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ANTIMICROBIAL EVALUATION AND PHYTOCHEMICAL ANALYSIS OF *SARACA ASOCA* AGAINST TETRACYCLINE RESISTANT *ESCHERICHIA COLI*

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ABSTRACT

Plants and their derivatives were used as therapeutic agents since ancient times. The magnificent uses of phytochemicals in therapy make them strong pillars in medicine. Their easy availability and less adverse effects make it as a choice for the treatment. The uses of plant compounds in combination with antibiotics to combat resistance are being explored. This study was designed to assess the resistance modulating potential of methanolic leaf extract of *Saraca asoca* or Indian Ashoka against tetracycline resistant *E. coli* isolates from clinical samples. Phytochemical analysis of the leaf extract was done qualitatively by colour reaction. The extract was also subjected to GC-MS analysis. The resistance modulating potential was assessed by Kirby-Bauer disc diffusion assay employing various concentrations of extract along with tetracycline. The phytochemical analysis revealed the presence of alkaloids, tannins, flavonoids, and saponins in the extract. There was a dose dependent increase in the zone of inhibition in the disc diffusion assay. Highest dose could significantly overcome the tetracycline resistance. Hence it can be concluded that *S. asoca* can be used as a promising agent to combat antibiotic resistance.

Key words: *Saraca asoca*, *Escherichia coli*, tetracycline, antimicrobial resistance, Kirby-Bauer Disc Diffusion assay

INTRODUCTION

In the current era, antimicrobial resistance (AMR) is a severe global issue that threatens both human and animal health. As a result, it compromises both the food security of the country and the financial stability of millions of rural farmers. The acquisition of newer resistant tactics by microbes against conventional antibiotics is the primary precursor

for the development of drug-resistant organisms. Due to the anticipated increase in medication resistance, phytochemicals appear to be a viable alternative to current antibiotics (Divya *et al*., 2023). People started using plants as medicines since ancient times. A major advance in health science was the use of phytochemicals in medicine since it can reduce the need for antibiotics. Nearly 80% of people in developed countries use traditional medicines, which contain ingredients derived from healing plants (Keerthika *et al*., 2022). Multidrug resistance (MDR) has been a growing problem among many bacterial pathogens, notably Gram-negative bacteria like *Escherichia coli* (Nisha *et al*., 2020). *E. coli* is a Gram negative pathogen predominantly found as the causative agent in many diseases affecting both human and veterinary medicine. The development of resistance among the organisms makes it difficult to treat the patient using conventional therapy (Arya *et al*., 2020). Bacteria exhibits different methods of drug resistance including drug inactivation or alteration, modification of drug binding sites or targets, changes in cell permeability that reduce intracellular drug accumulation (expression of efflux pumps), and biofilm formation (Arya *et al*., 2022). In a way to tackle this issue, plants and their derivatives can be used along with the conventional therapy. Natural plant products are anticipated to exhibit antibiotic resistance overwhelming ability thereby easing the therapy (AlSheikh *et al*., 2020; Vaou *et al*.,2021).

Saraca asoca is a tree in the Fabaceae family, popularly known as Asoka or Ashoka in India. A medium-sized evergreen and deciduous tree, it has numerous spreading and bending glabrous branches as well as fragrant orange or red blooms (Urumarudappa *et al*., 2023). The Indian medical system has been traditionally utilising *S. asoca* to treat a variety of conditions, including fever, pain, urogenital tract illnesses, and uterine, genital, and other reproductive diseases in women. Its qualities are listed in the Vedanasthapan (analgesic, antipyretic, and anti-inflammatory) category of the ancient Ayurvedic treatise Charaka Samhita (Gupta *et al*., 2014). Even though, the plant has got numerous activities, its resistance overcoming potential is scarcely documented. Hence, this study was undertaken to assess the resistance modulating potential of *S. asoca* against tetracycline resistant *E. coli* isolates from various clinical samples.

Materials and Methods

Procurement of plant material

The leaves of *S. asoca* were collected from the premises of College of Veterinary

and Animal Sciences, Mannuthy, Thrissur, Kerala.

Methanolic extraction of leaves of *S. asoca*

The collected plant leaves were shade dried and pulverized in an electric pulverizer. The coarse powder was then packed in a filter paper percolator and then fed into the Soxhlet apparatus using methanol as the solvent. After a week, the methanol containing the plant extract was subjected to a rotary vacuum evaporator to remove excess methanol. Finally, the filtrate was collected and stored in a sealed container under 4°C.

Phytochemical Screening

The phytochemical screening of methanolic extract of leaves of *S. asoca* was done qualitatively. The extract was subjected to different tests for alkaloids, saponins, flavonoids, terpenes, steroids, glycosides, phenols and tannins as per the procedure given by Harborne, 1993.

Gas Chromatography and Mass Spectrometry (GC-MS)

The quantitative phytochemical analysis of methanolic extract of leaves of *S. asoca*wasanalysed 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°C for 4 min and then increased to 280°C in 6 min. The injector temperature was 260°C 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. The column used was of Elite 5MS column with 30m length and 0.25µm thickenss. Major constituents were identified using mass spectrum library (NIST 11 and WILEY 8).

Evaluation of antimicrobial activity

Microorganisms studied

The test organisms used for the study was *Escherichia coli*. Biochemically identified *E. coli* isolates from various clinical samples were procured from the Laboratory at Teaching Veterinary Clinical Complex, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala. The organisms were collected on nutrient agar slants and sub cultured on brain heart infusion agar plates. The culture plates were then sealed and maintained at 4ºC.

Kirby-Bauer Disc Diffusion Assay

The antibacterial activity of the methanolic extract pf leaves of *S. asoca* was assessed using the Kirby- Bauer Disc Diffusion assay as per CLSI, 2012. Twenty-

four-hour old *E. coli* culture was used for the assay. The bacteria from the culture were grown in Brain heart infusion broth for attaining the prescribed turbidity of 0.5 Mc Farland standard. Then the bacterial isolates were evenly streaked onto Mueller Hinton (MH) agar plates using sterile cotton swab. Antibiotic disc of tetracycline (30 mcg) was placed on MH agar plate with gentle pressure. The test compound i.e. methanolic leaf extract of *S. asoca at* various concentrations viz. 80µg/ml, 40µg/ml, 20µg/ml, 10µg/ml and 5µg/ml was impregnated into sterile discs (6mm Hi-Media) and was allowed to dry. The discs were then placed on the agar plate with gentle pressure using sterile forceps. The effect of combination of methanolic leaf extract of *S. asoca* with antibiotic tetracycline was also evaluated. The combination was prepared by dissolving methanolic extract in 5% DMSO and 20 µL of the extract were added on the tetracycline disc (30 mcg) at above said concentrations. The discs were allowed to dry and placed on to the agar plates. The plates were incubated at 37°C for 18-20 hours. The diameter of zones of inhibition was measured for each treatment. All the tests were replicated three times.

RESULTS

Phytochemical Screening

The results of qualitative phytochemical analysis revealed the presence of alkaloids, tannins and saponins in the leaves of methanolic extract of *S. asoca*. The results of phytochemical analysis are summarized in table 1.

Table 1. Phytochemical screening of methanolic leaf extract of *S. asoca*. The presence of phytochemical is marked by "+" and absence is denoted by "-" sign

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

Gas Chromatography and Mass Spectrometry (GC-MS)

The GC-MS analysis of methanolic leaf extract of *S. asoca* revealed the presence of ten important components. The result of GC-MS is given in table 2. The chromatogram of the GC-MS analysis is shown in figure 1, which shows the peak of ten identified phytocomponents in the analysis.

Evaluation of antimicrobial activity

The antimicrobial activity of methanolic leaf extract of *S. asoca* is evaluated using Kirby Bauer disc diffusion assay on tetracycline resistant *E. coli*

Name of compound	Retention Time
Megastigmatrienone 2	22.176
Mome inositol	23.958
Neophytadiene	26.546
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	27.420
Methylpalmitate	28.318
9-octadecenoic acid (z)-, methyl ester	31.640
Phytol	31.880
Squalene	43.127
B-Tocopherol	46.392
Vitamin E	48.208

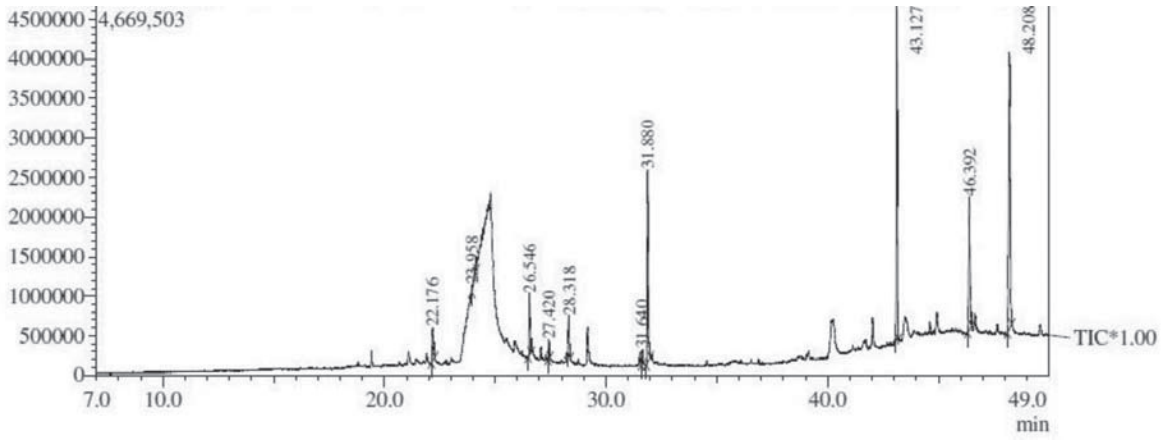

Fig. 1 Chromatogram of methanolic leaf extract of *S. asoca* after GC-MS analysis

isolates from various clinical samples. The assay revealed that extract of *S. asoca* when used alone did not show any zone of inhibition on disc diffusion assay. However, there was a dose dependent increase in the zone of inhibition when various concentrations of extract used in combination with tetracycline (30 mcg) disc. The maximum increase in the zone of inhibition was observed in the combination with highest dose of (80µg/mL) of extract which could overcome the tetracycline mediated resistance. The combination with least concentration of the extract did not show any significant difference with that of the drug when used alone. The zone of inhibition observed is given in table 3. The graph showing zone of inhibition is given figure 2.

DISCUSSION

The overwhelming and indiscriminate use of antibiotics globally has led to the emergence of resistant pathogens. The rise of resistance against antibiotics is alarmingly increasing. A solution to tackle

Table 3. Zone of inhibition (mm) for various concentration of methanolic extract of *S. asoca* in combination with tetracycline.

Treatment	Zone of Inhibition Mean ±SE (mm)
TE+ 80 µg/mL SA	20.67^{a} ±0.33
TE+ 40 µg/mL SA	18.33^{b} ±0.33
TE+ 20 µg/mL SA	16.67^{bc} ±0.33
TE+ 10 µg/mL SA	15.67^{c} ±0.33
TE+ 5 µg/mL SA	13.67^{d} ±0.33
TE Only	12.67^{d} ±0.33
p Value	<0.05

SA- *Saraca asoca*; TE- Tetracycline; All the tests were done in triplicates; n=6; r=3

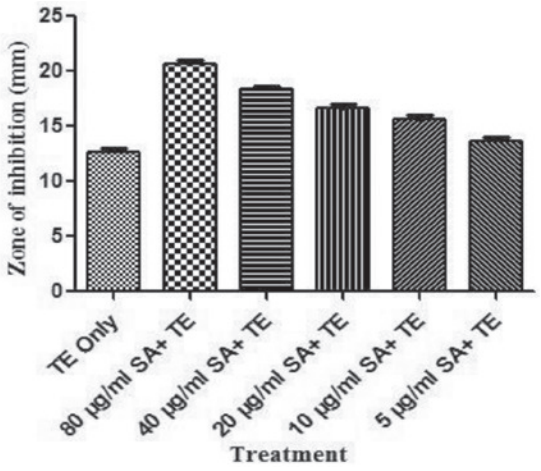

Fig. 2 Graph showing the result of Kirby Bauer Disc Diffusion Assay.

this catastrophe is the use of naturally obtained plants and their derivatives. These plant derivatives pose less side effects when compared to the antibiotics and are easily available. Tetracycline is a broad spectrum bacteriostatic antibiotic which is used to treat many infections. The development of resistance towards tetracycline makes the treatment difficult and fruitless. The bacteria acquire resistance towards tetracycline in many ways viz: chromosomal mutations that increase the expression of intrinsic resistance mechanisms, the acquisition of mobile genetic elements carrying tetracycline-specific resistance genes, and/or ribosomal binding site mutations (Grossman, 2016). The present study revealed the tetracycline resistance combating potential of methanolic extract of leaves of *S. asoca* against *E. coli*. This implies that plant compounds can be used as promising agents against resistant microorganisms in combination with antibiotics. The extract of *S. asoca* alone did not show any activity against resistant *E. coli.* This may be due to the presence of intricate resistance mechanisms active in the bacteria or the Gram negative cell wall. The resistance suppressing action of the extract may be the suppression or modulation of the resistance mechanisms acting in the bacteria that makes the antibiotic ineffective (Aanand *et al*., 2023). The phytochemical analysis revealed the presence of alkaloids, tannins, flavonoids and saponins which was consistent with the results of Athiralakshmy *et al*. (2016) and Divya *et al*. (2017). Additionally, similar results were reported by Rijo *et al*. (2020) in the GC-MS analysis of the extract. The antibiotic potentiating activity of the extract may be attributed to the presence of the active metabolites which were found in the phytochemical analysis.

CONCLUSION

The present study summarised that the leaves of *S. asoca* can be used as a resistance modifier against tetracycline resistance in *E. coli*. Even though, the extract did not show any activity against resistant isolates when used alone, but when combined with tetracycline, it potentiated the activity of tetracycline. Hence, it is concluded that the plant constituents can be used as adjuncts with antibiotics in therapy. Additionally, more research is needed in this area to assess the full potential of the extract.

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