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Extraction and antibiogram determination of extracted caffeine

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Abstract
Caffeine, a potent phytochemical, obtained from *Coffea robusta L* is a major component of most famous beverages preferred all over the world. Besides a component of food stuff, it is also a part of many prescription and non-prescription drugs for cold, allergy, pain relievers, Lipolytic, Antimigrane, Antimicrobial agent etc. This study is concerned about extraction, characterization and determination of antimicrobial activity of extracted caffeine. Caffeine extraction was done using ethyl acetate as solvent, giving 0.0457 %. Its conformation was done by Melting Point, λ_{max} and R.F. value determination. The broad spectrum nature of caffeine was confirmed using broth micro dilution method. Since the decades back, potent antimicrobial molecules are always a need of time. Hence using caffeine as a putative molecule can be a way out for the same. Considering other ADR related studies along with cautions about caffeine can point it a presumed biomolecule for various new therapeutic activities.

Keywords: broad spectrum antimicrobial, broth Micro dilution, caffeine, characterization, extraction, therapeutic

Introduction
Caffeine is a plant product that is most commonly found in coffee beans, tea, energy drinks, soft drinks, cocoa and chocolate. Caffeine is also found in some prescription and nonprescription drugs, including cold, allergy and pain relievers [1]. Caffeine (1,3,7-trimethylxanthine) is a plant alkaloid occurring naturally in some 60 plant species, of which cocoa beans, kola nuts, tea leaves and coffee beans are the most well-known. Its chemical structure is $C_8H_{10}N_4O_2$ and a molecular weight of 194.19KD. In pure form, it is a bitter white powder. Structurally, caffeine (and the other methylxanthines) resembles the purines. The mean half-life of caffeine in plasma of healthy individuals is about 5 hours. Upon ingestion, caffeine binds to adenosine receptors in the central nervous system (CNS), which inhibits adenosine binding. This inhibits the adenosine-mediated down regulation of CNS activity; thus, stimulating the activity of the medullary, vagal, vasomotor, and respiratory centers in the brain. This agent also promotes neurotransmitter release that further stimulates the CNS. The anti-inflammatory effects of caffeine are due the nonselective competitive inhibition of phosphodiesterases (PDEs). Inhibition of PDEs raises the intracellular concentration of cyclic AMP (cAMP), activates protein kinase A, and inhibits leukotriene synthesis, which leads to reduced inflammation and innate immunity [2].
There is also synthetic (man-made) caffeine, which is added to some medicines, foods, and drinks. For example, some pain relievers, cold medicines, and over-the-counter medicines for alertness contain synthetic caffeine. So do energy drinks and "energy-boosting" gums and snacks [3].
The FDA has approved caffeine for the use in the treatment of apnea of prematurity and prevention and treatment of broncho pulmonary dysplasia of premature infants Non-FDA approved uses of caffeine include treating migraine headaches and post-dural puncture headaches and enhancing athletic performance, especially in endurance sports. Caffeine has links with decreased all-cause mortality. It is also under investigation for its efficacy in the treatment of depression and neurocognitive declines, such as those seen in Alzheimer and Parkinson disease [4].
For this study, *Coffea robusta L* (*Coffee canephora*) is selected species for caffeine extraction. *Coffea canephora* also known as Robusta coffee is a variety of coffee, which has its origins in central and western sub-Saharan Africa. It is a species of flowering plant in the Rubiaceae family. The plant has a shallow root system and grows as a robust tree or shrub to about 10 metres. It flowers irregularly, taking about 10–11 months for cherries to ripen, producing oval-shaped beans. The robusta plant has a greater crop yield than that of C. arabica, and contains more caffeine - 2.7% compared to arabica's 1.5%. It is also said to be less susceptible to pests and disease, it therefore needs less herbicide and pesticide than Arabica [5]. Need of antimicrobial agent is always the thurst area for researchers. The diversified natural plant based molecules can be the forever solution of this. Considering the dose related issue consideration, this study encompasses the utilization of caffeine, a phytochemical and a food constituent, as a potent antimicrobial agent.

Materials and Methods
All the chemicals used in the study were procured from Himedia Pvt. Ltd. and Qualigens India Pvt. Ltd. and are of analytical grade.

Acquisition and Morphological Characterization of Selected Beans
Coffee beans, the seeds of *Coffea robusta L* were selected based upon their availability and ease of acquisition during August, 2019 to September 2019. All the coffee beans were washed, screened and shade dried for 10 days. Various

morphological parameters and structural features of selected coffee bean sample were studied *viz*. Odour, Colour, Shape and Size. All the coffee beans were inspected for the existence and patter of centre crease, split or midline and its related identification.

Sample processing

Fifty grams of dried beans were crushed directly by grinder without adding any solvent. Coffee beans of selected species were used for further analysis. Selected species coffee beans obtained were in roasted but intact form. Further the beans were utilized in order to process as a sample. Coffee beans were powered and further air dried in controlled condition. The powder is stored in an air tight container till its use.

Extraction of Caffeine

Single Solvent Extraction

Two solvents i.e. Ethyl acetate and Dichloromethane were used for single solvent extraction. When each solvent is used as sole solvent, using 20 Gm of coffee power as initial sample recovery obtained as caffeine.

Purification of Caffeine

After concentration of the organic phase there will be "brown gunk" in the bottom of the flask that was further purified using solvent as alcohol. The above brown gunk was purified by sublimation process as well.

Characterization of Caffeine

ph

Aqueous solution (1mg/1ml sterile distilled water) of extracted caffeine was used to determine the pH value [6]. pH value was determined by pH meter and pH strips as well.

Solubility Study

The isolated compound was taken in a test tube and the solubility study was carried out by considered the different solvents in a qualitative manner [6].The solubility of caffeine in water, ethyl acetate, ethanol, dichloromethane and acetone were determined by dissolving a 1 mg of extracted caffeine in respective solvent. Clear solution indicates solubility.

Melting Point

Melting point was determined by using a pre sealed capillary which is filled with extracted sample and assembled with thermometer. This assembly is placed in Thiele's tube and heated gradually [6]. The visual liquefiction of sample in capillary tube is considered as Melting point.

UV- Absorption Spectrum

UV- absorption spectrum of extracted purified crystalline caffeine was prepared using a UV absorption spectrophotometer from Thermo scientific Model for Ultra violet range [6].

Thin Layer Chromatography

Purity of caffeine was checked using TLC method**[6]**. The extracted caffeine from both plants were dissolved in chloroform, spotted on to the silica gel plates, Merck (Germany) 20 × 20 cm, 0.25 mm in thickness, and developed using the solvent system : ethyl acetate : methanol : water (10: 1.35: 1.0). The pre-coated TLC plates with Silica gel was first sprayed with 1 g Potassium Iodide and 1 g iodine dissolved in 100 ml ethanol, followed by spraying with a 1 : 1 mixture of 25% HCl : 96% ethanol. The caffeine zones were indicated by a dark-brown colour discernible in visible light. Then R_f value was calculated. Standard caffeine in references was compared for colour.

Antibiogram determination:

Procurement and Characterization of Bacterial Strains

Procurement of Bacterial Strains

A. Two bacteria *viz*. *Pseudomonas aeruginosa* and *Bacillus spp.* Were isolated from sewage sample near Govt. Medical College, Aurangabad and resistotyped by disc diffusion using commercial antibiotic discs of Himedia Pvt. Ltd. Mumbai, India[7].
B. **Broth Microdilution Assay:** Bacterial inoculums was prepared and grown overnight at 37^0C. The O.D. was adjusted (O. D. 0.1 at 500 nm) and serial addition of caffeine was done in test tubes as mg/ml to mg/ml. Bacterial culture (0.1 ml) was added to each tube of dilution. Separate set along with blank and positive control (streptomycin 10mg/ml) was prepared for each bacterium. The tubes were incubated at 37^0C for 24 hrs. After overnight growth, O.D was taken at 500nm and antimicrobial effect was determined [8].

Results and Discussion

Characterization of Coffee Beans

Coffee beans were procured and processed as per the method mentioned. Various morphological parameters and structural features of selected coffee bean sample were studied. The procured coffee beans were analysed for the typical smell and its intensity, *Coffea robusta L* hasmaximum smell at random. Price, caffeine content, availability etc. were considered for selecting *Coffea robusta L.* for further analysis. *C. Robusta* species was also selected by *Muthanna et. al*. [9] for caffeine extraction. Various coffee beans were observed for their typical colour shade. Average sized and shaped coffee beans were considered for processing. All these parameters selected species coffee beans obtained were in roasted but intact form which was also done by Shinde P. [10] for further study.

Sample Processing

Selected species of coffee beans S were used for further analysis. Selected species coffee beans were powered and was stored in an air tight container till gets utilized.

Single Solvent Extraction

When two solvents i.e. Ethyl acetate and Dichloromethane were used for single solvent extraction, ethyl acetate was found to be more efficient solvent giving higher recovery from same initial sample power. Two solvents were used for single solvent extraction are Ethyl acetate and Dichloromethane. When ethyl acetate was used as a sole solvent, 0.914 gm of caffeine was extracted from 20 gm of coffee powder. The process was further supplemented by purification of the extracted caffeine. The % recovery obtained was 0.0457. When dichloromethane was used as a sole solvent, 00.67 gm

of caffeine was extracted from 20 gm of coffee powder. The process was further supplemented by purification of the extracted caffeine. The % recovery obtained was 0.0335 (Table No. 1).

% Recovery = Grams of caffeine recovered / grams of sample taken.

After concentration of the organic phase there will be "brown gunk" in the bottom of the flask that was further purified using solvent as alcohol. The above brown gunk was purified by sublimation process as well.

Characterization of Caffeine

pH

1. When aqueous solution (1mg/1ml sterile distilled water) of extracted caffeine was used to determine the pH, the value was found to be 6.9. This indicates the near neutral nature of caffeine and is comparable with theoretical reference value. The ph is thus appropriate to be putative drug molecule.

2. **Solubility Study**
   When he isolated compound was tested for solubility in water, ethyl acetate, ethanol, dichloromethane and acetone, the solute is found to be soluble in all tested solvents in the given test conditions.

3. **Melting Point**
   Melting point for extracted caffeine was found to be near 238^0C.

4. **UV-Vis Spectra**
   When an UV- absorption spectrum of extracted purified crystalline caffeine was prepared the λ_{max} was found to be on 275 nm[11]. The same result was also reported by Md. Abdul Mumin.

Thin Layer Chromatography

After the complete run and spraying of indicator solution, the caffeine spots were observed by a dark-brown colour discernible in visible light. TLC using Ethyl acetate-methanol- water as solvent system and HCl-Iodine reagent reveals the single pointed brown spot as the presence of caffeine, as it is seen by Deepak P [6]. Chloroform –acetone – methanol solvent system was used by P. Muthanna [9] for the same.

Antibiogram determination

Procurement and Identification of Bacterial Strains

1. Isolated bacterial isolates were identified on various differential agars as per Bergey's [12] manual as *Pseudomonas spp. and Bacillus* sp. they are also correctly characterized using Grams staining

2. **Resistotyping**
   When the Resistogram was obtained for clinical isolates for antibiotics recommended by CLSI[13] guidelines "Disk diffusion Method" was used for resistotyping using commercial antibiotic discs of Himedia Pvt. Ltd. Mumbai, India, all the bacterial cultures show a variable biotyping data. The zone breaking point / IZD was considered as the parameter for determination of drug resistance. The Resistogram was as follows; *Pseudomonas* (Am^+, Ac^+, Va^-, Cu^-), and *Bacillus* sp (Am^+, Ac^+, Va-, Cu^+).

3. **Broth Micro Dilution Assay**
   When MIC determination was done using broth micro dilution assay, overnight incubated cultures were show a linear survival pattern as per the serial caffeine addition. O.D. was determined at 500 nm and less O.D. indicates maximum efficiency of caffeine (graph 1). Sensitivity was shown by *Pseudomonas aeruginosa* and *Bacillus Sp.* indicating the putative nature of caffeine as broad spectrum antimicrobial agent. The analysis was done in triplicate [14].

Tables and Graphs

Table 1: Recovery of caffeine using single solvent extraction method.

Sr.no	Solvent Name	Sample amount taken (in gms)	Yield (in gms)	% Recovery
1.	Ethyl acetate	20	0.914	0.0457
2.	Dichloromethane	20	00.67	0.0335

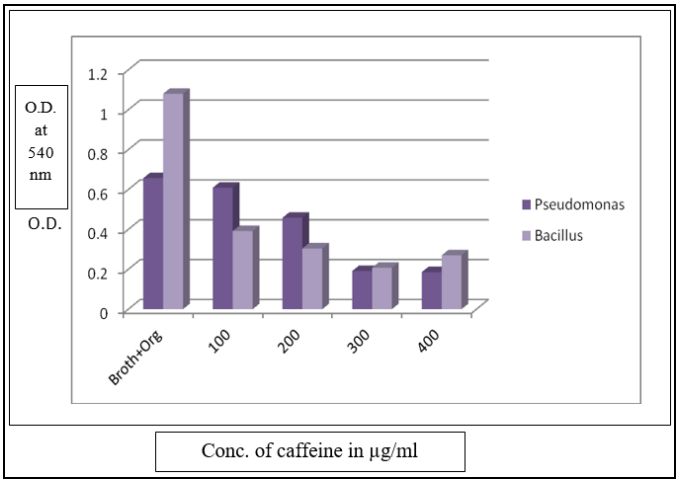

Fig 1: Broth Micro dilution assay

Conclusions

Caffeine is a stimulant drug is the world's most widely consumed psychoactive drug. Although there are beneficial side effects to caffeine intake, the negative effects clearly indicate that one should limit their caffeine consumption. Many major organ systems are adversely affected by high amounts of caffeine consumption, including the heart, stomach, respiratory, and reproductive organs. Age and diet are both factors in caffeine's effects on the body.

As concluding this study, Caffeine, a phytochemical obtained from *Coffea robusta L*, extracted using aqueous and organic solvents, found to be a potent antimicrobial agent against the microorganisms i.e. *Pseudomonas* sp. and *Bacillus* spp. under this study. Potent antimicrobial molecules are always a need of time since the decades back. Hence using caffeine as a

putative molecule can be a way out for the same. Further scale up formulation and other ADR related studies is the future perspective of this venture.

Reference

1. https://www.uhs.umich.edu/caffeine
2. Heckman MA. Caffeine (1, 3, 7-trimethylxanthine) in foods: a comprehensive review on consumption, functionality, safety, and regulatory matters. J Food Sci. 2010; 75:R77-87.
3. https://medlineplus.gov/caffeine.html
4. https://www.ncbi.nlm.nih.gov/books/NBK519490/
5. Nanyak Z Galam, Ibraheem M Gambo, Ahmed Rabiu, Nduruka Chinelo, Simi Dami. Anti-inflammatory Effect of Aqueous Extract of Coffee plant leaves (Coffea canephora) in Rats. Journal of Natural Sciences Research. 2013; 3(7):191-193.
6. Pradhan D, Biswasroy P, Pradhan R. Qualitative and quantitative analysis of caffeine in some commercial brands of tea consumed in India. Jar of Ayurvedic and herbal medicin. 2017; 3(4):200-204.
7. Ranade Y, Dharmadhikari S, Wadegaonkar P. Study of antimicrobial activity of *Andrographis paniculata* against ESBL existing urinary pathogens. Indian J of applied research. 2014; 4(12):57-60.
8. https://www.researchgate.net/publication/302558475_Evaluation_of_antibacterial_activity_of_caffeine
9. Muthanna J. Mohammed, Firas A. Al-Bayati. Isolation, Identification and Purification of Caffeine from *Coffea Arabica* L. and *Camellia Sinensis L*. A Combination Antibacterial Study. International J of Green Pharm. 2009, 52-56.
10. Shinde P, Shinde N. Extraction of Caffeine from Coffee and preparation of Anacin drug. International Research Publication House. 2017; 10(1):236-239.
11. Md Abdul Mumin, Kazi Farida Akhter, Md Zainal Abedin*, Md Zakir Hossain. Determination and Characterization of Caffeine in Tea, Coffee and Soft Drinks by Solid Phase Extraction and High Performance Liquid Chromatography (SPE - HPLC) Malaysian Journal of Chemistry, 2006, (8)1:045-051.
12. Bergey's manual of Determinative Bacteriology. 9th edition. Quail Williams and Wilkins, Baltimore MD, 1994.
13. CLSI. Performance Standards for Antimicrobial Susceptibility Testing, Sixteenth Informational Supplement. 2006, M100-S16.
14. Pawar P, Bikkad S, Kurhade S, Kadbane S. Evaluation of antibacterial activity of caffeine. Int Jar of Ayurveda and Pharmacy, 2011, 1354-1357.