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Investigation of Rf Value-Based Components From TLC of Cymbopogon Citrates for Antibacterial Activity Against E. Coli

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Abstract
Lemongrass commonly known as *Cymbopogon citrates* known for its lemon like aroma which carries various types for the essential oil i.e. including *nerols*, *myrcene*, *citroneliol*, *methyl heptanone*, *dipentene*, *geraniol*, *limonene* etc. Its compounds known for different types of anti-fungal, anti-bacterial, insecticidal, anti-septic, anti-dandruff, anti-oxidant, anti-inflammatory, pro-apoptotic, cytotoxic properties and also have various types of phytochemical components such as phenols, flavonoids, isoquercetin, naringin, caffeic acid, phytosterol etc. Current study focused on to isolate the component of the lemongrass (*Cymbopogon citrates*) based on TLC separation and investigate with respect to anti-bacterial activity against *E.coli*. Current finding indicates effective antibacterial activity found in four components separated during TLC.

Keywords: Thin Layer Chromatography (TLC), *Cymbopogon citrates*, anti-bacterial activity, solvent extraction, *E.coli*, *phytochemical* components, essential oil, and leukemia cells.

Introduction

Lemongrass is also known as *Cymbopogon citrates* which is also known for its lemon-like aroma as well as medicinal properties which contains a various types of essential oils including *nerols*, *myrcene*, *citroneliol*, *methyl heptanone*, *dipentene*, *geraniol*, *limonene* etc. (Kumar D *et. al*., 2017). These compounds are also known for its anti-fungal (Paranagama *et. al*., 2003), anti-bacterial (Behboud *et. al*., 2012), insecticidal (Moustafa Moataz A M *et. al*., 2021), anti-septic properties (Ambade S *et. al*., 2021), anti-dandruff properties (Chaisripipat *et. al*., 2015), anti-oxidant (Jose *et. al*., 2005), anti-inflammatory (Olorunnisola *et. al*., 2014), pro-apoptotic activity in several hematopoietic cell lines (Ganjewala *et. al*., 2009). Additionally, they have the abaility to kill P388 leukemia cell (Rodolph B, 1999). It also has been found to possess in protecting against bean worm, mosquitoes (Negrelle *et. al*., 2007). It is widely distributed in all over the world such as Malesia, South America, Central America, Cambodia, Vietnam, Laos, India, Sri Lanka, Burma, China, Myanmar, Bhutan, Bangladesh. India is currently one of the world's largest exporters, sending lemongrass essential oil to more than 80 nations. In several Indian states, including West Bengal, Jharkhand and Utter Pradesh, lemongrass is grown. It is extensively grown throughout the world's tropical and sub-tropical region (Singh SP *et. al*., 2022). It is easily cultivated on marginal land and in any type of the soil, for better yield it can also cultivate in sandy and

loam soil with a pH range 5 to 8.5. In India, Kerala is the hotspot region where lemongrass is grown as a semiarid crop (Singh and Shivraj 1999). While, under rainfed tropical condition it is grown as moist crop in Karnataka, Tamil Nadu, West Bengal, Chhattisgarh, Odisha, Uttar Pradesh, Assam, Jharkhand, Bihar, Maharashtra, Madhya Pradesh and Rajasthan about 250 tones are produced overall from about 4000 hectares. The current study is to isolate the component of the lemongrass (*Cymbopogon citratus*) that is responsible for anti-bacterial activity against *E.coli*.

Material and Methods

Sample collection

Lemongrass leaves (Fig1) were collected from the latitude 23.371888ºN and longitude 85.244429ºE. Extraction of sample: Using a mortar and pestle and acetone, the sample was mechanically ground until it was crushed. Estimated extract 40mg were used for further studies.

Thin Layer Chromatography: Thin Layer Chromatography experiment was performed using TLC extraction solvent (Bunshi Bioscience Pvt. Ltd.). Separation was performed on silica gel plate with thickness of 25 mm. Separation performed on TLC chamber presaturated with TLC running buffer for 30 minutes. TLC plate was spotted with acetone extract of *Cymbopogon citrates* young leaves, allowed to get separated for 40 minutes. Separated components were evaluated by R_f values.

The following formula was used to determine each separated pigment's Rf values:

$$Rf = \frac{\text{Distance travelled by solute (cm)}}{\text{Distance travelled by solvent (cm)}}$$

The TLC chromatogram was obtained and analyzed by comparing the R_f values of the separated pigments. Anti-bacterial test: Anti-bacterial test was performed using disc diffusion technique (Kumar D *et. al*., 2023). Plate was poured under sterile condition (LAF). *E.coli* Susceptibility were tested against each isolated component.

Phytochemical analysis

Phenol test: The presence of phenol was tested using Wagner's reagent test (Aniszewski 2007). Flavonoid test: The presence of flavonoid was tested using alkaline reagent test (Tiwari *et. al*., 2011).

Results

The distance travelled by different pigments of lemongrass (*Cymbopogon citratus*) acetone extracts and the TLC running buffer was noted (Figure2). Each pigment R_f value calculated using the formula which were mentioned earlier.

Figure2: TLC of Lemongrass extract

Figure1: Lemongrass

Figure3: Rf (0.39), (0.25), (0.53) and (0.62) of lemongrass against *E.coli*. Zone of inhibition was found to be Rf (0.39) is 1mm, Rf (0.25) is 1mm, Rf (0.53) is 2mm, Rf (0.62) is 2mm. *Refer to Table1.

Figure 4: Phytochemical analysis of lemongrass. Reddish-Brown (phenol) and Yellowish (flavonoid).

Distance travelled by TLC running buffer noted to be 12.8 cm.

Table1: *Rf* value obtained are evaluated for antimicrobial properties.

S.No	Lemongrass extract fragments after TLC	R_f value	Sample name on plates	Antibacterial activity	zone of inhibition found	Component as per comparative study	References
1	1.8	0.14	L1				
2	3.3	0.25	L2	+	1mm	Phenol	Muteeb G *et. al*., 2023
3	4.5	0.35	L3				
4	5	0.39	L4	+	1mm	Flavonoids	Chetty KM *et. al*., 2008
5	5.5	0.42	L5				
6	6.8	0.53	L6	+	2mm	Iso-quercetin	Tuberoso CIG *et. al*., 2009
7	8	0.62	L7	+	2mm	Naringin	L. Zang 2008, TH *et. al*., 2008
8	12.4	0.96	L8				

The result of this study finds eight components from lemongrass leaf extract (fig2), Rf comparative study indicates the presence of bioactive compounds like flavonoids, phenols, iso-quercetin and naringin. R_f value 0.25, 0.39, 0.53 and 0.62 indicates the presence of phenol, flavonoids, iso-quercetin and naringin respectively.

As per the R_f (0.25, 0.39, 0.53, 0.62) comparative study indicates the presence of flavonoid (0.39), phenol (0.25), naringin (0.62) and iso-quercetin (0.53) components from lemongrass leaf extract (Table1).

The presence of flavonoids in lemongrass is confirmed by the flavonoid test (fig. 4). Similarly, phenolic test (fig4) confirms the presence of phenol present in lemongrass and both having the anti-bacterial activity (fig3) and as per the R_f comparative study the presence of flavonoid (0.39) was tested using alkaline reagent (yellow) and phenol (0.25) was tested using Wagner's reagent (reddish-brown) by the help of phytochemical analysis and the result was found positive (fig4).

Discussion

Lemongrass is plant having various medicinal properties and having essential oil like *myrcene*, *nerols*, *limonene* etc. The components isolated using TLC from the lemongrass leaf extract was recorded (Table1). The lemongrass anti-bacterial components (phenol, flavonoid, naringin, iso-quercetin) can open up the opportunity for the pharmaceutical industry. Such natural sources can offer alternative treatments to common antibiotics which reduce the risk of antibacterial resistance and improving the overall potential of treatment. The current study isolated four components (0.25, 0.39, 0.53, 0.62) confirms anti-bacterial activity against *E.coli* (fig 3).

Conclusion

It is concluded that lemongrass is a source of phytochemical component like phenol (0.25), flavonoid (0.39), iso-quercetin (0.53), naringin (0.62) and others and having anti-bacterial activity against *E.coli.* Further studies are needed to identify and evaluate the efficiency of the bioactive compounds of lemongrass against several pathogens.

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