ORIGINAL RESEARCH PAPER
Volume - 12 | Issue - 10 | October - 2023 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

COFFEA ARABICA PHARMACOLOGICAL COMPONENT PRIMARY SCREENING BY THIN LAYER CHROMATOGRAPHY

Biotechnology

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ABSTRACT

Extraction of bioactive compound to study their properties is one of the priorities in this scientific world. Bioactive compound extraction from *coffea arabica* which possess antibacterial activity, antifungal activity, anti-cancer agent, anti-oxidants etc, is one of the area explored by many researchers all around the world working on *coffea arabica.* Proper understanding of solvents and their polarity can improve our knowledge to extract bioactive components from *coffea arabica* leaves. These extracted components can easily be separated using thin layer chromatography method. Each component can be extracted to study specific role in the area of antibacterial activity, antifungal activity, anti-cancer, antioxidants. Current study successfully extracted eleven components as per Rf value's from *coffea arabica* leaf and studied for their antibacterial activity against *E.coli.*

KEYWORDS

Thin layer chromatography (TLC), coffea arabica, antibacterial activity, extraction solvent, bioactive compound.

INTRODUCTION

The use of medicinal plants in treating various diseases has been widespread due to their favorable characteristic of causing fewer side effects compared to synthetic drugs. Natural plant extracts provide a rich source for discovering new drugs because of the remarkable diversity of chemicals they contain. The process of isolating bioactive compounds from plants is crucial for the development of pharmaceuticals. These isolated components possess biological activity and exhibit a range of beneficial properties, including anti-cancer, antimicrobial, antifungal, analgesic, antioxidant, and wound healing effects. Therefore, the extraction of bioactive compounds from medicinal plants plays a pivotal role in scientific research and investigation. Coffee, a highly popular beverage worldwide, is cultivated on a global scale and holds significant economic and agricultural importance, particularly in tropical regions of developing countries.

Coffee plants are native to tropical region of Africa, particularly Ethiopia and Sudan. Among the most widely cultivated and commercially relevant species are *Coffea arabica* and *Coffea canephora* (Robusta coffee).

Globally, over 70 countries including India are involved in coffee production. India contributes 4.5% of world coffee stands seventh among the world coffee producing countries (International coffee organization, 2020).

According to the USDA's office in India, coffee exports from India are anticipated to increase by 2% in the marketing year 2023-2024, which starts in October. This growth is expected to result in a total of 5.8 million bags of 60kg each.

Phytochemical Constituents of coffee

The chemical composition of coffee leaves and beans contains a large variety of substances include alkaloids (trigonelline, caffeine, theobromine, theophylline), polyphenols (chlorogenic acid, caffeic acid, etc.), fatty oil, carotenoids, enzymes, phytosterols (sitosterol, dihydro-sitosterol, stigmasterol), tannin, wax, carbohydrates and polymers (cellulose, hemicelluloses), and nonvolatile and volatile aliphatic acids (citric, oxalic, acetic, isovaleric, decanoic acids, etc), (Lepelley *et al*., 2007; Magalhães *et al.*, 2008; Eva Brigitta Patay *et al.*, 2016; Chen Ma Kitts 2018).

Chlorogenic acids (quinic acid derivatives) are esters of the trans-cinnamic acids whose amounts in green coffees are significantly reduced during the roasting process. CGA is a potent antioxidant and associated with reduced risk of chronic diseases, including cardiovascular disease and type 2 diabetes.

Other Polyphenols like kaempferol, quercetin, ferulic, synaptic, nicotinic, quinolic, tannic, pyrogallic acids offers significant health benefits (Eva Brigitta Patay *et al*., 2016).

Pharmacological properties of coffee

The reported pharmacological properties that are imparted by *coffea arabica* due to their phytochemical constituents include antidiabetic, antioxidant, anti-inflammatory, antibacterial & antimicrobial properties, immune-modulator, neuroprotective effect, DNA protective effect, hepatoprotective effect, cardioprotective effect, anti-obesity, anti-hypertension, free radicals scavenger and a central nervous system (CNS) stimulator.

This study therefore aimed to investigate the bioactive compounds of the of *coffea arabica* that are responsible for their antimicrobial activity against various pathogenic bacterial strains as well as to compare these finding with those reported worldwide.

Thin Layer Chromatography (TLC)

TLC is a basic versatile and effective chromatographic technique that is widely used for both qualitative and quantitative analysis. TLC can be used to analyze substances such as steroids, alkaloids, flavonoids, polyphenols (secondary metabolites), lipids, nucleotides, glycosides, carbohydrates, and fatty acids. Therefore, by using this simple technique bioactive compounds can be easily separated from coffee leaf extract.

It is believed that by adjusting the solvent system we can optimize the separation and visibility of various compounds present in coffee leaf extract. Like best known solvent mixtures are ethyl acetate/hexanes for less polar compounds and methanol/ dichloromethane for more polar compounds. So this study also focuses on improving the performance of TLC extraction by monitoring the polarity relationship between the target components and the solvent system.

MATERIALS AND METHODS

Sample collection

Coffea arabica plant were collected from Karnataka India and IFP, Maintained in Aakriti Biotechnology nursery, in Jharkhand. Middle aged leaf 4 cm length and 3cm width were selected for all extraction purpose.

Extraction of components

Leaves were cleaned by running tap water and then sterilized by ethanol. The leaf samples were cut into small pieces and processed for extraction using TLC extraction solvent (Bunshi Bioscience Pvt. Ltd.) with the help of sterilized mortar pestle. The sample was centrifuge at 5000 rpm for five minutes to remove the debris and supernatant was collected in pre weighed micro centrifuge tube (Tarson). Air dried sample were quantified for the further process.

Thin Layer Chromatography

TLC plates were prepared with G 400 silica (Loba Chemie). Extract of *Coffea arabica* were spotted and allowed to migrate in Glass TLC running Chamber (Qualigens). Migrated components were observed in visible 650 as well as UV 210 nm.

Recovery of Extracted components

All the separated bands were scooped out with the help of spatula and collected in centrifuge tubes. Scooped samples were again isolated from silica mixture by dissolving them in acetone followed by centrifugation at 5000 rpm for 5 minutes. Components were collected and dried to estimate the output.

Antibacterial Test

Antibacterial test was conducted using disc diffusion method (Kirby–Bauer 1940). Plates were prepared under the laminar condition. Sensitivity of bacteria were monitor for each components extracted for their antibacterial properties.

RESULT AND DISCUSSION

The TLC analysis of coffee leaf extract revealed maximum possible separation, indicating the presence of multiple distinct compounds of different Rf with varying polarities. The components obtained in chromatogram were analyzed by calculating the R_f value to aid in their identification (Table-1). Separated components of different Rf value were extracted from silica to evaluate for the antibacterial activity against the *E.coli* bacteria. Zone of inhibition obtained in three extracted components figure number 2.8, 2.10 and 2.11. Fig 2.11 had two components extracted together of R*f* value 0.1 and 0.13 shown in Table-1.

Table 1: R*f* value of each components and their Antibacterial activity

Coffee leaf extract fragments after TLC	Rf value	Antibacterial activity	Diameter of Zone
1	1	-	0
2	0.86	-	0
3	0.78	-	0
4	0.71	-	0
5	0.68	-	0
6	0.66	-	0
7	0.65	-	0
8	0.60	+	4mm
9	0.54	-	0
10	0.44	+	1.25mm
11	0.1/0.13	+	1.16mm

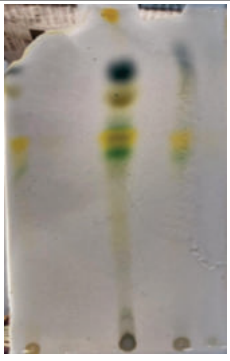

Figure 2: Antibacterial activity of coffee leaf extract on Agar plates

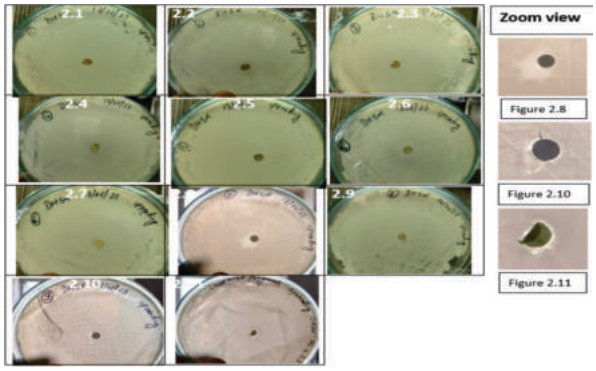

Fig 2.8 (extracted component RF 0.60), has partial antibacterial activity with a zone of inhibition 4mm. Fig 2.10 (extracted component RF 0.44) which has antibacterial activity with a zone of inhibition 1.25 mm. Fig 2.11 (extracted component RF 0.1 and 0.13) which has antibacterial activity with a zone of inhibition 1.16 mm. Zone of inhibition has not appeared in other eight plates.

The result of this study indicates the presence of bioactive compounds like flavonoids, alkaloids and chlorogenic acid (CGA). TLC plate of obtained Rf value range from 0.1 to 0.13 indicates the presence of chlorogenic acid (Kiattisin *et al*., 2016).

Obtained Rf value 0.44 of coffee leaf extract indicates the presence of flavonoids and trigonelline (alkaloid) in accordance with previous reported data (Karthikeyan Lakshmanan *et al.,* 2021), and finding suggest that Rf value of 0.60 also indicates the presence of alkaloid.

These Phenolic compounds in coffee leaves provide important biological functions. Chlorogenic acid (CGA) exhibits anti-diabetic and anti-lipidemic properties, neuroprotective effects, anti-obesity, DNA protective effect, hepatoprotective effect, cardioprotective effect cancer, and fatty liver disease. Trigonelline (alkaloid) act as antibacterial, antidiabetic, gastric stimulant, CNS stimulant, immunomodulator, hypoglycemic, hypolipidemic, anti-tumor and memory improving activities.

Coffee extracts have been found to exhibit potent antibacterial properties. Compounds like flavonoids, chlorogenic acids (CGA), trigonelline, caffeine (central nervous system stimulator) are the key contributors to the natural antibacterial activity against bacteria.

We can understand that their antimicrobial potential can open up the opportunity for developing the pharmaceuticals. Such natural sources may offer alternative treatments to conventional antibiotics and antifungal agents, reducing the risk of antimicrobial resistance and improving the overall efficacy of treatments.

CONCLUSION:

It is conclude that Coffea arabica is a rich source of phytochemical constituents and has antimicrobial activity against *E.coli* bacteria. Further studies are needed to identify and evaluate the efficiency of the bioactive compounds of coffea arabica against several pathogens.

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