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STUDY OF PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITIES OF *LIGUSTRUM SINENSE* (LOUR.)

M. Shakila Begam *, R. Mouli, P. Akilandeswari and B. V. Pradeep

Department of Microbiology, Karpagam Academy of Higher Education, Coimbatore - 641021, Tamil Nadu, India.

Keywords:

Medicinal plant, *Ligustrum sinense*, Phytochemical Activity, Antimicrobial Activity, GC-MS, TLC

Correspondence to Author:
M. Shakila Begam

Research Scholar,
Department of Microbiology,
Karpagam Academy of Higher Education, Coimbatore - 641021,
Tamil Nadu, India.

E-mail: shakilalinos@gmail.com

ABSTRACT: More attention on natural antimicrobials and antioxidants have increased. The medicinal plants production has high in Mediterranean region. WHO says many drugs can be obtained by using medicinal plants. Medicinal plants have many compounds of bioactive that gives more metabolic action on the human body. The congestive heart failure treatment and cardiac arrhythmia treatment can be treated by glycosides drugs. The glycosides have potent in curing many problems like cardiac insufficiency, coughs and circulatory problems. Glycosides acts as good sedatives and good antispasmodic properties. Saponins were found in *Ligustrum sinense* shows tendency for treating infections of fungal and yeast. The Ethyl acetate leaf mixed extract showed more effective of in phytochemical screening and against the tested microorganisms, Compare chloroform leaf extract.

INTRODUCTION: Plants from the Oleaceae family have been known for their medicinal properties for thousands of years. In both Chinese and Mediterranean folk medicine, these plants were used in the prevention of many no communicable diseases such as cardiovascular diseases, chronic inflammation, hypertension, type II diabetes and cancer [1]. Over the last few decades, numerous studies have demonstrated the bioactive effects of oleuropein, most of which are directly linked to its strong antioxidant activity [2]. Plants from Ligustrum L. are widely known for their health-promoting properties.

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In the last decade, a bitter-tasting herbal tea called Ku-Ding-Cha, known in southwestern China since 200 BC, has gained great popularity around the world, and among its ingredients are the dried leaves of various *Ligustrum species*, primarily *L. robustum*, but also *L. purpurascens*, *L. henryi*, *L. lucidum*, *L. sinense* and *L. japonicum*. This tea is believed to support weight loss, prevent diabetes, prevent high blood pressure and reduce inflammation [3].

Several studies described the phytochemical composition of *L. vulgare* extracts, where the main compounds were flavonoids, secoiridoids and phenylethanoids [4]. The oleoside-type secoiridoids are associated with various biological activities that have been extensively studied in recent years. Particularly important is the antioxidant activity, which is demonstrated primarily by secoiridoid derivatives with a hydroxytyrosol moiety [5]. Antioxidants have proven influence on the

occurrence of cardiovascular diseases, inflammation and diabetes. The results of our previous study showed significant radical scavenging activity of *L. vulgare* extracts, where the detected antioxidants were mostly from the group of iridoids, including oleuropein and its derivatives [6]. Several products around world are interested in studying biologically components mainly derived from medicinal plants. The investigation of antimicrobials and antioxidants has received more interest nowadays to increase demand of consumer for food products devoid of artificial chemical additions [7]. Due to its amazing diversity and role as a source of medicinal plant production, the Mediterranean region has garnered particular attention in this regard [8, 9, 10]. Tannins, carbohydrates, alkaloids, steroids, terpenoids and flavonoids are bioactive chemicals present in medicinal plants which shows effects on human body [11, 12].

Medicinal plants include phytochemicals that enhance human physiological equilibrium and the generation's knowledge [13]. Since ancient time, plant parts like root section, leaves area, barks, flowers, fruits and thick seeds parts were used in phytomedicines [14]. Because, they can arrest radical mediators and give electrons or hydrogen, plant substances are well known for acting as antioxidants. When the plants are eaten as food, phenolic chemicals also have beneficial effects on people [15]. In humans, plant extract's antioxidant capacity is often selective on minimum doses and also linked to the protection of cancer and cardiovascular problems [16]. In addition to other syndromes, the oxidative stress has a role in cancer, cardiovascular disease, inflammatory disease, diabetes mellitus, neurodegenerative illness development and their progression [17].

In the Indian subcontinent, medicinal plants have significant value in economical level. Nature has endowed us with an exceptionally rich botanical diversity, and numerous distinct plant species can be seen growing throughout the nation. In India, there are three types of biodiversity such as species level, genetic level and habitat level. Many medicinal plant's part to diagnose various problems has been familiar in India during ancient period. Today's indiscriminate use of commercial antimicrobial medications, which are frequently utilised in the diagnosis of diseases, has increase the multiple drug resistance development [18]. This stimulation encouraged researchers to look for fresh antibacterial compounds [19]. It is now clear that maintaining a healthy tissue homeostasis depends on maintenance between proliferation of cell and cell death. Any deviation in any of these two processes result in the variation of clonal expansion which is a hallmark of every neoplastic disease state [20, 21]. *Ligustrum sinense* - leaf portion is the focus of the current inquiry. Solvents were used to extract the plant material. The phytochemicals, antibacterial activity, TLC, and GC-MS of the crude extracts were assessed.

MATERIALS AND METHODS:

Plant Sample Collection and Processing: The plant *Ligustrum sinense* was collected from coimbatore. The *Ligustrum sinense* plant samples were collected and washed properly in tap water and also in distilled water. The samples were dried in the shadow for three days. The dried plant leaves were powdered using pestle and mortar then stored in airtight container to protect from sunlight.

Extraction of Plant Material: 20g of fresh plant samples were weighed and added into 100ml of the solvent Chloroform. The same amount of sample was also added into methanol and ethyl acetate. It was then stirred at every 30mins for 3hours and kept as such to stand for hours. The *Ligustrum sinense* leaf extract was filtered by filter paper using Whatman No.1 filter paper and filtrate were stored in conical flask at 4°C for further use.

Phytochemical Screening of *Ligustrum sinense*: The phytochemical screening test for the *Ligustrum sinense* leaf extract was done using the methodology described by RNS Yadav [22].

Qualitative Analysis:

Test for Proteins: 2ml of Million's reagent was added into crude extract and upon mixing the white precipitate was produced. Further it turned into red colour when heating. It confirmed the presence of proteins in crude *Ligustrum sinense* leaf extract.

Test for Carbohydrates: 2ml of iodine solution was added into crude extract which produced a dark blue or purple colour. It conformed the carbohydrates presence in extract.

Test for Phenols & Tannins: 2mL of 2 percentage soln. of $FeCl_3$ was added into crude *Ligustrum sinense* leaf extract which produced a bluish green or dark black colour. It confirmed the presence of phenols and tannins in extract.

Test for Flavonoids: 2mL of 2 percentage soln. of NaOH was added into crude *Ligustrum sinense* leaf extract which produced yellow colour solution. It turned into colourless upon adding diluted acid. It confirmed the presence of flavonoids in crude extract.

Test for Presence of Saponins: 5mL of deionized water was mixed well with crude *Ligustrum sinense* leaf extract in a glass test tube and shook well. The foam was formed. It conformed the saponins presence in extract.

Test for Glycosides: 2mL of chloroform and 2mL of acetic acid was mixed well with crude *Ligustrum sinense* leaf extract and cooled in an ice. While adding conc.H_2SO_4 into it, it produced a color change from violet into blue and further blue into green. It conformed the glycosides presence in crude *Ligustrum sinense* leaf extract.

Test for Terpenoids: 2ml of chloroform was mixed with crude extract and allowed to evaporate for dryness. 2mL of conc. H_2SO_4 was mixed into it and heated for two min. It produced a grey color. It confirmed the terpenoids presence in crude *Ligustrum sinense* leaf extract.

Test for Presence of Steroid: 2ml of chloroform was mixed well with crude extract and conc.H_2SO_4 was poured along the sides of the test tube. It produced a red color in the lower layer of chloroform. It confirmed the steroids presence in crude *Ligustrum sinense* leaf extract.

Test for Presence of Alkaloids: 2mL of 1 percentage HCl was mixed well with crude *Ligustrum sinense* leaf extract and heated mildly. While adding Mayer-Wagner's reagent to this mix, it produced a turbid precipitate. It confirmed the alkaloids presence in crude *Ligustrum sinense* leaf extract.

Quantitative Analysis:

Total Phenol Content: The phenolic content presence in the leaf extract was determined by Folin-Ciocalteau reagent test with few changes. 1ml of extract was mixed with 2.5mL of 10 percentage Folin-Ciocalteau reagent and 2mL of 2 percentage Na_2CO_3 solution. This was kept for an incubation for 15mins at RT. The OD of this sample was measured at 765nm with the standard Gallic acid (1mg/ml). The standard curve was plotted and the amount of phenol was calculated.

Measurement of Total Flavonoid Content: Flavonoid content was measured using aluminium chloride colorimetric method with some changes. 3mL of methanol, 0.2mL of 10 percentage aluminium chloride, 0.2mL of 1M potassium acetate and 5.6mL of distilled water was mixed onto 1ml of *Ligustrum sinense* leaf extract and kept at RT for 30mins. The OD value was measured at 420nm with standard quercetin (1mg/ml). The standard curve was plotted and the amount of flavonoid was calculated.

Antibacterial Activity of *Ligustrum Sinense* Leaf Extract: The antibacterial activity of *Ligustrum sinense* assessed using the disc diffusion technique. The antibacterial activity of *Ligustrum sinense* leaf extract was performed using Muller Hinton agar medium against *E. coli*, *Bacillus* and *S. aureus*. The bacterial culture *E. coli*, *Bacillus* and *S. aureus* streaked on nutrient agar plate to obtain the pure culture. The pure test organisms were rest reamed on the nutrient agar slants and stored at 4°C to keep the strains viable. Pure culture of bacterial strain was inoculated into 50ml of the nutrient broth to prepare the stock culture. 10ml of the sterile nutrient broth were aseptically inoculated with stock culture and incubated for 24 hrs at 37°C. The test cultures were swabbed on the air-dried Muller Hinton agar plates by using sterile cotton swab. The disc was loaded with varying concentration of plant extracts. Using a flame sterile forceps, the disc loaded with 20, 50, 100µl of plant extracts were kept on the surface of Muller Hinton agar plate swabbed with bacterial culture. Controls were also maintained in which, the discs were load with respective solvents. Then the plates were incubated for 24 hrs at 37°C. Antimicrobial activity of *Ligustrum sinense* leaf extract was analysed by calculating the zone of inhibition around each disc (excluding the disc's diameter) and recorded the diameter of the inhibitory zone in millimetre.

Thin Layer Chromatography (TLC): The *Ligustrum sinense* leaf extract was spotted on silica gel plate and kept in the chromatographic tank which was filled with the liquid mobile phase - methanol. It was allowed to run for 30mins and allowed to dry at 30-40°C using oven. The Rf value was calculated as follows,

Rf value = The distance travelled by the compound / The distance travelled by the solvent

GC-MS Analysis: The Gas Chromatography-Mass Spectrometry analysis was carried out by The Clarus-680GC, fused with column-silica and filled by Elite 5MS (5% biphenyl 95% dimethyl-polysiloxane, 30m × 0.25mm ID × 250µm df). The carrier gas used here is helium at a flow of 1mL/min was used to bring out the components.

The injector temperature was maintained at 260°C when chromatographic run. The components spectrums in the *Ligustrum sinense* leaf extract were compared with the database of known components stored in the GC-MS NIST (2008) library.

Statistical Method for Result Analysis: Each experiment was carried out for three times (N=3) and analysed through error bars.

RESULTS AND DISCUSSION:

Phytochemical Screening: Phytochemical analysis of leaf extract of *Ligustrum sinense* are tabulated in the **Table 1** and **2**. The leaf extract with ethyl acetate solvent showed positive results with all the compounds like alkaloids, glycosides, saponins, phenols and the leaf extract with chloroform solvent showed positive results with all the compounds like protein, flavonoids, saponins, steroid, terphenoids and alkaloids. The quantitative estimation of total phenolic content and flavonoids content was shown in the **Table 3.** Phenolic compounds are the important class of natural antioxidants and closely correlated to the antioxidant activities of all plants [23, 24, 25].

TABLE 1: PHYTOCHEMICAL ANALYSIS OF *LIGUSTRUM SINENSE* IN ETHYL ACETATE

S. no.	Tests	Results
1	Proteins	+
2	Carbohydrate	+
3	Phenols & Tannis	+
4	Flavonoids	+
5	Saponins	-
6	Glycosides	-
7	Steroids	+
8	Terpenoids	+
9	Alkaloids	+

TABLE 2: PHYTOCHEMICAL ANALYSIS OF *LIGUSTRUM SINENSE* IN CHLOROFORM

S. No.	Tests	Results
1	Proteins	+
2	Carbohydrate	+
3	Phenols & Tannis	+
4	Flavonoids	+
5	Saponins	-
6	Glycosides	-
7	Steroids	+
8	Terpenoids	+
9	Alkaloids	+

TABLE 3: PHYTOCHEMICAL SCREENING - QUANTITATIVE ANALYSIS OF *LIGUSTRUM SINENSE* LEAF EXTRACT

S. no.	Tests	Results	
		***Ligustrum sinense* leaf extract on Ethyl Acetate**	***Ligustrum sinense* leaf extract on Chloroform**
1	Total phenolic content	1	0.69
2	Total flavonoids content	1.53	0.83

Antibacterial Activity of *Ligustrum sinense* Leaf Extract: The Antibacterial activity of fresh of *Ligustrum sinense* extract with ethyl acetate and chloroform were recorded. The leaf extract with

ethyl acetate showed the maximum inhibitory activity on *Staphylococcus aureus*, *Bacillus* and *Escherichia coli*. They showed 10mm in 50µl concentration, 10mm in 100µl concentration and 14mm in 20 µl concentration. The leaf extract with chloroform showed the maximum inhibitory activity on *Bacillus* and *Escherichia coli*. They showed 8mm in 25µl concentration, 6mm in 5µl concentration, 5mm in 20µl concentration *Staphylococcus* showed 9mm in 25 µl concentration **Fig. 1**, **Table 4** & **5**, Graph 1 & 2). A variety of TCMs have shown bactericidal effects against respiratory pathogens by directly killing or inhibiting their growth [26, 27, 28].

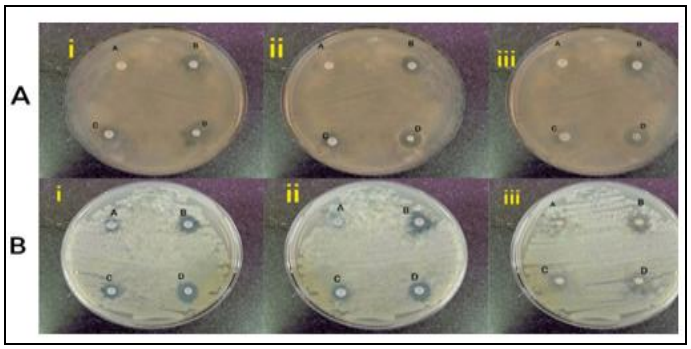


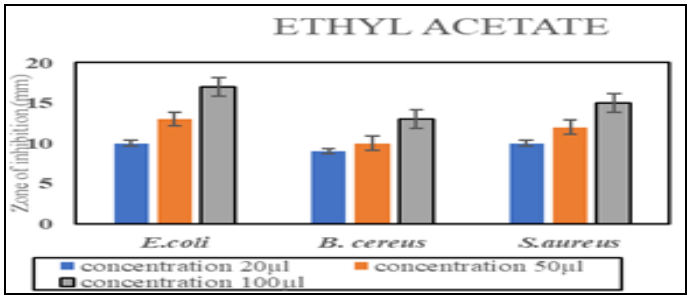
FIG. 1: ANTIBACTERIAL ACTIVITY OF *LIGUSTRUM SINENSE* LEAF EXTRACT WITH ETHYL ACETATE AND CHLOROFORM. A - Ethyl Acetate; B – Chloroform. (i - *Bacillus cereus*, ii - *Staphylococcus aureus*, iii - *E. coli*)

TABLE 4: ANTIBACTERIAL ACTIVITY OF *LIGUSTRUM SINENSE* LEAF EXTRACT WITH ETHYL ACETATE

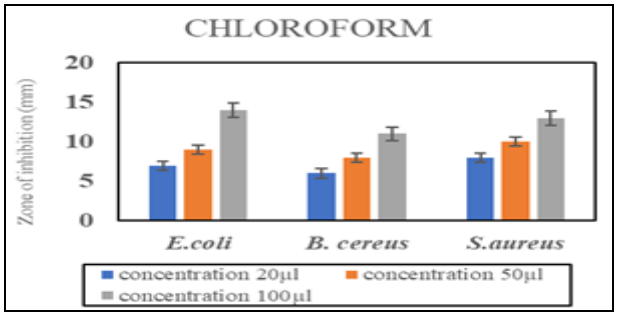
S. no.	Pathogens	Concentration of *Ligustrum sinense* leaf extract on ethyl acetate	Zone of Inhibition in diameter
1	*Staphylococcus aureus*	20µl	10mm
		50µl	12mm
		100µl	15mm
2	*Bacillus sp.*,	20µl	9mm
		50µl	10mm
		100µl	13mm
3	*Escherichia coli*	20µl	10mm
		50µl	13mm
		100µl	17mm

TABLE 5: ANTIBACTERIAL ACTIVITY OF *LIGUSTRUM SINENSE* LEAF EXTRACT WITH CHLOROFORM

S. no.	Pathogens	Concentration of *Ligustrum sinense* leaf extract on chloroform	Zone of Inhibition in diameter
1	*Staphylococcus aureus*	20µl	8mm
		50µl	10mm
		100µl	13mm
2	*Bacillus sp.*,	20µl	6mm
		50µl	8mm
		100µl	11mm
3	*Escherichia coli*	20µl	7mm
		50µl	9mm
		100µl	14mm



GRAPH 1: ZONE OF INHIBITION OF LEAF EXTRACT WITH ETHYL ACETATE SOLVENT AGAINST *BACILLUS CEREUS, STAPHYLOCOCCUS AUREUS AND E. COLI.*



GRAPH 2: ZONE OF INHIBITION OF LEAF EXTRACT WITH CHLOROFORM SOLVENT AGAINST *BACILLUS CEREUS, STAPHYLOCOCCUS AUREUS AND E. COLI.*

GC-MS (Gas Chromatography- Mass Spectrometry): The GC-MS chromatogram of the leaf extract of *Ligustrum sinense* have various compounds with different peaks at different retention time. The structure of the compounds identified in the extract as shown in **Fig. 2** & **3**, **Table 6** & **7**, Graph 3 & 4. The experimental extracts totally revealed 77 compounds in GC-MS analysis and all the extracts showed anti-inflammatory activity in an *in-vitro* assays [29].

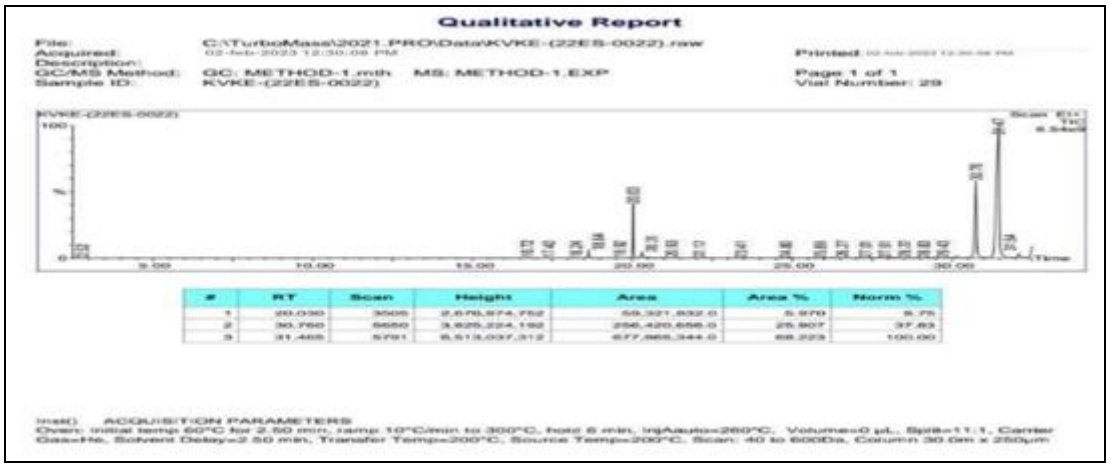


FIG. 2: QUALITATIVE REPORT OF *LIGUSTRUM SINENSE LEAF EXTRACT ON ETHYL ACETATE*

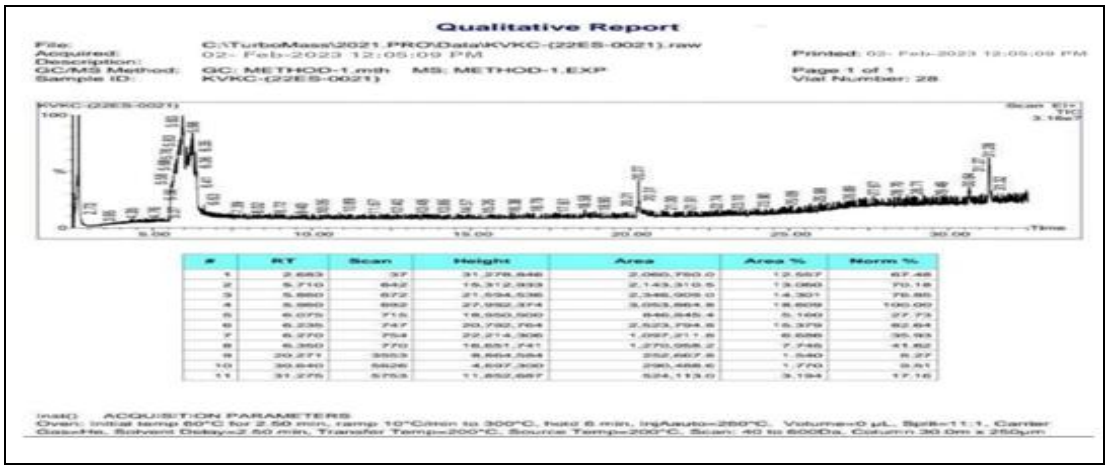
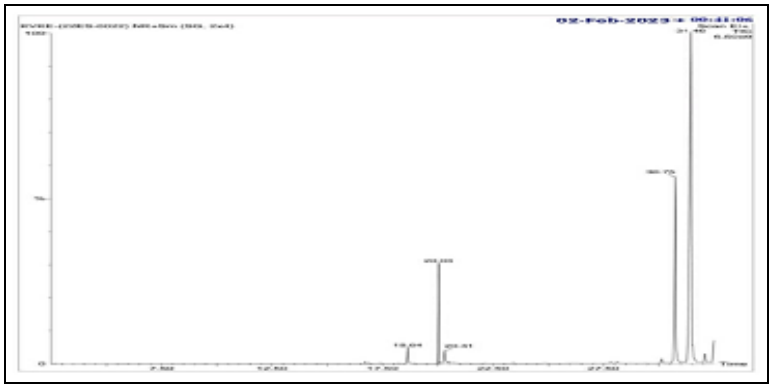


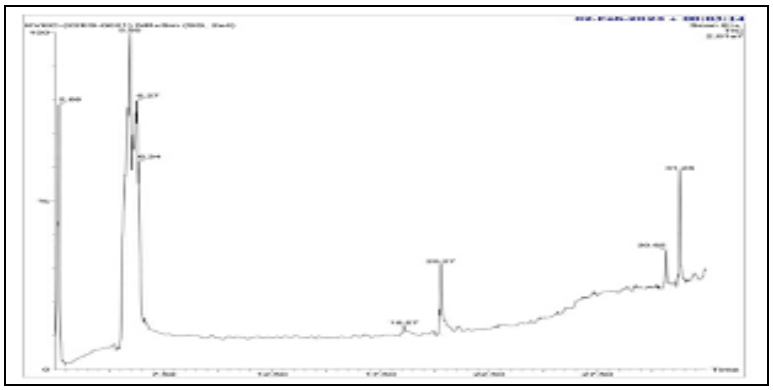
FIG. 3:1 QUALITATIVE REPORT OF *LIGUSTRUM SINENSE* LEAF EXTRACT ON CHLOROFORM

TABLE 6: GC-MS ANALYSIS OF CHROMATOGRAM OF *LIGUSTRUM SINENSE* LEAF EXTRACT ON ETHYL ACETATE

S. no.	Compounds	Retention time (min)
1.	Heptadecane	18.64
2.	Nonadecane, 2-methyl	20.03
3.	n-Hexadecanoic acid	20.31
4.	Tetratriacontane	30.75
5.	Eicosane	31.46



GRAPH 3: GC-MS ANALYSIS OF CHROMATOGRAM OF *LIGUSTRUM SINENSE* LEAF EXTRACT ON ETHYL ACETATE



GRAPH 4: GC-MS ANALYSIS OF CHROMATOGRAM OF *LIGUSTRUM SINENSE* LEAF EXTRACT ON CHLOROFORM

TABLE 7: GC-MS ANALYSIS OF CHROMATOGRAM OF *LIGUSTRUM SINENSE* LEAF EXTRACT ON CHLOROFORM

S. no.	Compounds	Retention time (min)
1.	Cyclohexane	2.68
2.	o-Toluidine	5.95
3.	o-Anisidine	6.27
4.	4-Chloroaniline	6.34
5.	Heptadecane	18.57
6.	Heptadecane, 9-hexyl	20.27
7.	Tetratriacontane	30.65
8.	Tetratriacontane	31.28

TLC (Thin Layer Chromatography): The leaf extract of *Ligustrum sinense* with ethyl acetate and chloroform solvent were used for thin layer chromatographic analysis. It showed three spots with Rf values ranging from 0.45 - 0.53. It shown in the **Fig. 4** and **Table 8**. The chromatogram was developed in a previously saturated ADC2-TLC chamber using the mixture of 1-butanol, 2-propanol and boric acid (5 mg/mL), 30:50:10 (*v*/*v*/*v*), as a mobile phase [30].

FIG. 4: *LIGUSTRUM SINENSE* LEAF EXTRACT ON THIN LAYER CHROMATOGRAPHY (TLC) PLATE OF VARIOUS MOBILE PHASE

TABLE 8: THE RF VALUES OF *LIGUSTRUM SINENSE* LEAF EXTRACT ON VARIOUS MOBILE PHASE

Sample	Rf value
1	0.78
2	0.84
3	0.45
4	0.53
5	0.63
6	0.53
7	0.68
8	0.53

Summary: *Ligustrum sinense* plant was collected. The leaves were dried and powdered. Then the crude extract of leaves was prepared using methanol and ethyl acetate and filtered. It was then purified and analysed in TLC and GC MS. This leaf extract was treated for phytochemical screening such as qualitative and quantitative analysis. The antibacterial activity of *Ligustrum sinense* leaf extract was done against *Staphylococcus aureus, Bacillus* and *Escherichia coli*. The *Ligustrum sinense* leaf extract showed maximum inhibitory activity on those pathogenic microorganisms.

CONCLUSION: Plants are valuable sources of potentially bioactive components for the creation of novel chemotherapeutic drugs. The nutritional profile and phytochemical screening are the first step towards achieving this goal. Phytochemicals, which are typically present in plants. Phytochemicals are plant compounds with non-nutritive, disease protective or disease preventive characteristics. Antioxidants, control of detoxifying enzymes, reduces inflammation, control of steroid metabolism, antibacterial and anti-viral activities in humans are just a few of the complementary and overlapping actions that phytochemicals can have. According to my research, *Ligustrum sinense* has a variety of chemical elements in its methanolic extract, including alkaloids, glycosides, terpenoids, flavonoids, saponins, and phenols. Alkaloids are also employed in medicine to lower fever and headache symptoms. They are said to have analgesic and antibacterial properties. Terpenoids have therapeutic benefits for anticancer effects, antiparasitic effects, antimicrobial effects, antiallergic effects, antihyperglycemic effects, anti-inflammatory effects and an immunomodulatory activity. As consumers shift to functional foods with specific health benefits, they discovered that an edible member of the Oleaceae family have potent antioxidant activity. Thus, the food manufacturing industries brought the attention in the usage of phenolic compounds. They make up one of the main classes of substances that operate as main antioxidants or minor radical terminators. Glycosides are inherently cardioactive and are used to diagnose and treat congestive heart failure.

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