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Antimicrobial Activity of Coffee Beans

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ABSTRACT

About 30% - 40% individuals daily consume the coffee. The Himalayan coffee beans were collected from the local market of Karad, Maharashtra, India. The coffee beans were crushed into the powder and methanolic extract was prepared. Methanolic extract of coffee beans was found to be having antimicrobial activity against Shigellasonnei and Pseudomonas aeruginosa. The minimum inhibitory concentration of methanolic extract of coffee beans was found to be 70mg/mL against Shigellasonnei and Pseudomonas aeruginosa. The coffee beans have been found to have antimicrobial potential.

Key words: *Coffee beans, Shigellasonnei, Pseudomonas aeruginosa, Methanolic extract*

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INTRODUCTION

Coffee, one of the important export items, in 2000 and 2010, exports increased by 4.7% and 14.7% respectively [1]. Along with the sensory properties, the valuable stimulant effects of coffee on mental and physical activities have led to its worldwide consumption [2]. The coffee melanoidins derived from Maillard reaction or carbohydrate caramalization and thermolysis of organic compounds give brown colour to coffee [3,4]. The antibacterial activity of Mallard reaction products has been reported in many literatures [5,6,7] The extracts of coffee beans and coffee by-products, in combination treatment with tetracycline, has been promoted as an alternative therapeutic agent to treat drug-resistant *V. cholerae* infections [8]. So the aim of the present study was to investigate the antimicrobial activity of coffee beans.

MATERIALS AND METHODS [8]

Collection of samples-

The Himalayan coffee beans were collected from the local market of Karad Maharashtra, India. Coffee beans were crushed in to powder. The different solvents such as acetone, methanolic, ethanolic, hot water and cold water were used for extraction of active agents from coffee beans.

Preparation of Various Extracts of Coffee Beans

Preparation of Water Extract

The aqueous extract was prepared by 5 g of air-dried powder of coffee beans was suspended in 20 mL of sterile distilled water. The 0.5 mL extract was added to 5mL ofhot water to make the final concentration (0.1 g/mL) and stored as stock solution.The aqueous extracts were then filtered and rewashed with small volume of sterile distilled water and added to filtrate and used immediately.

Preparation of Ethanol Extract

5g of airdried powder of coffee beans was suspended in 20mL of ethanol and kept overnight at room temperature for evaporation. The 0.5 mL extract was added to5mL of ethanol to make the final concentration (0.1 g/mL) and stored as stock solution.

Preparation of Methanol Extract

5-g of air dried and coarsely powder of coffee beans was suspended in 20ml of methanol and kept overnight at room temperature for evaporation. The 0.5 mL extract was dissolved in 5mL of methanol to make the final concentration (0.1 g/mL) and stored as stock solution.

Preparation of Acetone Extract

5-g of air dried and coarsely powder of coffee bean was suspended in 20mL of acetone and kept overnight at room temperature for evaporation. The 0.5 mL extract was added to5mL of acetone make the final concentration (0.1 g/mL) and stored as stock solution.

Preparation of Hot Water Extract

The hot water extracts were prepared by adding 5 g of air dried powder of coffee beans in 20 mL of sterile, boiled distilled water. The 0.5 mL extract was added to 5mL of hot water to make the final concentration (0.1 g/mL) and stored as stock solution.

The aqueous extract was then filtered and rewashed with small volume of sterile distilled water and added to filtrate and is used immediately.

Preparation of Test Organisms

Preparation of standard bacterial suspension- The 18 h old bacterial culture of *E. coli, Staphylococcus aureus, Enterobacter aerogenes, Shigella sonnei, Proteus mirabilis, Candida albicans and Pseudomonas aeruginosa* were inoculated into sterile saline tubes. The turbidity of bacterial culture adjusted with 0.5 MacFarland standard (10^8 CFU/mL).

Determination of Antimicrobial Activity of Coffee Bean Extracts

Preparation of Paper Discs

The discs were prepared by punching the Whatman filter paper no. I with the help of punching machine these discs were taken into screw capped tube and sterilized in an autoclaved at 121°C for 20 min.

Determination of Antimicrobial Activity (Primary Screening)

Disc diffusion method was used to study the antimicrobial activity of the prepared extracts. The Muller Hinton agar medium was prepared and autoclaved at 120°C for 15 min. Then medium was poured in sterile petri plates and allowed to solidify 0.1 mL of standardized bacterial culture suspensions were spread on sterile Muller-Hinton agar plates with the help of spreader aseptically. The plates were allowed to diffuse for five minutes before applying the discs. Then discs were soaked into ethanolic, methanolic, acetone aqueous, hot water extracts. Then discs were aseptically kept on the agar.

Similarly, discs were soaked in the pure ethanol, methanol, acetone solvent and sterile water and these discs were kept on agar medium plates by using sterile forceps. They were considered as controls. Then plates were allowed to diffuse at refrigerator for 10 min. The plates were incubated in upright position at 37 °C for 24 h. After incubation plates were observed for zones of inhibition and zones of inhibition were measured and noted down.

Determination of Minimum Inhibitory Concentration (MIC)

Dilution method was used to determine the MIC bean extracts against bacterial strains. Cold water, methanol, ethanol, acetone and hot water extracts were diluted. Dilution was prepared as the final concentration of extracts in tubes were 10 mg/mL. 20 mg/mL. 30 mg/mL, 40 mg/mL, 50 mg/mL, 60 mg/Ll, 70 mg/mL, 80 mg/mL and90 mg/mL. Muller Hinton agar plates were prepared. 0.1 mL of standardized bacterial culture suspensions were spread on sterile Muller-Hinton agar plates with the help of spreader aseptically. The plates were allowed to diffuse for five minutes before applying the discs.

Then Whatman paper discs were soaked in all the respective dilutions (10mg/mL to 90mg/mL) of ethanolic, methanolic, acetone, cold water, hot water extracts. Then discs were aseptically kept on the agar.

Similarly, discs were soaked in the pure ethanol, methanol, acetone solvent and these discs were kept on agar medium plates by using sterile forceps. These were considered as controls. All the plates were allowed to diffuse at refrigerator for 10 min. The plates were then incubated in upright position at 37 °C for 24 h. After incubation plates were observed for zone of inhibition and zone of inhibition was measured and noted down.

RESULTS AND DISCUSSION

Results of primary screening for antimicrobial activity of various extracts of coffee beans against laboratory strains of known pathogens are as follows:

From Table 1, all the ethanolic, methanolic, acetone, cold and hot water extracts of coffee beans showed antimicrobial activity against the *Pseudomonas aeruginosa.* The ethanolic extracts of coffee bean showed antimicrobial activity against *Pseudomonas aeruginosa* (1mm), *Proteus mirabilis* (3mm), *Enterobacter aerogenes* (6mm) and *Escherichia coli* (2mm). The methanolic extract of coffee beans showed the antimicrobial activity against *Pseudomonas aeruginosa* (13mm) and *Shigella sonnei* (7mm). The acetone extract of coffee beans showed antimicrobial activity against *Pseudomonas aeruginosa* (8mm). The hot water extract of coffee beans showed the antimicrobial activity against *Pseudomonas aeruginosa* (1mm). The cold water extract of coffee beans showed the antimicrobial activity against *Pseudomonas aeruginosa* (3mm).

Results of evaluation of minimum inhibitory concentration of coffee beans extracts against the test organisms *Pseudomonas aeruginosa* and *Shigella sonnei* are shown in Table 2.

The minimum inhibitory concentration of methanolic extract of coffee beans against *Pseudomonas aeruginosa* and *Shigella sonnei* was found to be 70 mg/mL.

Table 1 Results of Antimicrobial Activity of Various Extracts of Coffee Beans

Name of the test organisms	Diameter of zone inhibition (in mm) with extracts of				
	Ethanol	Methanol	Acetone	Cold water	Hot water
Stapylococcus aureus	-	-	-	-	-
Pseudomonas aeruginosa	1	13	8	3	1
Proteus mirabilis	3	-	-	-	-
Candida albicans	-	-	-	-	-
Enterobacter aerogenes	6	-	-	-	-
Escherichia coli	2	-	-	-	-
Shigella sonnei	-	7	-	-	-

- :- Indicates no zone of inhibition

Table 2:- Results Of Evaluation Of Minimun Inhibitory Concentration of Coffee Beans Extracts.

Sr. No.	Name of the extracts	Test organism	Concentration of extract (in mg/ml)								
			10	20	30	40	50	60	70	80	90
1	Methanolic extract	*Pseudomonas aeruginosa*	-	-	-	-	-	-	+	+	+
2	Methanolic extract	*Shigella sonnei*	-	-	-	-	-	-	+	+	+

+ :- Indicates presence of zone of inhibition

- :- Indicates no zone of inhibition

CONCLUSION

The coffee beans were collected from local market of Karad, coffee beans were crushed into powder. Ethanolic, methanolic, acetone, cold and hot water extracts of coffee beans powder were prepared as (100 mg/mL) concentration.

Antimicrobial potential and minimum inhibitory concentration of all the extracts of coffee beans were tested against the laboratory strains of *Escherichia coli, Staphylococcus aureus, Enterobacter aerogenes, Shigella sonnei, Proteus mirabilis, Candida albicans* and *Pseudomonas aeruginosa* by disc diffusion method.

Methanolic extract of coffee beans was found to be having antimicrobial activity against *Shigella sonnei* and *Pseudomonas aeruginosa*.

The minimum inhibitory concentration of methanolic extract of coffee beans was found to be 70mg/mL against *Shigella sonnei* and *Pseudomonas aeruginosa*. The coffee is consumed by almost every individual. Coeffee beans have the antimicrobial potential. Consumption of coffee would be having health benefits however more study regarding the antioxidant properties should boost their health benefits and medicinal use.

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