{g}) + 0.009.$$

Total flavonoid content was estimated using a colorimetric method [11]. The extracts were diluted with distilled water to a volume of 3.5ml, followed by the addition of 150 μ l of 5% sodium nitrite solution. After incubation of 5 min, 300 μ l of 10% of aluminium chloride solution was added to each extract mixture. This was followed by addition of 550 μ l of the distilled water after incubation of 6 min at room temperature. The absorbance was measured at 510nm. Quercetin was used as standard.

Determination of Antioxidant Activity

DPPH Radical Scavenging Assay

Free radical scavenging ability of the extracts was tested by DPPH radical scavenging assay as described by Blois [12]. The hydrogen atom donating ability of the plant extractives was determined by the decolorization of methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). A solution of 0.1 mM DPPH in methanol was prepared, and 2.4 ml of this solution was mixed with 1.6 ml of each plant crude extract in methanol at different concentrations (12.5–150 μ g/ml). The reaction mixture was vortexed thoroughly and incubated in the dark for 30 min at 37°C. The absorbance of the mixture was measured spectrophotometrically at 517 nm. BHT was used as reference. Decrease in absorbance indicated increased radical scavenging activity which was determined by the following equation:

$$\text{Inhibition (\%)} = \frac{(\text{abs. of control} - \text{abs. of test solution}) \times 100}{\text{abs. of control}}$$

Where, absorbance of control = total radical activity without inhibitor and absorbance of test = activity in the presence of test compounds.

Ferric reducing antioxidant power (FRAP Assay)

Total antioxidant capacity was determined using FRAP assay [13]. The FRAP reagent included 10 mM TPTZ

solution in 40 mM HCl, 20 mM FeCl₃ solution and 0.3 M acetate buffer (pH 3.6) in ratio of 1:1:10 (v/v). 100 µl of all three pre-prepared extracts of coffee beans were mixed with 900 µl of freshly prepared FRAP reagent (pH 3.6) and incubated for 4 minutes at room temperature. Absorbance of the colored product (ferrous tripyridyltriazine complex) was measured at 593 nm using trolox as the standard solution.

Antimicrobial activity

Five bacterium strains i.e. *Bacillus subtilis*, *Alcalygens denitrificans* and *Klebsiella pneumonia*, *Camphylobacter jejuni*, *Micrococcus luteus* were incubated for 24 hours at 37°C on nutrient agar medium and then maintained at 4°C. Two fungal strains *Fusarium oxysporum* and *Bipolaris specifera* were grown on PDA medium at 28°C and maintained at 4°C.

In vitro antibacterial activity was observed for chloroform, methanol, and aqueous extracts using agar well diffusion assay [14]. The agar plates were seeded with indicator test strains (bacterial). Stock solution of each plant extract was prepared at a concentration of 1 µg / µl in different solvents viz. Methanol, chloroform

and aqueous. 100 µl of each plant solvent extracts was added into wells using sterile syringe and allowed to diffuse at room temperature for 3 hrs. The plates were incubated at 37°C for 18- 24 hours for bacteria growth. The effect was compared with that of positive reference (ampicillin) to determine the sensitivity of bacterial growth.

The antifungal activity was determined using poisoned food technique [15]. Five-day old fungal cultures of *Fusarium oxysporum* and *Bipolaris specifera* were punched aseptically with a sterile cork borer of 7mm diameter. The fungal discs were then put on the gelled agar plates that were prepared by impregnating desired concentration of plant extract. The plates were then incubated at 26±1°C temperature. Colony diameter was recorded by measuring the two-opposite circumference of the colony growth. Percentage inhibition of mycelial growth was evaluated by comparing the colony diameter of poisoned plate (with plant extract) and non-poisoned plate (with distilled water) and calculated using the formula given below [16].

$$\% \text{ Mycelial inhibition} = \frac{\text{Mycelial growth}(\text{control}) - \text{Mycelial growth}(\text{treated})}{\text{Mycelial growth}(\text{control})}$$

RESULTS AND DISCUSSION

Qualitative analysis of different extracts of *Coffea robusta L.*

The results for the qualitative estimation of different crude extracts of coffee are given below in table 1

Table 1: Phytochemical analysis for the methanolic, aqueous and chloroform extracts of *C. robusta*

Phytochemical Tests	Methanol extract	Chloroform extract	Water extract
Carbohydrates	++	-	+
Proteins	++	-	+
Flavanoids	++	-	+
Phenolics	++	-	+
Terpenoids	++	+	-
Saponins	++	+	-
Steroids	++	+	-

++: Present in high amount +: Present in traces -: Absent

Quantitative phytochemical Analysis

Antioxidant and antimicrobial properties of various extracts from many plants have recently been of great interest in both research and the food industry. The presence of phytochemicals as natural additives have tendency to replace synthetic antioxidants and antimicrobials with natural ones.

Total Phenolic and flavonoid Content

Total phenolic content was determined according to Folin- Ciocalteu method. The concentration of phenolic

compounds in test samples was calculated from the Standard Gallic acid curve.

The total phenolic content in different solvent extracts is shown in Table 2. The highest amount of phenolics were observed in methanolic extract (16.84±0.12µgGAE/ g dry wt), while the aqueous extract has only 4.857±0.23µg GAE/ g dry wt (Table 2). The total phenolic content in plant depends on the type of extract, i.e. the polarity of solvent used in extraction. High solubility of phenols in polar solvents provides high

concentration of these compounds in the extracts obtained using polar solvents for the extraction [17]. The results of the present study showed that much different between the amounts of flavonoids was not recorded in different solvent extracts. The crude methanolic extract had highest amount of flavonoids (76.04 ± 0.23 mgQEs/g) as compared to chloroform and aqueous extracts which had 68.89 ± 0.87 mgQEs/g and

51.65 ± 0.45 mgQEs/g of flavonoids respectively (Table 2). Hecimovic et al. [18] observed a good correlation in the content of total flavonoids and phenolics. Their results indicated that roasting affects the polyphenolic compounds of coffee, and confirmed that light and medium roasting are more favourable in terms of preserving these beneficial compounds during coffee roasting.

Table 2: Total phenol and flavonoid content in different solvent extracts of *C. robusta*

Extracts	Total Phenolics ($\mu\text{g GAE/g dry wt}$)	Total flavonoids (mg QE/g dry wt)
Aqueous	4.857 ± 12	68.98 ± 0.87
Methanol	16.84 ± 23	76.0 ± 0.23
Chloroform	10.91 ± 23	51.65 ± 0.45

Antioxidant activity

DPPH Assay

DPPH assay is a simple and quick method for determining the ability of anti-oxidants to scavenge the free radicals. Among all the solvent extracts, methanolic extract of *C. robusta* was found to be the most potent antioxidant (IC_{50} $89.50 \pm 1.1 \mu\text{g/ml}$), followed by aqueous extract (IC_{50} $241.66 \pm 0.5 \mu\text{g/ml}$). Chloroform extract showed poor radical scavenging activity (IC_{50} $425.92 \pm 0.3 \mu\text{g/ml}$), whereas BHT was taken as reference antioxidant (IC_{50} $23.5 \pm 0.5 \mu\text{g/ml}$) (Table 3).

Pérez-Hernández et al. [19] observed a high antioxidant activity in Green Arabica and robusta coffee beans. But our studies show lower value as compared that of green coffee beans. These changes in the antioxidative capacity of coffee upon roasting are associated with the degradation of chlorogenic acid [20]. A linear correlation has been obtained by comparing the antioxidant activity and polyphenols and flavonoid content of the extracts. The methanol extract contained

good amount of phenols and flavonoids and thus possess potential antioxidant activity. Previous studies have also reported positive correlation between phenolic and flavonoid content and DPPH scavenging activity of plant extracts

FRAP assay

The ferric reducing antioxidant activity is shown in Table 3. The FRAP assay showed difference in all the three crude extracts. The results showed that the FRAP activity was high in methanol extract (58.02 mg Fe^{2+}/g of extract) whereas both chloroform and aqueous extracts showed poor FRAP activity viz. 16.79 ± 34 mg Fe^{2+}/g of extract and 12.79 ± 0.76 mg Fe^{2+}/g of extract respectively. Budryn and Nebesny [21] observed higher antioxidative efficacy in the extracts of *robusta* coffee had than those from *arabica* coffee beans. This is due to Progressive reduction in the antioxidant during brewing of coffee beans that causes degradation of phenolic compounds and the generation of Maillard's reaction products [22].

Table 3: Antioxidant activity of extracts of *Coffea robusta* using DPPH and FRAP assay

S.No.	Extracts	DPPH assay IC_{50} in $\mu\text{g/ml}$	FRAP assay (mg Fe^{2+}/g of extract)
1	Aqueous	241.665 ± 0.52	12.79 ± 0.76
2	Methanol	89.502 ± 1.1	58.02 ± 0.12
3	Chloroform	425.92 ± 0.3	16.79 ± 0.34
4	BHT	23.5 ± 0.5	-----

Antimicrobial Assay

Antibacterial activity

The results of antibacterial activity assay of three extracts of *Coffea robusta* were obtained by agar

diffusion method. Zone of inhibitions of three extracts that is aqueous, chloroform and methanol against five bacterial strains is shown in the table 4. The highest zone of inhibition was shown against *Alcaligenes*

denitrificans which was 22.5mm while with *Camphylobacter jejni*, the zone of inhibition was 10.2mm. However, no antibacterial activity was observed against *Bacillus subtilis*, *Micrococcus luteus* and *Klebsiella pneumonia*.

Huck et al., 2005 confirmed the presence of an alkaloid caffeine in roasted coffee and the rough green beans in both Arabica and Robusta coffee and observed that the antimicrobial activity of coffee against several

microorganisms is due to presence of caffeine which inhibits the bacterial growth [23]. Murthy and Manonmani [24] observed antimicrobial activity against the growth of *E.coli*, *S. enterica* and *S. aureus*, using the disc diffusion method. Almeida et al. [25] observed that the high amount of phenolics in coffee also exert antimicrobial activity by altering the structure of the cytoplasmic membrane, disrupting the proton motive force and electron flow.

Table 4: Antibacterial activity of the aqueous, chloroform and methanolic extracts of *Coffea robusta*

Bacterial strains	Diameter of zone of inhibition in (mm)				
	Concentration mg/ml	Aqueous extract	Methanol extract	Chloroform extract	Standard (Chloramphenicol)
<i>Bacillus subtilis</i>	20.0	-	-	-	39.0
<i>Micrococcus luteus</i>	20.0	-	-	-	35.0
<i>Alcaligenes denitrificans</i>	20.0	22.5	27.5	-	35.0
<i>Camphylobacter jejni</i>	20.0	10.2	19.0	-	39.0
<i>Klebsiella pneumonia</i>	20.0	-	-	-	31.0

Antifungal activity

The results of antifungal activity of methanolic, aqueous extracts at different concentrations of *Coffea robusta* against pathogenic fungus *Bipolaris specifera* and *Fusarium oxysporum* was studied using poisoned food technique and the percentage inhibition is given in

table 5. It was observed that as the extract concentration was increased to 0.9ml, the colony diameter decreased, and the percentage inhibition increased in all the three extracts. However maximum inhibition was obtained in aqueous extracts (Table 5).

Table 5: Antifungal activity of *Coffea robusta* against different fungal strains at different concentrations

S. No	extract concentration (ml)	<i>Bipolaris specifera</i>		<i>Fusarium oxysporum</i>	
		Growth diameter (cms)	%age inhibition	Growth diameter (cms)	%age inhibition
Aqueous extract					
1.	Control	3.5	0	3.0	0
2.	0.3	2.5	28.5	2.2	26.7
3.	0.6	2.0	42.8	1.9	36.6
4.	0.9	1.3	62.9	1.6	46.6
Methanol extract					
1.	Control	3.5	0	3.0	0
2.	0.3	2.8	20.0	2.4	20.2
3.	0.6	2.5	28.5	2.1	30.0
4.	0.9	1.9	45.7	1.7	40.1
Chloroform extract					
1.	Control	3.5	0	3.0	0
2.	0.3	2.2	16.1	2.1	18.6
3.	0.6	1.5	23.8	1.4	22.5
4.	0.9	1.1	35.1	0.8	33.1

CONCLUSIONS AND FUTURE PROSPECTS

The presence of high amounts of phenolic and flavonoids in *Coffea robusta* revealed that it could be

used as a potential source of natural antioxidants. Since the present study involves crude plant extract to study the partial presence of active compounds in the total

extract, further investigations are still needed for the presence of bioactive molecules and their structural elucidation.

Acknowledgement

The authors are thankful to School of Biotechnology, University of Jammu, Jammu for providing basic facilities to carry out this present research work. The authors are also thankful to Ms Shivani Koul for helping in phytochemical assays.

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