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Identify the Role of Coffea Arabica and Camellia Sinensis Aqueous Extract on Pathogenic Cultures

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Abstract*—The contemporary study compacts with the aqueous extracts of Filter Coffee Arabica Aqueous Extract [FCAAE], Instant Coffee Arabica Aqueous Extract [ICAAE], Camellia Sinensis Aqueous Extract [CSAE] and Lipton Green Tea Aqueous Extract [LGTAE] on desired pathogenic cultures. The FCAAE, ICAAE, CSAE and LGTAE withholds enormous of both macro and micro nutrients, such as tannins, Flavonoids, Saponins, Phenols, Steroids, Caffeine etc… which play vital role in several phytochemical applications. FCAAE, ICAAE, CSAE and LGTAE commonly display the presence of Terpenoids, Saponins, Tannin, Flavonoids and Alkaloids in the preliminary screening study. It also confirms that the presence of above said phytochemicals in GC-MS analysis of FCAAE, ICAAE, CSAE and LGTAE. Evidently LGTAE exhibits more antibacterial and antifungal property when compare to FCAAE, ICAAE and CSAE. Unfortunately, all of the above extracts fail to demonstrate its role on anticoagulant effect, which intern confirms that FCAAE, ICAAE, CSAE and LGTAE have no role plasma re-calcification time. Moreover, FCAAE, ICAAE, CSAE and LGTAE did not hydrolyze RBC cells suggested its non-toxic property.*

***Keywords*—** *Filter Coffee Arabica Aqueous Extract [FCAAE], Instant Coffee Arabica Aqueous Extract [ICAAE], Camellia Sinensis Aqueous Extract [CSAE], Lipton Green Tea Aqueous Extract [LGTAE], GC-MS, Antibacterial property, Antifungal property and Non- toxic property.*

I. INTRODUCTION

The Scientific terminology of Coffee is nothing but *Coffea Arabica*. *Coffea Arabica* is basically falls under Rubiaceae family. Majorly Coffee is classified into two types namely *Coffea Robusta* and *Coffea Arabica* [1]. Similarly, scientific name of Tea is *Camellia*, which includes more than 82 species based on origin and locality. These are most probably native to high altitude lands of east and south India [2]. Both *Coffea Arabica* and *Camellia Sinensis* come under the group of non-alcoholic beverages which is consumed all over the world irrespective of gender and age bar [3]. Both Coffee and tea powder exhibits many therapeutical applications due to the presence of numerous phytochemicals such as polyphenols, flavonoids, alkaloids, tannins and catechins etc., [4]. The most leading coffee producer and supplier country throughout the world wide is none other than Brazil [5]. The major bioactive compound present in coffee powder is Caffeine [6]. Caffeine is widely used in food and beverage industries as a major element or active compound due to its anti-oxidative property as it drastically decrease oxidative stress [7]. Biological synthesis of caffeine undergone several steps which includes hydrolytic release of 7 methyl xanthine from the ribose nucleoside to 3-methylations producing theobromine and caffeine [8, 9]. Similarly, Tea also very popularly consumed beverage throughout the world wide. China, India, Kenya, Sri Lanka and Turkey were the largest producers of tea in the world [10]. Due the presence of Flavonoids in the tea it attributes to strong antioxidant property [11]. More or less tea withholds essential oils, enzymes, tannins, poly phenolic compounds, caffeine and other minerals [12]. Flavin 3-ol or Flavan 3,4-diol flavonoid falls under the category of tannins broadly present in the tea [13]. Even though, tea and coffee exhibits enormous therapeutical applications, this study deals with the antimicrobial property of FCAAE, ICAAE, CSAE and LGTAE on desired cultures.

II. MATERIALS AND METHODS

All the chemicals used were of analytical grade. Fresh microbial cultures were purchased by ATCC and MTCC.

Preparation of Extracts

Filter Coffea Arabica, Normal Coffea Arabica, Camellia sinensis and Lipton green tea were purchased from local market in the form of powder. From the purchased powders secondary metabolites were extracted by the purified HPLC grade water using Soxhlet extraction method. The finally obtained extracts were termed as FCAAE, ICAAE, CSAE and LGTAE. These extracts were utilized for further assays.

Preliminary phytochemical screening of FCAAE, ICAAE, CSAE and LGTAE

FCAAE, ICAAE, CSAE and LGTAE were screened for alkaloids, anthroquinones, flavonoids, steroids, terpenoids [14], glycosides, saponins [15], phytosterol [16], tannins [17], phenols [18], carbohydrates and protein/amino acids [19, 20].

GC-MS analysis of FCAAE, ICAAE, CSAE and LGTAE

FCAAE, ICAAE, CSAE and LGTAE were analyzed in GC-MSD, model number 5977B, Agilent Make on single quadrupole mass spectrometers in the Electron Impact Ionisation Total Ion Chromatography (EITIC) mode with capillary column (30m lengthX0.25mm ID, 0.25µm film thickness, composed of 5% Phenyl methyl poly siloxane). Helium (99.999%) gas was used as carrier gas at the flow rate of 1ml/min and the injection volume of 2µl. Split ratio of 10:1, temperature program was set as follows, injector temperature

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350°C; Auxiliary temperature 250°C, oven temperature initially 50°C (4min hold) with an increase in temperature of 10°C/min to 150°C (4min hold), thereafter 20°C/min to 200°C (4min hold), 25°C/min ramp to 250°C (4 min hold), 30°C/min ramp to 280°C (4 min hold). Total run time 35.5 min. Sample was analyzed in GC-MSD, model 5977B Agilent Make. Mass spectrum was taken at 70ev; a scan interval of 2.92s [21].

Preparation of Platelet Rich Plasma (PRP) and Platelet Poor Plasma (PPP)

The PRP and PPP were prepared as described by Ardlie and Han [22]. The platelet concentration of PRP was adjusted to 3.1×10^8 platelets/mL with PPP. The PRP has to be used within 2hr from the time of blood drawn at 37°C. All the above preparations were made in plastic containers or silicified glass containers.

Plasma re-calcification time of FCAAE, ICAAE, CSAE and LGTAE

The plasma re-calcification time was determined according to the method of Quick [23]. Briefly, the FCAAE, ICAAE, CSAE and LGTAE (1-10µg) were pre-incubated with 0.2mL of citrated human plasma in the presence of 10mM Tris HCl (20µL) buffer pH 7.4 for 1min at 37°C. Clotting time was recorded after the addition of 20µL $CaCl_2$ (0.25M) to the pre-incubated mixture.

Direct hemolytic activity of FCAAE, ICAAE, CSAE and LGTAE

Direct hemolytic activity was determined by using washed human erythrocytes. Briefly, packed human erythrocytes and Phosphate Buffer Saline (PBS) (1:9v/v) were mixed; 1mL of this suspension was incubated independently with the various concentrations of FCAAE, ICAAE, CSAE and LGTAE (0-100µg) for 1hr at 37°C. The reaction was terminated by adding 9mL of ice cold PBS and centrifuged at 1000g for 10min at 37°C [24]. The amount of hemoglobin released in the supernatant was measured at 540nm. Activity was expressed as percent of hemolysis against 100% lysis of cells due to the addition of water (positive control), whereas PBS served as negative control.

Antimicrobial assay of FCAAE, ICAAE, CSAE and LGTAE

The bacterial cultures (E. coli, Salmonella, Pseudomonas, Shigella and S. aureus) were grown in Muller Hinton nutrient agar medium that contain peptone (1%), beef extract (1%) and NaCl (1%) at pH 6.8. Sterile nutrient agar petri plates were prepared and 0.1mL of the overnight grown bacterial culture was spread on the solidified agar plates evenly with the help of a glass spreader. Wells were made in the solidified agar with a cork borer. The test solution was made by dissolving 100mg of FCAAE, ICAAE, CSAE and LGTAE in 1.0mL of water to get 100mg/mL concentration followed by sonication for 2min. The 10µL of this test solution containing 1mg of FCAAE, ICAAE, CSAE and LGTAE were added into the respective wells by varying the concentration (1-10mg). The standard antibiotic drug Amoxycillin was kept as positive control and tested against all the pathogens. These plates were incubated at 37°C for 24hr. The diameter of 'zone of inhibition' at each well was measured and recorded [25]. Minimum inhibitory concentration (MIC) determination was performed in triplicate and mean values are reported.

III. Results and Discussion

Physical and chemical Characterization of FCAAE, ICAAE, CSAE and LGTAE

FCAAE, ICAAE, CSAE and LGTAE were observed to found to be present of terpenoids, saponins, tannins, flavonoids, alkaloids as per preliminary screening of all above said extracts (Table 1).

TABLE 1. Preliminary Phytochemical analysis of FCAAE, ICAAE, CSAE and LGTAE

SL No	Phytochemical analysis	FCAAE	ICAAE	CSAE	LGTAE
1	Terpenoids	+	+	+	+
2	Saponin	+	+	+	+
3	Phytosterol	-	-	-	-
4	Tannin	+	+	+	+
5	Phenol	-	+	+	-
6	Flavonoid	+	+	+	+
7	Glycoside	+	+	-	-
8	Carbohydrates	-	-	-	-
9	Proteins	-	-	-	-
10	Alkaloid	+	+	+	+
11	Steroid	-	-	+	+

GC–MS Analysis of FCAAE, ICAAE, CSAE and LGTAE

As per GC-MS analysis of FCAAE and ICAAE shows 5 major peaks at the retention time of 23.6, 13.8, 13.1, 12.6 and 3.4min in FCAAE. 25.3, 13.10, 13.69, 14.39 and 3.41min in ICAAE respectively. Whereas CSAE and LGTAE demonstrate 7 major peaks at the retention time of 25.5, 14.7, 13.9, 13.5, 9.6, 10.5 and 12.14min in CSAE. 25.2, 13.14, 10.8, 9.5, 7.9, 3.17 and 2.73min in LGTAE (Fig. 1-4).

FCAAE, ICAAE, CSAE and LGTAE role on plasma re-calcification time

To assess the part of FCAAE, ICAAE, CSAE and LGTAE on plasma re-calcification time, plasma coagulation time was performed using both human Platelet Rich Plasma (PRP) and Platelet Poor Plasma (PPP). Unfortunately, FCAAE, ICAAE, CSAE and LGTAE don't show any role on PRP and PPP. In recent studies emerging scientists demonstrate that few herbal extracts exhibits anticoagulant and procoagulant properties which intern play a pivotal role in treating the thrombotic disorders without any side effects [26-28].

Additionally, FCAAE, ICAAE, CSAE and LGTAE did not hydrolyze RBC suggested its nontoxic property (Fig. 5).

FCAAE, ICAAE, CSAE and LGTAE role on pathogenic cultures

FCAAE, ICAAE, CSAE and LGTAE antimicrobial property were performed with both gram negative pathogenic bacterial strains namely *E.coli*, *S. aureus*, *Salmonella*, *Pseudomonas* and *Shigella*. Surprisingly, LGTAE exhibits more antibacterial and antifungal property when compare to

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FCAAE, ICAAE and CSAE. All extracts Minimum Inhibitory of Concentration value was demonstrated individually (Fig. 6). Many researchers demonstrates that herbal extracts and nanoparticle conjugated extracts shows more broad antibacterial spectrum when compare to synthetic molecules [29].

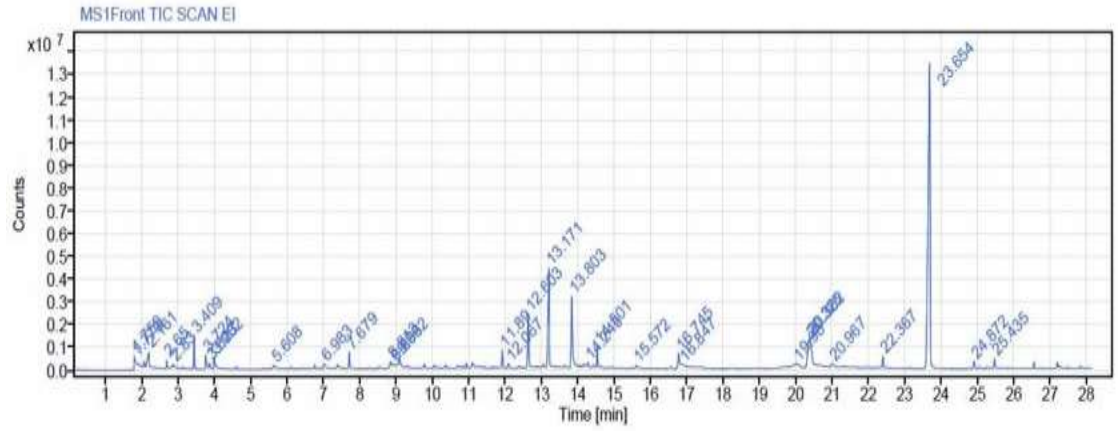

Fig:01

Fig. 1. GC-MS Chromatogram of FCAAE

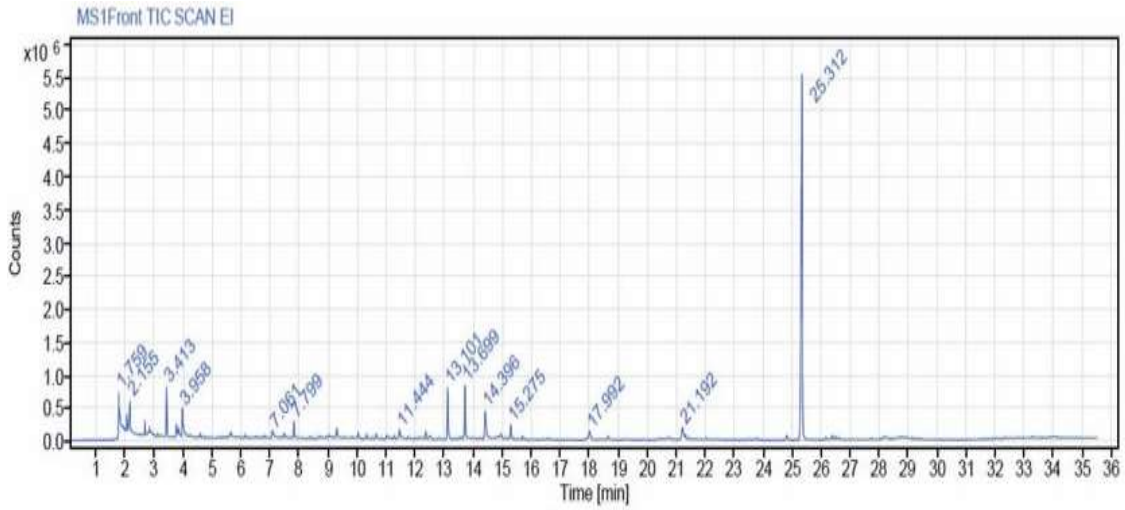

Fig:02

Fig. 2. GC-MS Chromatogram of ICAAE

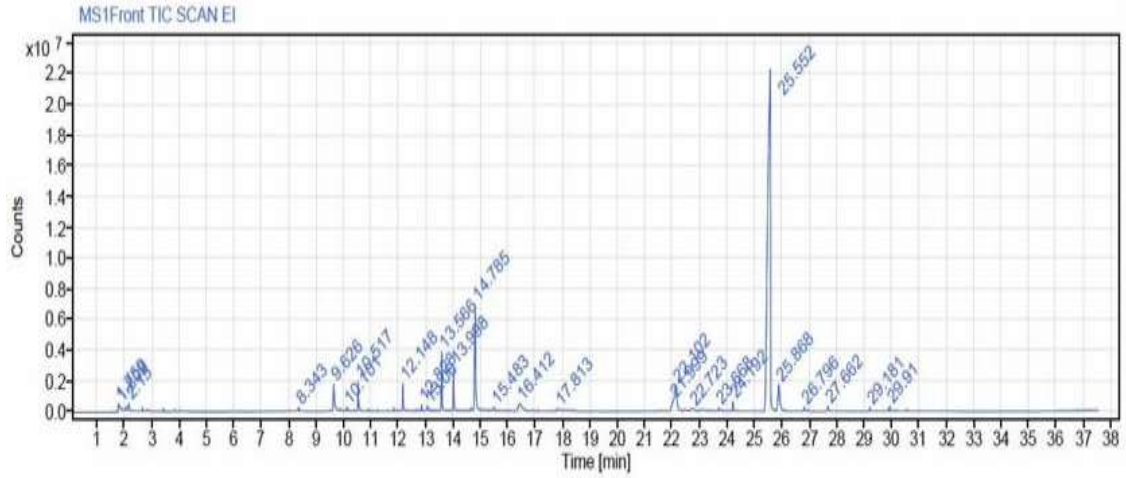

Fig:03

Fig. 3. GC-MS Chromatogram of CSAE

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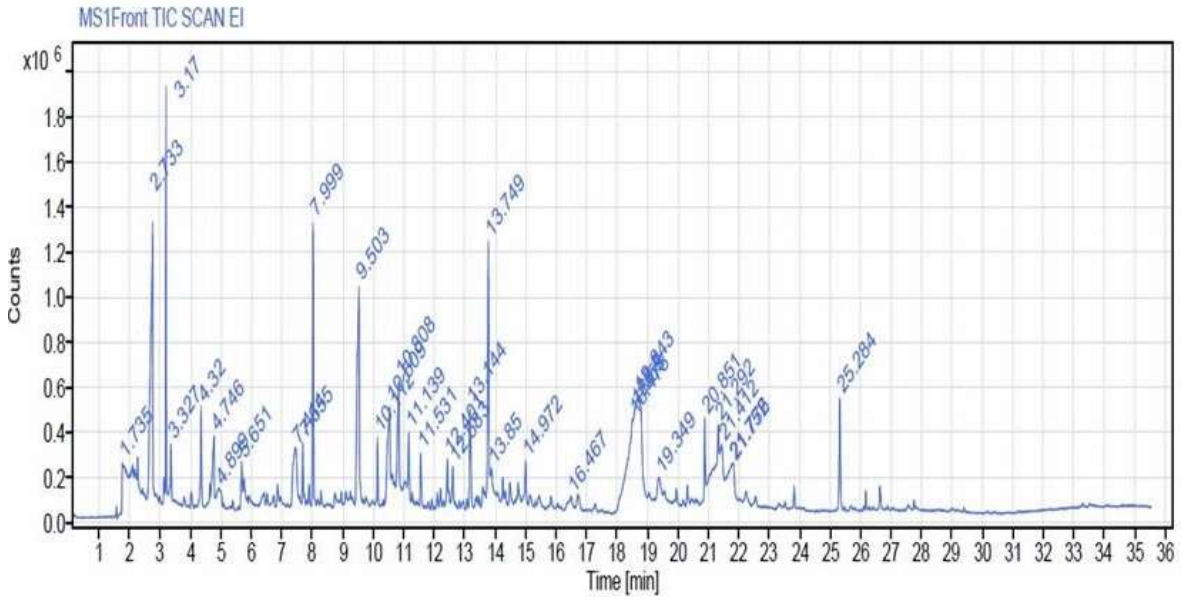

Fig:04

Fig. 4. GC-MS Chromatogram of LGTAE

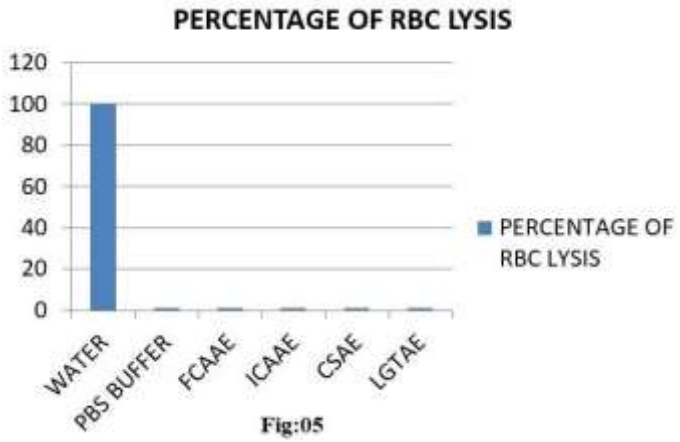

Fig. 5. Hemolytic activity of FCAAE, ICAAE, CSAE and LGTAE

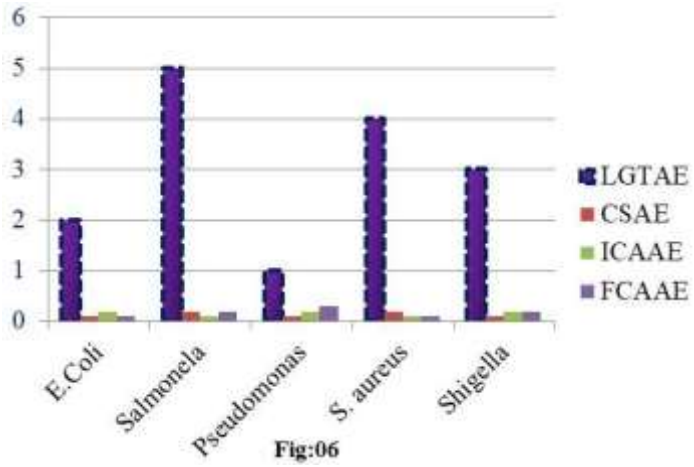

Fig. 6. Minimum Inhibitory Concentration of FCAAE, ICAAE, CSAE and LGTAE against pathogenic cultures in mg

IV. Conclusion

FCAAE, ICAAE, CSAE and LGTAE show different biomolecules in the preliminary study. LGTAE demonstrate its strong antimicrobial property compare to FCAAE, ICAAE and CSAE.

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Declaration of Conflict of Interest

The authors declared no potential conflict of interest with respect to the authorship and publication.

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