

Comparative Antibacterial activity of *Azadirachta indica* and *Ocimum sanctum* against *Escherichia coli* and *Staphylococcus aureus*

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Abstract – Medicinal plants have attracted significant attention for their healing and antimicrobial properties. This study aimed to compare the antibacterial activity of aqueous and ethanolic extracts of *Azadirachta indica* (Neem) and *Ocimum sanctum* (Tulsi) against *Escherichia coli* and *Staphylococcus aureus*. Fresh leaf samples of Neem and Tulsi were collected from a local village market (Kanis Hatto) and tested in the Department of Biotechnology Lab at Khallikote Unitary University, Brahmapur, Odisha, India. The antibacterial activity was assessed using a disc diffusion test and Minimum Inhibitory Concentration (MIC) analysis. Additionally, Gas-Liquid Chromatography (GLC) analysis was performed to identify the key bioactive compounds responsible for these antibacterial effects.

The results demonstrated that both Neem and Tulsi extracts possessed significant antibacterial activity against the tested bacterial strains. Ethanolic extracts were more effective against *E. coli*, whereas aqueous extracts exhibited better efficacy against *S. aureus*. Among the samples, Tulsi extracts generally showed stronger antibacterial potential than Neem in the zone of inhibition tests. However, MIC analysis indicated that bacterial growth inhibition was concentration-dependent, with Neem extracts demonstrating slightly better effectiveness in specific cases. GLC analysis confirmed the presence of eugenol in Tulsi and azadirachtin in Neem, which likely contribute to their antimicrobial properties. This study suggests that both medicinal plants hold promising antibacterial potential and may provide natural alternatives for managing bacterial infections.

Key Words: *Azadirachta indica*, *Ocimum sanctum*, Antibacterial activity, *Escherichia coli*, *Staphylococcus aureus*, MIC

1. INTRODUCTION

The usage of medicinal plant in traditional medicine has been described in literature dating back several thousand of years. The use of medicinal plants in traditional medicine has been described in literature dating back several (Chang, J. D., et al., 2016). In modern complementary and alternative medical practice, plants are the primary source of therapeutics and each part of the plant, including the seeds, root, stem, leaves, and fruit, potentially contains bioactive components (Jiang, W., et al., 2014; Jiang, B., et al., 2015; Mandave, P., et al., 2014; Sun, X., et al., 2014). Plants have many biologically active compounds which have latent for development as medicinal agents. Herbal medicines already form the basis of beneficial use in the developing countries, but of recent, there has been rise in the use of herbal medicines in the developed world (Moga, M. A., et al., 2018). Plants provide an alternate approach in search for new treatments. There is a plenty of

plants reputed in traditional medicine to hold protective and therapeutic properties (Alzohairy M.A., 2016).

Azadirachta indica A. Juss belong to family Meliaceae is an evergreen tree commonly found in the Indian subcontinent, which is famous by the local name 'Neem', it has been considered to provide freedom from all diseases, and used for thousands of years in Indian and African continents. Different parts of the plant including flowers, leaves, seeds and bark have been used to treat both acute and chronic human diseases, and used as an insecticide, antimicrobial, larvicidal, anti-malarial, antibacterial, antiviral, and spermicidal Gupta, S.C., et al., 2017). Neem tree contains large number of biologically active compounds that are diverse and complex. More than 140 compounds including alkaloids, flavonoids, triterpenoids, carotenoids and nimbin have been isolated from different parts of the plant (Ganguli, S., 2002; Subapriya and R, Nagini, S., 2005). Azadirachtin is the most important limonoid or teranotriterpenoid. Nimbidin is the crude principle that is extracted from the seed kernels of neem and from this crude base, some more teranotriterpenes like nimbin, nimbinin, nimbidinin, nimbolide, and nimbidic acid have been reported to be isolated (Kudom, A. A., et al., 2011).

Tulsi "Queen of herbs" is described as sacred and medicinal plant in ancient literature. It is an important symbol of the Hindu religious tradition. The name Tulsi is derived from "Sanskrit", which means "matchless one" (Ghosh, G.R., 1995). *Ocimum sanctum* L. belongs to family Lamiaceae has two varieties i.e. black (Krishna Tulsi) and green (Rama Tulsi), their chemical constituents are similar. Both the varieties also have common medicinal properties.

According to Das, S.K. and Vasudevan, D.M., (2006), Fresh leaves and stems of *Ocimum tenuiflorum* L. or *Ocimum sanctum* L. extract yielded some phenolic compounds (antioxidants) such as cirsilineol, circimaritin, isothymusin, apigenin and rosmarinic acid and appreciable quantities of eugenol (Uma Devi P., 2001). Tulsi is also known as "the elixir of life" since it promotes longevity. Chewing of Tulsi leaves also cures ulcers and infections of mouth (Prajapati, N.D., 2003).

The present study was designed to comparatively evaluate the antibacterial activity of aqueous and ethanolic extracts of *Azadirachta indica* and *Ocimum sanctum* against *Escherichia coli* and *Staphylococcus aureus* using disc diffusion assay and Minimum Inhibitory Concentration (MIC) analysis. The study also aimed to compare the effectiveness of major phytochemicals constituents such as eugenol and azadirachtin in relation to their antibacterial potential. Hence the main objectives of the study were as follows.

- 1) To comparatively evaluate the antibacterial activity of aqueous and ethanolic extracts of *Ocimum sanctum* and *Azadirachta indica* against *Escherichia coli* and *Staphylococcus aureus* using disc diffusion assay and MIC analysis.
- 2) To analyze the influence of extraction solvents on the antibacterial efficacy of Neem and Tulsi extracts and compare their inhibitory potential against Gram-positive and Gram-negative bacteria.
- 3) To identify and compare the major bioactive phytochemicals constituents responsible for antibacterial activity through Gas Liquid Chromatography (GLC) analysis.

2. MATERIAL AND METHOD

Collection of Plant Materials

Fresh leaves of *Azadirachta indica* (Neem) and *Ocimum sanctum* (Tulsi) were procured from local village market (Kanisi Hatto), Berhampur, Odisha. The plant materials were cleaned, shade dried, and ground into fine powder using a mechanical grinder.

Preparation of Plant Extracts

Aqueous and ethanolic extracts of Neem and Tulsi were prepared using solvent extraction method. About 10g of powdered plant material was mixed with 100mL of distilled water and ethanol separately in a ratio of 1:10 (w/v). The mixtures were kept under continuous shaking for 72 hours at room temperature. The extracts were filtered using Whatman No.1 filter paper and concentrated using rotary evaporation. The concentrated extracts were stored at 4°C until further experimental analysis.

Test Microorganisms

The bacterial strains used in the present study were *Escherichia coli* (MTCC-443) and *Staphylococcus aureus* (MTCC-1430). Pure cultures were obtained from the Regional Institute of Biotechnology (RIB), Brahmapur, and subcultures onto solidified nutrient agar medium using a sterile inoculation loop and incubated at optimum temperature for growth. The cultures were subcultures every 2–3 days to maintain sterility and active growth.

Preparation of Culture Media

Nutrient agar and nutrient broth media were prepared and sterilized by autoclaving at 121°C for 15 minutes. The sterilized media were cooled and poured aseptically into sterile Petri plates for antibacterial assay.

Preparation of Bacteria Inoculum

Fresh bacterial inoculum was prepared by inoculating bacterial colonies into sterile nutrient broth followed by incubation at 37°C for 18–24 hours. The turbidity of bacterial suspension was adjusted to match McFarland standard for uniform inoculation.

Preparation of Antibacterial Disc

Sterile paper discs were prepared from thick chromatography paper and sterilized using 70% ethanol followed by ultraviolet (UV) irradiation inside a laminar airflow chamber. Uniform circular discs were punched out aseptically using a sterile paper puncher. Crude extracts of Neem and Tulsi were prepared at a stock concentration of 100 mg/ mL. Working concentrations of 10mg/ mL and 5mg /mL were prepared separately for both aqueous and ethanolic extracts using sterile distilled water and ethanol respectively. The sterile paper discs were immersed in the prepared extract solutions for approximately 15 minutes to

ensure proper impregnation. After soaking, the discs were removed aseptically using sterile forceps and dried inside the laminar airflow chamber before use in antibacterial assay.

Antibacterial Activity of Disc Diffusion Method

The antibacterial activity of aqueous and ethanolic extracts of *Ocimum sanctum* and *Azadirachta indica* was evaluated using disc diffusion assay by pour plate technique. Sterile molten nutrient agar medium was inoculated with bacterial cultures and poured aseptically into sterile Petri plates. After solidification of the medium, sterile filter paper discs impregnated with plant extracts were carefully placed on the agar surface using sterile forceps. Standard antibiotic discs were used as positive control, while solvent-treated discs served as negative control. The plates were incubated at 37°C for 24 hours, and antibacterial activity was determined by measuring the diameter of zones of inhibition.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of plant extracts was determined using standard serial broth dilution method. The first test tube contained 10 mL sterile nutrient broth, while subsequent tubes contained 9 mL broth. Stock extract (100mg/ mL) was diluted to prepare 10 mg/ mL working solution. Serial two-fold dilutions were carried out to obtain concentrations of 10mg/ mL, 5 mg/ mL, 2.5mg/ mL, 1.25mg/ mL, 0.625 mg/ mL, and 0.312 mg/ mL. Each tube was inoculated with standardized bacterial suspension and incubated at 37°C for 24 hours. Following incubation, bacterial growth was assessed by measuring optical density (OD) at 600nm using a spectrophotometer. The MIC was considered as the lowest concentration showing no visible growth or significant reduction in turbidity compared to the control.

Gas Liquid Chromatography (GLC) Analysis

Gas Liquid Chromatography (GLC) analysis was performed to identify the major phytochemicals constituents present in Neem and Tulsi extracts. The chromatographic peaks obtained were analyzed to identify major bioactive compounds responsible for antibacterial activity, including eugenol in Tulsi and azadirachtin in Neem.

3. RESULT FINDING

3.1 Zone of Inhibition against *Escherichia Coli*

The comparative study of *Azadirachta indica* A. Juss (Neem) and *Ocimum sanctum* L. (Tulsi) against *E. coli* showed that both plants have strong antibacterial activity. However, *Ocimum sanctum* L. had larger zones of inhibition than *Azadirachta indica* at both concentrations. The ethanolic extracts of both plants demonstrated greater antibacterial activity than the aqueous extracts. This suggests that ethanol is a better solvent for extracting active compounds. Among all the samples tested, the ethanolic extract of *Ocimum sanctum* L. at a concentration of 10 had the largest zone of inhibition at 1.5cm, while the ethanolic extract of *Azadirachta indica* measured 1.4 cm. The results also showed that higher concentrations increased antibacterial activity. Therefore, the study indicates that Tulsi has stronger antibacterial potential against *E. coli* compared to Neem under the tested conditions.

Table 1: Zone of Inhibition of aqueous extracts of Neem and Tulsi against *Escherichia coli*

Concentration	Tulsi Extract (Aqueous)	Neem extract (Aqueous)
10mg	14mm	8mm
5mg	12mm	7mm
Vancomycin	13mm	14mm

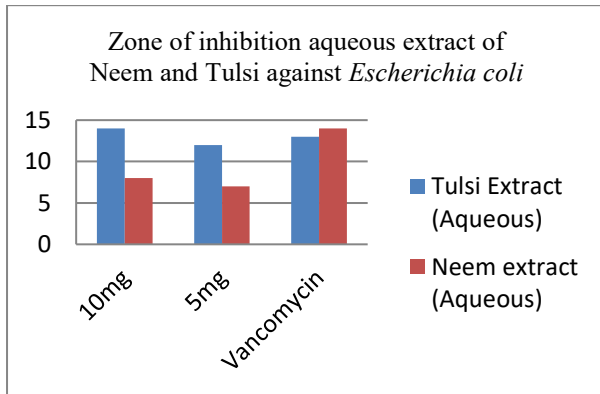


Table 2: Zone of Inhibition of ethanolic extracts of Neem and Tulsi against *Escherichia coli*

Concentration	Tulsi extract (Ethanol)	Neem extract (Ethanol)
10mg	14mm	14mm
5mg	14mm	11mm
Vancomycin	13mm	12mm

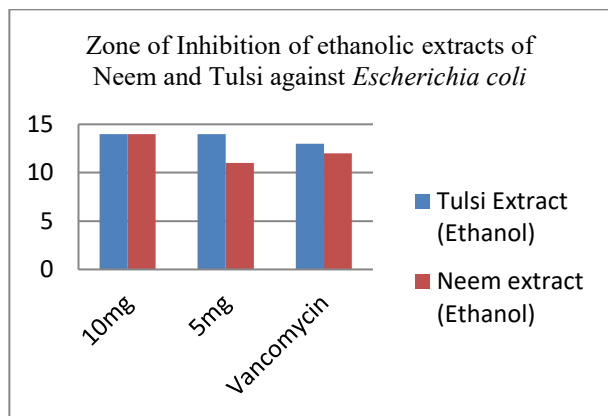


Fig.1



Fig. 2



Fig.3



Fig. 4

Fig.1: Zone of Inhibition of aqueous extract of Neem against *Escherichia coli*

Fig.2: Zone of Inhibition of aqueous extract of Tulsi against *Escherichia coli*

Fig.3: Zone of Inhibition of ethanolic extract of Neem against *Escherichia coli*

Fig.4: Zone of Inhibition of ethanolic extract of Tulsi against *Escherichia coli*

3.2 Zone of Inhibition against *Staphylococcus aureus*

The study comparing *Ocimum sanctum* L. (Tulsi) and *Azadirachta indica* (Neem) against *Staphylococcus aureus* found that both plant extracts have antibacterial properties. Tulsi had slightly better inhibition than Neem. In the aqueous extracts, Tulsi showed inhibition zones of 1.2cm and 1.1cm at concentrations of 10mg and 5mg, respectively. In contrast, Neem showed 1.1cm and 1.0cm at the same concentrations. For the ethanolic extracts, Tulsi had inhibition zones of 0.8cm and 0.5cm, while Neem showed 0.8cm and 0.6cm at 10mg and 5mg, respectively. The standard antibiotic Vancomycin had the highest antibacterial activity in both tests, with inhibition zones of 1.4cm and 1.3 cm. Overall, the aqueous extracts were more effective than the ethanolic extracts against *S. aureus*, and Tulsi demonstrated slightly stronger antibacterial potential than Neem under the tested conditions.

Table 3: Zone of Inhibition of aqueous extracts of Neem and Tulsi against *Staphylococcus aureus*

Concentration	Tulsi Extract (Aqueous)	Neem extract (Aqueous)
10mg	12mm	11mm
5mg	11mm	10mm
Vancomycin	14mm	14mm

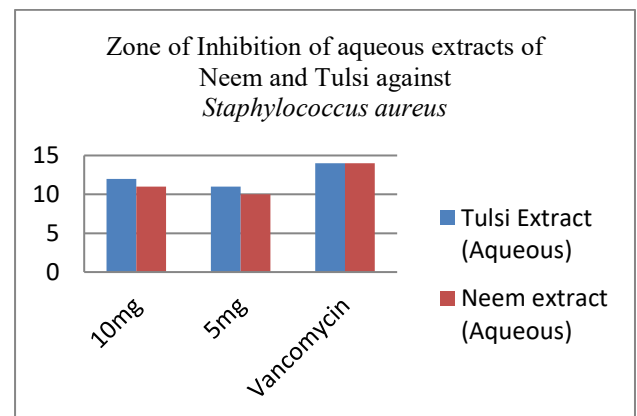


Table 4: Zone of Inhibition of ethanolic extracts of Neem and Tulsi against *Staphylococcus aureus*

Concentration	Tulsi extract (Ethanol)	Neem extract (Ethanol)
10mg	8mm	8mm
5mg	5mm	6mm
Vancomycin	13mm	13mm

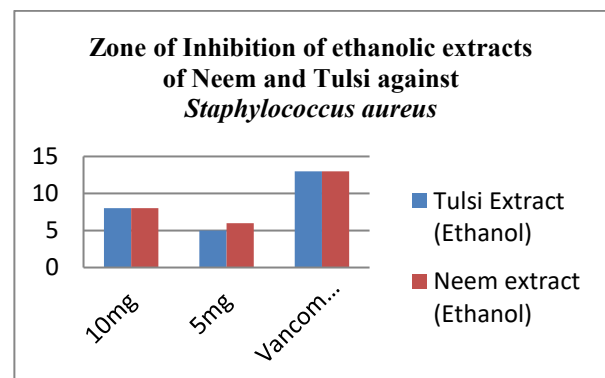




Fig.5



Fig. 6



Fig.7



Fig. 8

Fig 5: Zone of Inhibition of aqueous extract of Neem against *Staphylococcus aureus*

Fig 6: Zone of Inhibition of aqueous extract of Tulsi against *Staphylococcus aureus*

Fig 7: Zone of inhibition of ethanolic extract of Neem against *Staphylococcus aureus*

Fig 8: Zone of Inhibition of ethanolic extract of Tulsi against *Staphylococcus aureus*

A comparison of the antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* showed differences in how effective Tulsi and Neem extracts were. For *Escherichia coli*, the ethanolic extracts had stronger antibacterial activity, with both Tulsi and Neem reaching a maximum zone of inhibition of 14 mm at a 10 mg concentration. However, for *Staphylococcus aureus*, the aqueous extracts showed better activity. Tulsi and Neem had inhibition zones of 12mm and 11 mm, respectively, at the same 10 mg concentration. Tulsi generally showed stronger antibacterial potential than Neem for both bacterial strains. Higher concentrations of the extracts resulted in larger zones of inhibition in all cases, which indicates that antibacterial activity depended on concentration. Overall, the study suggests that how effective the extracts are varies depending on the bacterial species and the solvent used for extraction.

3.3 Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) analysis demonstrated that ethanolic extracts of both Neem and Tulsi exhibited greater bacterial growth inhibition compared to aqueous extracts. Optical density (OD) values measured at 600nm showed reduced bacterial growth with increasing extract concentration. Neem extracts exhibited lower optical density values slightly lower than Tulsi extracts against both *Escherichia coli* and *Staphylococcus aureus*, indicating comparatively stronger antibacterial efficacy. The inhibitory effect was concentration dependent, with higher extract concentrations showing greater bacterial growth suppression.

Table 5: MIC of aqueous extracts of Tulsi and Neem on *Escherichia coli*

Concentration	Tulsi Extract (Aqueous)	Neem extract (Aqueous)
10mg	0.51	0.69
5mg	0.59	0.72
2.5mg	0.63	0.84
1.25mg	0.67	0.86
0.62mg	0.84	0.93
0.31mg	0.87	0.95

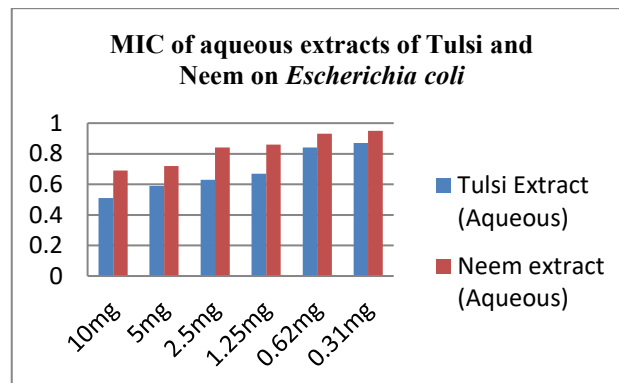


Table 6: MIC of ethanolic extracts of Tulsi and Neem on *Escherichia coli*

Concentration	Tulsi extract (Ethanol)	Neem extract (Ethanol)
10mg	0.56	0.76
5mg	0.58	0.78
2.5mg	0.62	0.81
1.25mg	0.64	0.83
0.62mg	0.71	0.87
0.31mg	0.84	0.92

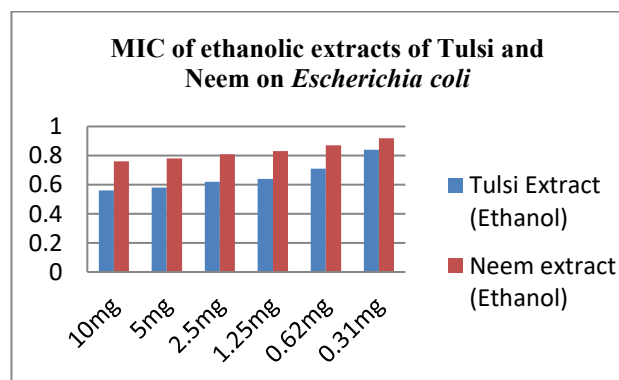


Table 7: MIC of aqueous extracts of Tulsi and Neem on *Staphylococcus aureus*

Concentration	Tulsi Extract (Aqueous)	Neem extract (Aqueous)
10mg	0.73	0.59
5mg	0.81	0.61
2.5mg	0.84	0.72
1.25mg	0.86	0.78
0.62mg	0.90	0.90
0.31mg	0.92	0.92

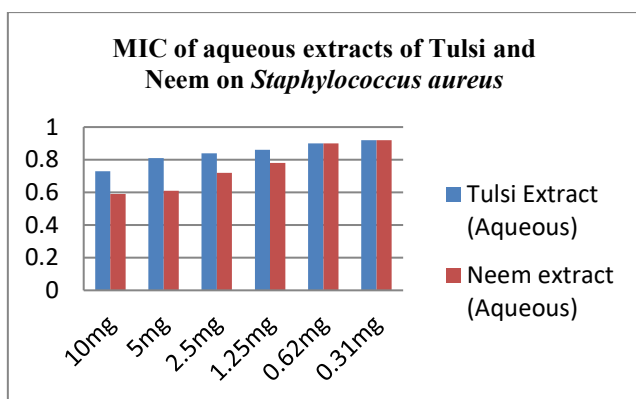
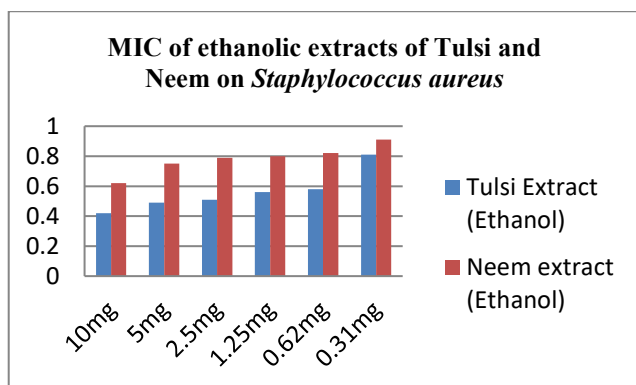


Table 8: MIC of ethanolic extracts of Tulsi and Neem on *Staphylococcus aureus*

Concentration	Tulsi extract (Ethanol)	Neem extract (Ethanol)
10mg	0.42	0.62
5mg	0.49	0.75
2.5mg	0.51	0.79
1.25mg	0.56	0.80
0.62mg	0.58	0.82
0.31mg	0.81	0.91



3.4 Gas Liquid Chromatography (GLC) Analysis

Gas Liquid Chromatography (GLC) analysis confirmed the presence of major phytochemicals constituents in both medicinal plants. Eugenol was identified as the predominant bioactive compound in Tulsi extract, whereas azadirachtin was identified as the major compound present in Neem extract. The chromatographic analysis suggested that the antibacterial activity observed in the present study may be associated with these bioactive phytochemicals constituents. The higher antibacterial activity of Neem extracts compared to Tulsi extracts may be attributed to the comparatively stronger antimicrobial action of azadirachtin.

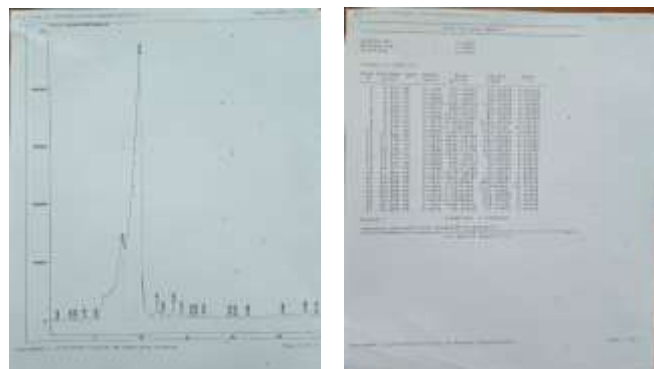
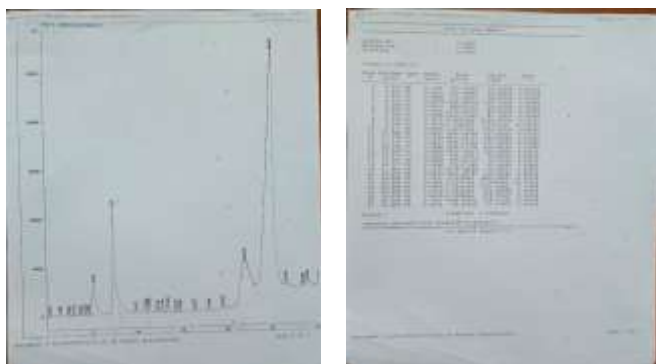


Fig. 10: GLC Analysis of Neem extract

4. DISCUSSION

The use of medicinal plants in traditional medicine has been described in literature dating back several thousand of years (Chang, J. D., et al., 2016). Plant-derived antimicrobial compounds have gained considerable scientific interest because of their natural origin and broad-spectrum biological activities (Cowan, M. M., 1999). Several researchers have long demonstrated the antimicrobial properties of neem extract. Aqueous and alcoholic extracts of the plant parts were subjected to different assays to understand their activity against bacteria, (Khan, M., and Wassilew, S.W., 1987) documented the antibacterial activity of the extracts of the leaves, seeds, and bark of neem. The extracts exhibited inhibition against a broad spectrum of gram-negative and gram-positive microorganisms.

Reports of nimbolide showing effectiveness against *Staphylococcus aureus* and *S. coagulase* have been given by Schmutterer, H., and Singh, R. P., (1995); Govindachari, T. R., et al., (1998) first reported about the polyphenolic flavonoids - Quercetin and β - sitosterol, which were purified and isolated from fresh neem leaf extract and known to have antifungal and antibacterial properties.

Biologically active principles isolated from different parts of the plant include: azadirachtin, meliacin, gedunin, salanin, nimbin, valassin, and many other derivatives of neem seed oil (Iyevhobu K.O., et al., 2022). Active components play role in cure of diseases through activation of antioxidants enzymes, by breaking the cell wall of bacteria and act as chemo preventive through regulating cellular pathways (Kumar, P.S., et al., 2010).

Ocimum sanctum, Tulsi, or Holy Basil from the family Lamiaceae has been described as the "Queen of plants" and the "mother medicine of nature" due to its perceived medicinal qualities. It has been one of the most valued and holistic herbs used over years in traditional medicine in India and almost every part of the plant have been found to possess therapeutic properties (Singh V., et al., 2010).

Tulsi consists highly complex chemical composition, containing many nutrients and biologically active compounds. Due to the botanical nature and inherent biochemical complexity, the standardization of the active component of Tulsi is very complicated. The best-known active component of Tulsi leaves the source of essential oil, i.e., eugenol and ursolic acid. The major constituents identified and extracted from Tulsi are none other than the ursolic acid. The different parts of the Tulsi plant give eugenol through the extraction process, and it is a phenolic compound and significant

constituent in the essential oil. The therapeutic potential of the extracted essential oils from the fresh leaves of the *Ocimum sanctum* is mainly due to substantial constituents, i.e. Eugenol (Deepika Gulati, et al., 2015).

The findings of this investigation showed that *Azadirachta indica* and *Ocimum sanctum* have significant antibacterial properties against the tested bacterial pathogens. The evaluation of the extracts revealed clear inhibitory effects, which varied depending on the type of extract and the bacterial strain. We recorded a steady increase in antibacterial activity with higher extract concentrations, indicating a dose-dependent effect. The antimicrobial potential of neem and tulsi may come from various compounds, including azadirachtin, nimbin, and flavonoids in neem, along with eugenol, tannins, and phenolic compounds in tulsi. These results emphasize the promise of both medicinal plants as natural sources of antibacterial agents for healthcare, pharmaceutical formulations, and food preservation. Further studies focusing on isolating active compounds and understanding how they work could help develop new plant-based antimicrobial treatments.

5. CONCLUSIONS

The present study demonstrated that *Azadirachta indica* (Neem) and *Ocimum sanctum* (Tulsi) possess significant antibacterial activity against the tested bacterial pathogens. Antimicrobial efficacy increased with extract concentration, as confirmed by the MIC analysis, indicating a clear dose-dependent inhibitory effect, with Neem showing slightly greater effectiveness in some cases. GLC analysis verified the presence of bioactive compounds, including azadirachtin in Neem and eugenol in Tulsi, which are known to contribute to their antimicrobial properties. Tulsi contains a complex phytochemical profile rich in biologically active constituents such as eugenol, ursolic acid, phenolic compounds, and tannins, while Neem is abundant in azadirachtin, nimbin, flavonoids, and other antimicrobial phytochemicals. The synergistic action of these compounds is likely responsible for the observed antibacterial effects. Overall, the findings highlight the potential of Neem and Tulsi as natural sources of antibacterial agents with promising applications in healthcare, pharmaceutical formulations, and food preservation. Further research aimed at isolating, characterizing, and evaluating the mechanisms of action of their active constituents may facilitate the development of effective plant-based antimicrobial therapies.

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