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# Phytochemical investigation of the leaves of *Gliricidia sepium* and its antimicrobial properties

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**Abstract**
*Gliricidia sepium* is a leguminous tree and belongs to the family *Fabaceae*. It is a medium-sized tree and can grow to from 10 to 12 meters high used in many tropical and sub-tropical countries for various purposes such as live fencing, fodder, coffee shade, firewood, green manure and rat poison. The leaves of the plant were under investigation. The leaves of the plant was collected and extracted using petroleum ether, ethyl acetate and ethanol solvent. The antibacterial screening of these extracts was carried out by disc diffusion method. The extract was tested against four pathogenic bacterial stains of both *gram positive* and *gram negative* organism. The ethyl acetate extract of *Gliricidia sepium* shows high antimicrobial activity.

**Keywords:** *Gliricidia sepium*, antimicrobial activity, *gram positive* and *gram negative* bacteria, Disc diffusion method

## Introduction
*Gliricidia sepium* is a leguminous tree and belongs to the family *Fabaceae.* The tree is used in many tropical and sub-tropical countries for various purposes such as live fencing, fodder, coffee shade, firewood, green manure and rat poison. *Gliricidia sepium* is a medium-sized tree and can grow to from 10 to 12 meters high. The bark is smooth and its color can range from a whitish gray to deep red-brown. It has composite leaves that can be 30 cm long. Each leaf is composed of leaflets that are about 2 to 7 cm long and 1 to 3 cm wide. The flowers are located on the end of branches that have no leaves. These flowers have a bright pink to lilac color that is tinged with white. *Gliricidia sepium* (Jacq.) Steud (Leguminosae) is a tropical, multipurpose, fast-growing legume that has been recommended for use in reclaiming derelict ecosystems and in agroforestry [1]. Toxicity well known in cases, where the leaves or the ground bark, mixed with cooked maize are used as a rodenticide. There are many compounds in *Gliricidia sepium*. Major compounds of the leaf oil are found to be propylene glycol, coumarin, hydroquinone, myrtenol etc. The most researched one are the tannins. *Gliricidia* was found to contain 40.7g of condensed tannins/kg dry matter. The insecticidal efficacy of the legume against the late third instar larvae of *Anopheles stephansi*, *Aedes aegypti*, and *Culex quiquefasciatus* were reported by Nirmal S and *et al* [2].
*Gliricidia*, which literally means "Ratpoison" originated in central America and its plantation, has spread to many parts of the world specifically South Asia. The plant is used for fuel wood, animal feed, green manure, shade, living fences and as support plants [3]. The leaves can be used as fodder for cattle that improves their health and increases milk yield. It is an ideal for fattening cattle fold, the valuable feed resources improve the weight. Nematicidal property of extract was observed in different concentration, mosquito's repellent activity has been studied against *Aedes aegypti* and the antibacterial activity of ethanol extract against *E. coli*, *S.aureus*, *Pseudomonas* spp., *S.typhi*, *Klebsillia* spp were reported by Rahila N and *et al*. [4].
Plant material have been shown to posses potential for development as new antimicrobial agents and they may have the advantage over conventional type in terms of low mammalian toxicity, rapid degradation and local availability. Hence the chemical compounds present in them are likely to be biologically active even at lower concentrations. No similar work had been reported on the antimicrobial activity in the petroleum ether, ethyl acetate and ethanol extracts of the leaves of *Gliricidia sepium* collected from Thrissur district, Kerala, India. The present work reports the antibacterial activity of *Gliricidia sepium* in the petroleum ether, ethyl acetate and ethanol extracts of the leaves of *Gliricidia sepium*.

## Materials and Methods

### Plant material

Leaves of *Gliricidia sepium* plant was collected from different areas of Thrissur district. The leaves of plant were collected in the middle of January 2017. Leaves of the plant were collected were shade dried. After drying the plant materials were powdered. Powders were used for extraction in petroleum ether, ethyl acetate and ethanol. Fifty gram of powder was used for extraction.

### Method of extraction

**Extraction:** The leaves collected were shade dried. Coarsely powdered 50g of the plant material was extracted with 500 ml of petroleum ether, ethyl acetate and ethanol. The extraction was carried out in a round bottom flask by boiling the material in the solvent with a water condenser. Refluxed the material until the solvent started to boil and the hot content was left standing overnight. Then filtered and collected the extract and added fresh solvent to residue. The process is repeated three times to complete the extraction. The combined extract collected was reduced to 20 ml.

**Identification:** Thin-layer chromatography is conducted for the ethyl acetate fraction.IR spectra (KBr) were taken on a JASCO FT-IR spectrometer. GC-MS analysis of this extract was conducted to identify the components present in it. GC/MS analyses were carried out using a Perkin Elmer Clarus 500 GC equipped with a Clarus 500 mass spectrometer, capillary column (0.32µm film thickness). 1µL of each sample was diluted with 300µL of $Et_2O$ and injected (0.5µL) in the "split" mode (1:30) with a column temperature program of 40 °C for 5 min, then increased to 250 °C at 4 °C/min and finally held at this temperature for 10 min. Injector and detector were set at 150 and 270 °C, respectively, and the carrier gas was He with a head pressure of 12.0 psi. Mass spectra were acquired over 40-500amu range at 1scan/sec with ionizing electron energy 70eV, ion source 230 $^oC$. The transfer line was set at 250 $^oC$, while the carrier gas was He at 1.0mL/min.

### Biological activity

The antibacterial screening of the extract was carried out by determining the zone of inhibition using standard method [4]. The extract was tested against four pathogenic bacterial strains of gram positive and gram negative organism by disc diffusion method [5]. The test microorganisms of *gram positive* bacteria: *Staphylococcus aureus, Bacillus subtilis, Streptococcus faecalis, Staphylococcus albus*. *gram negative* bacteria: *Escherichia coli, Pseudomonas aeruginosa, Protieus vulgaris, Klebsiella aerogenes*. Previously prepared paper discs were dispensed onto the surface of the inoculated agar plate. Each disc was pressed down firmly to ensure complete contact with the agar surface. The discs were placed on the medium suitably apart and the plates were incubated at 5 °C for 1h to permit good diffusion and then transferred to incubator at 37 °C for 24h. After completion of 24h, the plates were inverted and placed in an incubator set to 37 °C for 24h.

## Results and Discussion

The activity of each compound against the microorganism under study can be concluded from their respective zone of inhibition diameter which is given in Table 1. It was observed that all the extract exhibit biological activity, hampering the growth of one or the other organism. Table 2 shows the minimal inhibitory concentration.

GC-MS analysis of *Gliricidia sepium* report shows that it contains coumarins, propylene glycol, hydroquinone in major percentage. The higher antimicrobial activity in ethyl acetate extract may be due to the presence of compounds present in it or due to the synergistic effect of the major and minor components. Usually the major components are responsible for the antimicrobial activity of plant extract, but the minor components also play major role making the whole extract more active than the combination of major components in synergism.

**Table 1:** Antimicrobial screening of extract

| Sl. No. | Test organisms | Diameter of zone of inhibition (mm) at different concentrations | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Petroleum ether extract | | | Ethyl acetate | | | Ethanol | | | STD |
| | **Gram +ve bacteria** | **1µ g/l** | **2.5 µg/l** | **5µ g/l** | **1µ g/l** | **2.5 µg/l** | **5 µg/l** | **1µ g/l** | **2.5 µg/l** | **5 µg/l** | **2µg/disc** |
| 1 | *Staphylococcus aureus* | 09 | 10 | 11 | 15 | 16 | 17 | 09 | 10 | 11 | 20 |
| 2 | *Bacillus subtilis* | 10 | 11 | 12 | 14 | 15 | 16 | 11 | 11 | 12 | 19 |
| 3 | *Streptococcus faecalis* | 11 | 12 | 13 | 13 | 14 | 15 | 10 | 11 | 12 | 19 |
| 4 | *Staphylococcus albus* | 10 | 09 | 11 | 12 | 13 | 14 | 11 | 12 | 13 | 18 |
| | **Gram -ve bacteria** | **1 µg/l** | **2.5 µg/l** | **5 µg/l** | **1 µg/l** | **2.5 µg/l** | **5 µg/l** | **1 µg/l** | **2.5 µg/l** | **5 µg/l** | **2µg/disc** |
| 1 | *Escherichia coli* | 09 | 10 | 10 | 15 | 16 | 17 | 08 | 10 | 12 | 18 |
| 2 | *Pseudomonas aeruginosa* | 10 | 11 | 11 | 14 | 15 | 17 | 11 | 12 | 14 | 19 |
| 3 | *Klebsiella aerogenes* | 10 | 11 | 12 | 14 | 14 | 15 | 10 | 11 | 12 | 19 |
| 4 | *Protieus vulgaris* | 11 | 12 | 13 | 13 | 15 | 16 | 11 | 12 | 13 | 19 |

Standard (STD) – Ciprofloxacin 2µg/disc

Solvent – DMSO (Shows nil effect against the micro organisms under test)

**Table 2:** Minimal inhibitory concentration

| MIC - Determination : µg/ml | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sl. No. | Tested organism | Diameter of zone of inhibition (mm) at different concentrations | | | | | | | | | | | | | | | |
| | | Petroleum ether extract | | | | | Ethyl acetate extract | | | | | Ethanol extract | | | | | STD |
| | **Gram +ve bacteria** | 800 | 600 | 400 | 200 | 100 | 800 | 600 | 400 | 200 | 100 | 800 | 600 | 400 | 200 | 100 | |
| 1 | *Staphylococcus aureus* | 07 | 07 | 06 | 06 | 06 | 17 | 16 | 15 | 14 | 13 | 12 | 11 | 10 | 10 | 09 | 20 |
| 2 | *Bacillus subtilis* | 07 | 07 | 05 | 05 | 04 | 16 | 15 | 14 | 13 | 12 | 11 | 11 | 10 | 09 | 09 | 19 |
| 3 | *Streptococcus faecalis* | 08 | 07 | 07 | 06 | 06 | 18 | 17 | 16 | 15 | 15 | 14 | 13 | 12 | 11 | 11 | 19 |
| 4 | *Staphylococcus albus* | 07 | 07 | 06 | 06 | 06 | 16 | 15 | 14 | 14 | 13 | 13 | 12 | 11 | 10 | 10 | 18 |
| | Gram –ve bacteria | 800 | 600 | 400 | 200 | 100 | 800 | 600 | 400 | 200 | 100 | 800 | 600 | 400 | 200 | 100 | |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *Escherichia coli* | 07 | 06 | 06 | 05 | 04 | 14 | 13 | 13 | 12 | 11 | 11 | 10 | 09 | 08 | 07 | 18 |
| 2 | *Pseudomonas aeruginosa* | 06 | 05 | 03 | 03 | 03 | 15 | 14 | 13 | 12 | 11 | 10 | 09 | 07 | 07 | 06 | 19 |
| 3 | *Klebsiella aerogenes* | 07 | 05 | 05 | 04 | 04 | 14 | 14 | 13 | 13 | 12 | 09 | 08 | 07 | 06 | 05 | 19 |
| 4 | *Protieus vulgaris* | 08 | 06 | 05 | 05 | 04 | 13 | 13 | 12 | 11 | 10 | 09 | 07 | 06 | 05 | 05 | 19 |

Standard (STD) – Ciprofloxacin 2µg/disc

## Conclusion

The antimicrobial activity in the petroleum ether, ethyl acetate and ethanol extracts of the leaves of *Gliricidia sepium* collected from Thrissur district, Kerala, India. Therefore, leaves extract of *Gliricidia sepium* in ethyl acetate solvent, a potentially useful antimicrobial agent against *gram positive* and *gram negative* bacteria. This antimicrobial activity can be attributed to the compounds present in the extract or due to the synergistic effect of the major and minor components. Usually the major components are responsible for the antimicrobial activity of plant extract, but the minor components also play major role making the whole extract more active than the combination of major components in synergism. Hence it can be used as a potential anti microbial agent.

## References


1. Nair PKR, Fernandese ECM, Wombugn. Multipurpose leguminous trees and shrubs for agroforestry. Agroforestry. 1984; 2:145-163
2. Nirmal S, Qadry JS, Subramanium B, Verghese T, Rahman SJ, Sharma SK, Jalees S. Larvicidal activity of *Gliricidia sepium a*gainst mosquito larvae of *Anopheles stephansi*, *Aedes aegypti* and *Culex quinquefasciatus*. Pharmaceutical Biology. 1998, 36(1):3-7.
3. Csurhes S, Edwards. Potential environmental weeds in Australia; candidate species for preventative control. Queens Land Department of Natural Resources, 1998, 164.
4. Rahila N, Mussarat A, Shagufta A, Abdul Hameed S, Nighat S. Insecticidal, nematicidal and antibacterial activities of *Gliricidia sepium* Pak. J Bot. 2008; 40(6):2625-2629.
5. NCCLS (National Committee for Clinical Laboratory Standards), Performance standards for antimicrobial disc susceptibility tests. Approved. Standard, M2-A7., 2000a.
6. Bayer AW, Kirby MDK, Sherris JC, Trick M. Antibiotic Susceptibility testing by standard single disc diffusion method. Am J Clinical Pathol. 1986; 45:493.