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QUANTIFICATION AND ANTIBACTERIAL ACTIVITY OF FLAVONOIDS IN COFFEE SAMPLES

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Abstract

Background: Flavonoids are the phenolic substances widely found in fruits, vegetables, grains, bark, roots, stems, flowers, tea and coffee.
Methodology: In the current study quantity of flavonoids and antibacterial activities were determined in different coffee samples namely Nescafe classic, Nescafe gold, Nescafe martina, original, creamy and cafe soluvel by using standard methodology available in literature.
Results: Nescafe original and gold showed highest content of flavonoid while cafe soluvel showed lowest content of flavonoid. Cafe soluvel, gold and classic showed good antibacterial activities.
Conclusion: This study showed that coffee is good source of flavonoids and had excellent antibacterial potential.

Key words: Coffee, flavonoids, antibacterial activities**.**

Introduction

The main constituents of coffee have been known for almost half a century and particularly caffeine has been the subject of extensive studies. The stimulant effect of coffee is attributed to the pharmacological activity of caffeine, acting as an antagonist at adenosine receptors in brain (Fredholm et al 1999). Although a central stimulant drug caffeine is not generally considered to have abuse potential because pure caffeine elicits a dose-dependent, subjective feeling of anxiety, even at low doses (Kaplan et al 1997). Beverages made from roasted coffee, on the other hand, are able to elicit a feeling of well being that seems to increase with the strength of the brew, but not with its caffeine content. Brewed and instant coffees contain pharmacologically active compounds, other than caffeine, in concentrations sufficient to cause significant effects with normal consumption of coffee. Kalsner reported that decaffeinated coffee contains a vasoconstrictive substance, later proposed but never identified as a muscarinic acetylcholine receptor agonist (Quinlan et al 2000, Paulis et al 2002)**.**

Materials and Methods

Extraction of Flavonoids

All studies were carried out at the department of chemistry and department of pharmacy Kohat University Science & Technology KUST Khyber Pukhtunkhwa Pakistan.

Solvents: All commercial grade solvents used were distilled before use and in some occasions analytically grade solvents were used.
Samples: Nescafe classic, Nescafe gold, Nescafe matinal, Nescafe original, Nescafe creamy and Café soluvel were collected from Kohat .

Methodology

Five g of the coffee sample was put in 80% aqueous methanol (200 mL) for 24 h. The same process was repeated thrice. The solution was filtered and concentrated using rotary apparatus. The concentrated filtrate was put in the vials (of known weight) and dried on the water bath at 70 °C. The weights of crude flavonoids were calculated (Hussain et al 2011).

Antibacterial Activity

All study was carried out at the department of chemistry and department of pharmacy Kohat University of Science & Technology KUST Khyber Pukhtunkhwa Pakistan. All commercial grade solvents used were distilled before use in some occasions. Test samples include Nescafe classic, Nescafe gold, Nescafe matinal, Nescafe creamy, Nescafe original and café soluvel while the reagents include Nutrient agar, distilled water, petry dish, autoclaved machine, cork borer, micro pipette, oven, DMSO, sample (crude flavonoids).

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Bacterial strains: *S.aureus* 284, *S.aureus* 294, *P.aerogonisea (*ATTC), *E.coli.*

Sample Preparation: 0.2 gm of sample (flavonoids) was dissolved in 2 ml of DMSO.

First of all nutrient agar was prepared then kept in autoclaved machine at 121C for 15 minutes. A loop full of bacterial strain was inoculated in 30 mL of Nutrient broth in a conical flask and incubated for 72 hrs to get active strain by using agar well diffusion method. Nutrient Agar was poured into Petri dishes. After solidification 0.25 mL of test strains were inoculated in the media separately .The experiment was performed under strict aseptic conditions. After the medium solidified, a well was made in the plates with sterile borer (5mm).The extract compound (10 µL) was introduced into the well and plates were incubated at 37°C for 72 hrs. All samples were tested in triplicates. Microbial growth was determined by measuring the diameter of zone of inhibition and results were recorded (Hussain et al 2011).

Results and Discussion

Flavonoids are a major class of oxygen-containing heterocyclic natural products that are widespread in green plants Generally, they are found as plant pigments in a broad range of fruits andvegetables. Flavonoids have been recognized as having a protective effect in plants against microbial invasion by plan pathogens. Flavonoid-rich plant extracts have been used for centuries to treat human disease**.** Isolated flavonoids have been shown to possess a host of important biological activities, including antifungal and antibacterial activities (Treutter et al 2006, Galeotti et al 2008, Sathiamoorthy et al 2007, Alarcon et al 2008).

Table 1 : Quantitative determinations of crude Flavonoids in Nescafe Gold , Nescafe matinal , Nescafe original , Nescafe creamy , Nescafe classic and Cafe soluvel

S.no	Coffee samples	Coffee in gm	Wt. of crude flavonoids	Percentage (%)
1	Nescafe Gold	10 gm	2.00 gm	20.0%
2	Nescafe Matinal	10 gm	1.88 gm	18.8%
3	Nescafe Original	10 gm	2.18 gm	21.8%
4	Nescafe Creamy	10 gm	1.94gm	19.4%
5	Nescafe Classic	10 gm	1.56 gm	15.6%
6	Café soluvel	10 gm	1.02 gm	10.2%

Higher content of flavonoids were found in Nescafe original which was 21.8% followed by Nescafe Gold, Nescafe creamy , Nescafe matinal , Nescafe classic and café soluvel which were 20.% , 19.4% , 18.8% , 15.6% and 10.2% respectively .
The quantified flavonoid content of Nescafe classic i.e. 15.6%, was in close agreement to the flavonol from dried tea leaves which was 15.60% (Chey et al 2007). The cafe soluvel has flavonoid content 10.2% which is similar to the published data in which flavonol content for fresh tea leaves was 10.50% (Chey et al 2007). The Nescafe original has flavonoid content 21.85% which is in close agreement to the documented data in which the flavonoid content of grean tea i.e. epicatechin gallate was 21.69%. The flavonoid content of Nescafe gold which is 2.00 mg/g, is similar to the vanillin (catechin) from Vietnam variety which was 2.28 mg/g (Hecimovic et al 2011).

Table: 2: Antibacterial activity of flavonoids (zone of inhibition in mm).

Sample	*S.aureus 284*	*S.aureus 294*	*P.aerogonisea (ATTC)*	*E.coli*
Matinal	13 mm	17 mm	15 mm	00 mm
Creamy	15 mm	19 mm	16 mm	03 mm
Classic	11 mm	14 mm	12 mm	00 mm
Gold	16 mm	18 mm	14 mm	05 mm
Original	13 mm	16 mm	14 mm	00 mm
Café soluvel	15 mm	20 mm	13 mm	04 mm
Tetracycline	32 mm	28 mm	• mm	30 mm

Gold shows high activity against *S.aureus 284* with zone of inhibition of 16mm followed by creamy and café soluvel. While classic show low activity against *S.aureus 284* with zone of inhibition of 11mm. Café soluvel show high activity against *S.aureus 294* with zone of inhibition of 20mm while classic show low activity against *S.aureus 294*. All the samples show very less activity against *E.coli*. Creamy show high activity against *P.aerogonisea (ATTC*) while classic show less activity as compared to creamy. Café soluvel has zone of inhibition 20mm against *S.aureus 294* which is in close agreement to the literature (Manimozhi et al 2012). Similarly, gold has zone of inhibition 18mm against *S.aureus 294* which is in close agreement to the documented data in flavonoid glycoside from fresh tea leaves had zone of inhibition 18mm against *L.monocytogenes* **(**Chey et al 2007). Gold ,original and creamy have zone of inhibition 16mm against *S.aureus 284, S.aureus 294 and P.aerogonisea* respectively which was similar to the documented data (Manimozhi et al 2012)**.** Matinal, creamy and café soluvel have zone of inhibition 15mm against *P.arogonisea, s.aureus 284 and S.aureus 284* respectively which is in close agreement to the zone of inhibition of hydrolyzed flavonoid from dried tea leaves against *L.monocytogenes* which was 15.00mm.The zone of inhibition of classic against P.aerogonisea is 12mm which is similar to the zone of inhibition of flavonoid glycoside from fresh tea leaves against *E.coli O157:H7* was 12mm (Chey et al 2007).

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Conclusion

This study showed that coffee is good source of flavonoids.

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