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PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL SCREENING OF *TERMINALIA CATAPPA* AGAINST TETRACYCLINE RESISTANT *ESCHERICHIA COLI*

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ABSTRACT

Antimicrobial resistance is a global epidemic posturing huge loss to the economy of a nation. In this context, a novel therapeutic regime consisting of antibiotics and plant natural products can be selected as antibacterial resistance modifying agents. This study was undertaken to assess the antibacterial activity of methanolic extracts of *Terminalia catappa* against tetracycline resistant *Escherichia coli*. Phytochemical screening was done to assess the phytochemical constituents in the methanolic plant extract. Antibacterial activity was evaluated by Kirby-Bauer disc diffusion assay. The results indicated that the methanolic extracts of *Terminalia catappa* significantly increased the zone of inhibition of tetracycline in a dose dependent manner against tetracycline resistant *E. coli*. Phytochemical analysis indicated the presence of saponins, tannins, steroids, flavonoids and phenols. The results of the study revealed that combination of phytochemicals with antibiotics can combat the antimicrobial resistance.

Key words- Antimicrobial resistance, *E. coli*, tetracycline, *Terminalia catappa*, Kirby Bauer disc diffusion assay

INTRODUCTION

Plants have been used for medicinal use since ancient times. the use of phytochemicals in medicine was a breakthrough in health science as it can minimise the use of antibiotics. In developed nations, almost 80% of people utilise traditional medicines, which contain substances derived from medicinal plants (Keerthika *et al*., 2022). The presence of plant secondary metabolites also known as phytochemicals attribute4 to the medicinal property of plants. In this era, the rise of antimicrobial resistance (AMR) is posing a serious threat to both human and animal health sector. The successful therapy

using antibiotics against bacteria, virus, fungi etc. is now fruitless due to emerging AMR (Nisha *et al*., 2021). However, the development of a novel compound is indispensable to address this issue. In a way, plant natural products can be used as a viable candidate in combating AMR. *Escherichia coli*, a Gram negative bacteria belonging to enterobacteriaceae family is a common clinical pathogen. The bacteria can be isolated from various clinical samples from animals and humans. The emergence of resistant *E. coli* failed the antibiotic therapy used against them (Arya *et al*., 2020).

Terminalia catappa L., also known as Indian almond is a large monoecious tree with large leaves having many medicinal properties. The extracts of its leaves are reported to have anti-inflammatory, anticancer, hepatoprotective activity, antipyretic, hemostatic, also the leaves are food for tussar silk worms (Krishnaveni *et al*., 2015; Allyn *et al*., 2018). Hence this study was designed to assess the phytochemical analysis and antibacterial screening of *T. catappa* against tetracycline resistant *E. coli*.

MATERIALS AND METHODS

Plant Material

T. catappa L. leaves were collected from the premises of College of Veterinary and Animal Sciences, Mannuthy, Thrissur.

Extraction of Leaves

The leaves of *T. catappa* L. were shade dried and powdered in a pulverizer. About 100gm of the powdered leaves was fed into a soxhlet apparatus using methanol as solvent and ran for about a week. The obtained sample was extracted from solvent by keeping it in a rotary vacuum evaporator overnight. The filtrate was collected and stored in a sealed container at 4°C.

Screening for Phytochemical Constituents

The obtained extract was subjected to both qualitative and quantitative phytochemical analysis. The qualitative phytochemical analysis assessed the presence of various constituents like flavonoids, alkaloids, phenols, steroids, terpenoids, saponins, tannins, etc. using standard procedures as described by Harborne *et al*. (1993). Salkowski's test, Libermann-Bruchardt Reaction, test for Alkaloids, Dragendroff's test, Wagner's test, Mayer's test, Hager's test, Test for Glycosides, Sodium Hydroxide test for Phenols, Ferric Chloride test, Test for Tannins, Ferric Chloride test, Gelatin test, test for Flavonoids, Ferric Chloride test, Lead Acetate test, test for Diterpenes, test for Triterpenes, Salkowski's test,

Libermann-Bruchardt Reaction, test for Saponins and Froth test were done for phytochemical analysis

Gas Chromatography and Mass Spectrometry

The quantitative phytochemical analysis of methanolic extract of leaves of T. *catappa was* analysed using GC-MS system of Centre for Analytical Instrumentation-Kerala (CAI-K), Kerala Forest Research Institute (KFRI), Peechi, Kerala. Gas chromatography Mass Spectrometer (Shimadzu GC-MS, Japan, QP2010S) with a mass range of 1.5- 1000 m/z was used. Helium was used as the carrier gas at flow rate of 1 mL/ min. The oven temperature was maintained at 80 0C for 4 min and then increased to 280 0C in 6 min. The injector temperature was 260 0C and total analysis time was 50 min. Aliquots of extracts (0.4 µL) were injected into the chromatographic column after a clear baseline was obtained. Major constituents were identified using mass spectrum library (NIST 11 and WILEY 8).

Screening of antibacterial activity

Clinically important *E. coli* isolates were procured from Teaching Veterinary Clinical Complex, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala. The isolates were grown in the nutrient broth and maintained on Brain Heart Infusion (BHI) agar slants at 4°C.

Antibacterial Assay

The antibiotic susceptibility testing was carried out for the isolates of *E. coli* by Kirby Bauer disc diffusion assay. The bacterial culture was prepared by growing the bacteria in Mueller Hinton (MH) broth (M/s Hi media labs, Mumbai, India). A loopful of culture was taken from the primary culture plate and added to the broth which was incubated at 37°C for 3-4 hours. Then the culture was adjusted to 0.5 McFarland standards and was evenly spread on MH agar using sterile cotton swab. Antibiotic discs of tetracycline (30 mcg) were placed on MH agar plates with gentle pressure. The test compound i.e. methanolic leaf extract of *T. catappa* L. alone and various concentrations viz. 80µg/ml, 40µg/ml, 20µg/ml, 10µg/ml and 5µg/ml along with tetracycline was impregnated into sterile discs (6mm Hi-Media) and was allowed to dry. Methanolic leaf extract of *T. catappa* L. and antibiotic combination was prepared by dissolving methanolic extract in 5% DMSO and 20 µL of the extract were added on the antibiotic disc at above said concentrations. The plates were incubated at 37°C for 18-20 hours. The diameter of zones of inhibition was measured for each treatment.

RESULTS

Phytochemical screening

The results of phytochemical analysis revealed the presence of tannins, saponions and alkaloids in the methanolic extract of leaves of *T. catappa*. The results of phytochemical analysis are given in Table 1 and figure 1.

Test	Methanolic extract of *T. catappa*
Steroids	+
Alkaloids	-
Glycosides	+
Phenols	+
Tannins	+
Flavonoids	+
Terpenes	-
Saponins	+

Gas Chromatography Mass Spectrometry

The results of GC-MS are given in table 2. The chromatogram is given in Figure 2.

Antimicrobial Screening

The results of Kirby Bauer disc diffusion assay revealed that *T. catappa* alone have no antimicrobial activity against tetracycline resistant *E. coli*. But when various concentrations of extract used in combination with tetracycline, the zone of inhibition was found to increase in a dose dependent manner. The maximum zone of inhibition was at the higher dose of 80µg/ml (20.33±0.33), which could overcome the tetracycline mediated resistance. The zone of inhibition of extracts at various

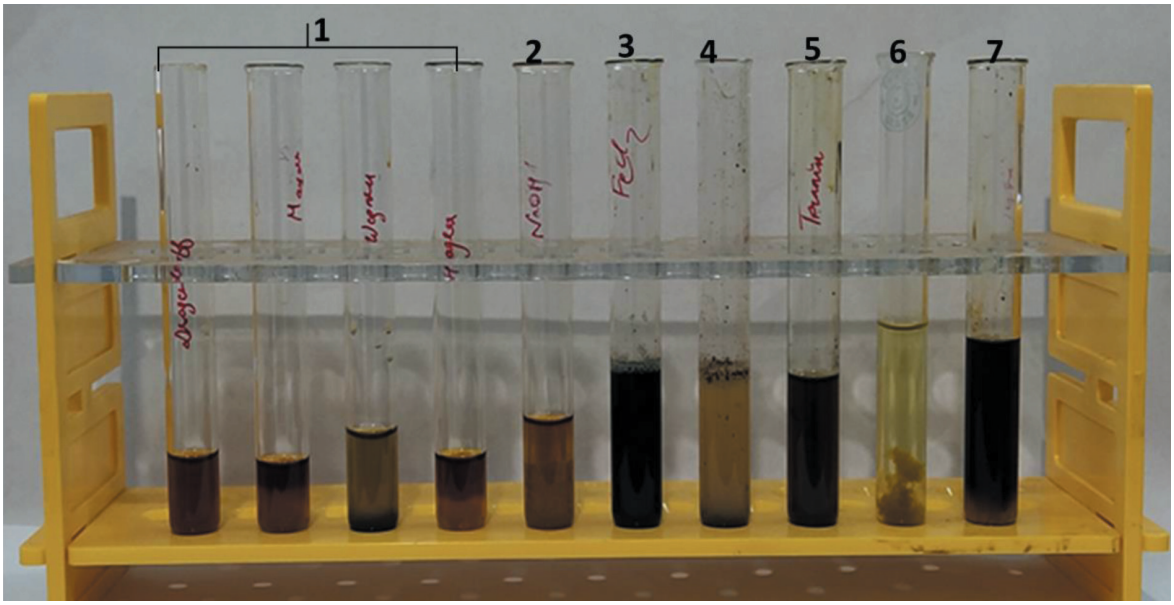

Figure 1. Showing the results of qualitative phytochemical screening of methanolic extract of *T. catappa* 1- Alkaloid test, 2- test for glycosides, 3- Test for flavonoids, 4- Test for steroids, 5- test for tannins, 6-, 7- Test for phenol

Table 2. Results of GC MS analysis

Name of compound	Retention time
Neophytadiene	26.551
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	27.423
Methylpalmitate	28.339
Dibutyl phthalate	29.181
Linolenic acid, Methyl Ester	31.674
Phytol	31.886
Squalene	43.125
Lupeol	43.564
Vitamin E	48.209

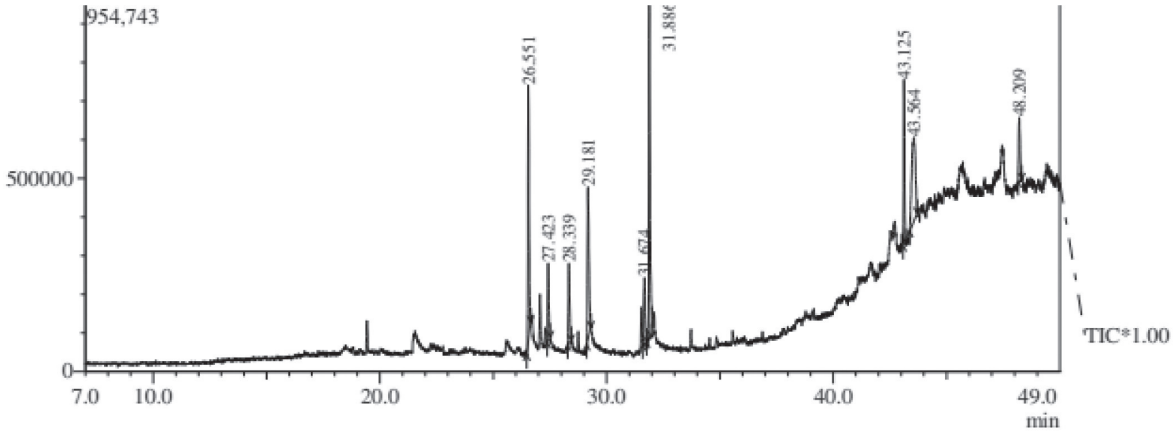

Fig 2. Graph showing GCMS analysis results

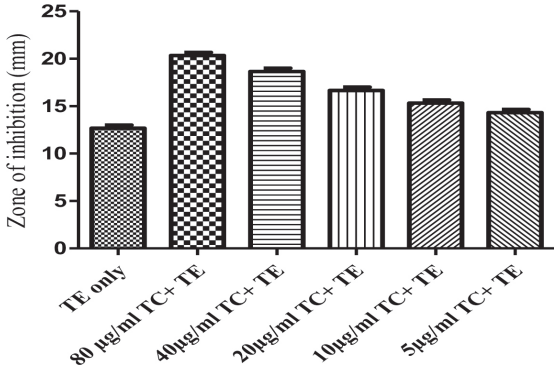

Figure 3. Graph showing the results of Kirby Bauer Disc Diffusion Assay

concentration in combination is given in table 3

Table 3. Zone of inhibition (mm) for various concentration of methanolic extract of T. catappa in combination with tetracycline. The values are expressed as Mean± SE. n=6, r=3

Concentration	Zone of inhibition Mean± SE (mm)
80µg/ml TC + TE	20.33^{a}±0.33
40µg/ml TC+ TE	18.67^{a}±0.33
20µg/ml TC+ TE	16.67^{b}±0.33
10µg/ml TC+ TE	15.33^{bc}±0.33
5µg/ml TC+ TE	14.33^{cd}±0.33
TE alone	12.67^{d}±0.33
P Value	< 0.0001

DISCUSSION

Plants and plant derived compounds have been used globally for treatment purpose since time immemorial. The therapeutic use of these plant compounds have paved the way for identification of new compounds and their therapeutic purpose. The parts of *T. catappa* tree exhibits several pharmacological properties of importance viz antidiabetic, anticancer, hepatoprotectant, antiviral, antifungal etc. (Tercas *et al*., 2017). The phytochemical analysis of the methanolic leaves extract of *T. catappa* showed the presence of alkaloids, tannins, flavonoids and saponins which may be responsible for the antimicrobial activity of the extract. Krishnaveni *et al*., 2015 reported the presence of propane, 1, 1-diethoxy and t-Butyl hydrogen phthalate in the stem of *T. catappa* and the antifungal activity of the stem extract. Also, Allyn *et al*., 2018 reported that the antimicrobial activity of the leaf extract of *T. catappa* is due to the prtesence of flavonoid compound presence in the extract. Additionally, the antimicrobial screening on both Gram positive and Gram negative bacteria confirmed that the extract is more potent against Gram positive bacteria. A higher concentration of extract was needed for acting against Gram negative bacteria. This study is also in correlation with the previous study confirming the less activity of extract against resistant *E. coli* when used alone. This may be due to the Gram negative cell wall of the organism. In the present study, the extract potentiated the activity of antibiotic tetracycline by increasing the zone of inhibition. Thus, a synergistic activity can be noticed. The resistance potentiating action of the plant extract against resistant *E. coli* may be due to the suppression of the resistance mechanism present in bacteria (Arya *et al.,* 2020). As reported by Nisha *et al*., 2020, the main resistance mechanism in *E. coli* is the over expression of AcrAB-TolC efflux pump which confer tetracycline resistance in *E. coli.* The antimicrobial potentiating action of the methanolic extract may be due to action of extract on the resistance mechanism pathways in bacteria.

CONCLUSION

The present study concludes that the methanolic leaf extract of *T. catappa* showed no antimicrobial activity when used alone. But when used in combination with tetracycline potentiated the action of antibiotic against tetracycline resistant *E. coli*. Thus the plant extracts can be used as future candidates as an adjunct along with antibiotics. This study also paved the way for further exploitation of the plant extract to the next level.

REFERENCES

Allyn OQ, Kusumawati E, and Nugroho RA. 2018. Antimicrobial activity of *Terminalia catappa* brown leaf extracts against *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853. F1000Res. **4**:7-9. doi: 10.12688/f1000research.15998.1.

Arya Mohan, Nisha A.R, Keerthika V and Usha P.T.A. 2020**.** Methanolic extracts of *Tectona grandis* and B*iophytum sensitivum* extracts potentiate the effect of tetracycline on bacterial isolates from bovine mastitis. *J. Indian Vet. Assoc*. **13**: 716-725.

Harborne, J.B. (1973). Phytochemical Methods-Guide to Modern Techniques of Plant Analysis. (2nd Ed.).London, LDN: Chapman and Hall

Keerthika, V., Nisha, A.R., Suresh, N. N., Preethy, J., Ramnath, V., Shankar, R. and Arya,M. 2022. Phytochemical analysis and biosynthesis of silver nanoparticles from aqueous extract of seeds of *Sesamum indicum*. *J. Vet. Anim. Sci.* **53**: 262-268 DOI: https://doi.org/10.51966/jvas.2022.53.2.

Krishnaveni M, G. Krishna Kumari, M. Kalaivani, and C. Ragina Banu. 2015. A Preliminary Study on *Terminalia catappa* L. Leaf, Stem. *J. Pharm. Tech*. **8**: 1635-1639.

Krishnaveni M., Kumari G. Krishna, Banu C. Ragina, and Kalaivani M. 2015. Phytochemical Analysis of *Terminalia catappa* Stem using GC-MS/MS. *J. Pharm. Tech*. **8**: 1281-1283.

Nisha A.R., Vanishree, Preethy J., Keerthika V. and Arya M. 2021. Effect of capsaicin on tol C, mdf A and nor E genes in tetracycline resistant *Escherichia coli*. *J. Indian Vet. Assoc*. **19**: 72-80.

Nisha AR, Preethy John, Vanishree, V Keerthika and Arya M. 2020. Effect of capsaicin in inhibiting efflux pump AcrAB-TolC of tetracycline resistant *Escherichia coli*. *Pharma Innov. J.* **9**: 39-45.

Terças A. G., Monteiro Andrea de Souza, Moffa Eduardo B., Santos Julliana R. A. dos, Sousa Eduardo M. de, Pinto Anna R. B., Costa Paola C. da Silva, Borges Antonio C. R., Torres Luce M. B., Barros F. A. K. D., Fernandes E. S., and Monteiro C. de Andrade. 2017. Phytochemical Characterization of Terminalia catappa Linn. Extracts and Their antifungal Activities against Candida spp. *Front. Microbiol.* **8**: 1-5.