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REVIEW ARTICLE

Antimicrobial Potential of *Tylophora indica* and its Future Considerations in Health and Food Industry

Charu Khanna, Shalini Singh, Manish Vyas[1]

Department of Microbiology, School of Bioengineering and Biosciences, Lovely Professional University, [1]Department of Ayurveda, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India

ABSTRACT

One of the most interesting areas in scientific research has been drug discovery. The discovery of antibiotics led to the improvement of the overall health status of the general population, but in the present scenario, its misuse has caused the development of microbial drug resistance. The focus has shifted toward the use of natural drugs as a source of treatment for various ailments to improve the health of individuals. A climber, identified as *Tylophora indica,* has been in use for a very long time for the treatment of ailments such as asthma and diarrhea. *T. indica* possesses compounds such as phenanthroindolizidine alkaloids and nonalkaloid components which may be responsible for its biological activity. *T. indica* is an endangered species, and its genetic preservation is a matter of concern. Literature reports that the different plant parts of *T. indica,* especially the leaves, possess a wide range of activities against different Gram-positive and Gram-negative bacteria. The experimental observations suggest that the methanolic extracts are more effective as compared to the aqueous extracts. Further studies are needed to explore the potential of *T. indica* extracts for its antimicrobial activities on various microbial pathogens including bacterial species, protozoal parasites, and viruses. This may further open an investigation into healthy and natural food preservation techniques. The article makes an attempt to review the antimicrobial activities of *T. indica,* its potential in the food and health sectors, and the future scope with emphasis on overcoming the limitations such as microbial contamination of food and use of synthetic preservatives.

Key words: Antmool, Asclepiadaceae, phenanthroindolizidine alkaloids, *Tylophora indica*

INTRODUCTION

The use of synthetic and semi-synthetic drugs for the treatment of infectious diseases is on the rise these days. Their misuse has led to the development of drug-resistant microbial species which has further elevated treatment failures in the clinical cases. Looking at this aspect, scientists are now more focused on investigating the use of natural compounds derived from plants. Medicinal plants have always been in use since the ancient era and their commendable and reproducible effects can be observed even today. Ayurveda has discussed the role of herbs to combat various infectious diseases. Chainani-Wu (2003) reported the role of *Curcuma longa* as an anti-inflammatory drug[1] whereas Samy *et al*. discussed the various bioactive compounds found in plants such as *Azadirachta indica* and *Solanum xanthocarpum*[2] used in the treatment of various pathological conditions of the body.

According to Madhavi *et al*., *Tylophora indica (T. indica)* is known for its role as treatment for asthma.[3] *T. indica* is part of the Asclepiadaceae family and native to India. It can be found in the Himalayas and the sub-Himalayan tract, where it is found growing from Uttar Pradesh to Meghalaya, Orissa, and Bengal, in various plains, forests, and hilly areas of the country. *T. indica* is also known as "Antmool" and is found at altitudes of up to 1260 m as per Harmanjit and Karanveer (2012).[4] Sunila and Priya reported that *T. indica* is a perennial and slender climber and possesses fleshy, long, and knotted roots. It is well branched with a long and twining stem. This semi-shrubby presenting plant inhabits well-drained soil, preferably on sandy soil, and has restricted growth in arid areas. *T. indica* has been included in the Bengal Pharmacopoeia since 1884. The main parts which are used are the roots and the leaves.[5] As stated by Suhas *et al*., watery latex is found in the plant. The leaves are opposite, elliptic-oblong to ovate-oblong, glabrous and with an acute apex. The leaf has a petiole which is about 6–13 mm long and glabrous and possesses a characteristic pleasant odor. Under the microscope, it has a single-layered epidermis with a thin cuticle. The mesophyll can be differentiated into the palisade and spongy parenchyma with 2–3 layers and 6–8 layers, respectively. Rosettes of calcium oxalate are also found in the spongy parenchyma. The flowers are pale yellow with a purple color within and present in the lateral cymes. The follicle of fruits occurs in pairs and tapers to a fine point in the apex.[6] Anwar *et al*. shared the fact that the wide and careless approach to collecting the plant for its use, the low-germinating capability of the seeds, and the lowered potential of vegetative cuttings for propagation has placed *T. indica* in the category of an endangered species.[7] The macroscopic view of *T. indica,* collected from Ayushya Vatika of Lovely Professional University, has been illustrated in Figures 1-5.

Correspondence:
Dr. Shalini Singh,
Department of Microbiology, School of Bioengineering and Biosciences, Lovely Professional University, Jalandhar-Delhi G.T. Road, Phagwara - 144 411, Punjab, India.
E-mail: shalinisingh.iit@gmail.com

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Figure 1: Image illustrates *Tylophora indica* growing in the Ayushya Vatika of Lovely Professional University

Figure 2: Image illustrates various parts of *Tylophora indica*

Figure 3: Image illustrates the arrangement of leaves of *Tylophora indica*

Figure 4: Image illustrates the roots of *Tylophora indica*

Harmanjit and Karanveer have reported the various chemical components of *T. indica*. Various phenanthroindolizidine alkaloids and nonalkaloid compounds have been isolated which are responsible for its biological activities. The various phenanthroindolizidine alkaloids found were tylophorine, tylophorinine, tylophorinidine, and septidine which have been isolated from the root and leaves. Other than this, some more alkaloids named tyloindicines A–J, tylophorindine, desmethyltylophorine, desmethyltylophorinine, desmethyltylophoridine, and anhydrous dehydrotylophorinine have also been isolated and studied for their different activities. Other reported alkaloids are isotylocrebrine and 4, 6-des-methylisodroxy-o-Methyltylophorinindine.[4] Figure 6 illustrates the various chemical structures of alkaloids found in *T. indica*.[5] The nonalkaloid constituents that have been reported are kaempferol, quercetin, α- and β-amyrins, tetratriacontanol, octaosanyl octacosanoate, sigmasterol, β-sitosetrol, tyloindane, cetyl-alcohol, wax, resin, coutchone, pigments, tannins, glucose, calcium salts, and potassium chloride.[4]

The plant *T. indica* has been in used in many pathological conditions. The plant has been found to exhibit different biological activities such as antimicrobial activities, antifungal effects, and antiviral effects. Its various constituents in the different extracts have shown various pharmacological activities in different models, some of which has been enlisted in Table 1.

ANTIMICROBIAL POTENTIAL OF *TYLOPHORA INDICA*

Various scientists have evaluated different extracts of various parts of *T. indica* for their antimicrobial properties against a number of microbial species.

TYLOPHORA INDICA ROOTS

Reddy *et al.* studied the crude, methanolic root extracts and observed it to be effective against *Bacillus subtilis, Staphylococcus aureus,* and *Micrococcus luteus* with a zone of inhibition of (ZOI) <5 mm diameter. No activity was found against *Escherichia coli* and *Pseudomonas aeruginosa*. In this study, the extract was effective against *Aspergillus niger* and *T. viridae* but not against *Aspergillus fumigatus*.[23] Balasubramanian *et al.* evaluated the ethyl acetate root extract dissolved in dimethyl sulfoxide against *Klebsiella pneumoniae, E. coli, S. aureus, Salmonella typhi,* and *P. aeruginosa* at different concentrations (1 mg/ml, 10 mg/ml, and 50 mg/ml). The 10 µl of 1 mg/1 ml extract showed no antibacterial activity while other concentrations showed zones of inhibition with against all

Figure 5: Image illustrates stem of *Tylophora indica*

microbes with ZOI above 10 mm. The maximum activity was found against *K. pneumoniae* with ZOI of 18 mm. The 10 µl of 10 mg/10 ml of methanolic root extract was effective against *K. pneumoniae* and *S. typhi* while resistance was shown against the other microbes. Maximum ZOI (16 mm) was observed against *S. typhi.*[24] Sangeeta *et al.* investigated methanolic, petroleum ether, and aqueous root extracts of *T. indica* against *E. coli, Pseudomonas flavescens,* and *Micrococcus roseus*. The methanolic extract was able to inhibit the growth of all three species while the aqueous extract was only effective against *E. coli*. The petroleum ether extract was not found to be effective against any of the three species.[25]

As shown by Gami and Parmar, developments in the fields of biotechnology have enabled the plant to be grown and biomass of the plant to be available throughout the year. In their experimental work, the comparison of the antimicrobial activity against *Bacillus cereus, M. luteus, E. coli,* and *S. typhi* was observed for the roots developed under natural conditions and *in vitro*. Different extracts (aqueous, methanol, petroleum ether, and chloroform) were made for conducting the study. The ZOI was found to be larger for the naturally grown extracts as compared to the *in vitro* developed root extracts.[26]

TYLOPHORA INDICA LEAVES

Reddy *et al.* conducted an antibacterial and antifungal assay with *T. indica* crude methanolic leaf extract and reported that the extract was effective against *B. subtilis, S. aureus, M. luteus, P. aeruginosa, A. niger, T. viridae,* and *A. fumigatus* with ZOI of <5 mm. No activity was observed against *E. coli*.[23] Reddy *et al.* investigated the antimicrobial activity of the crude ethanolic extract of dried *T. indica* leaves at different concentrations (20 mg/ml, 30 mg/ml, and 40 mg/ml) against *K. pneumoniae, S. aureus, S. typhi, Proteus vulgaris, P. aeruginosa, E coli, A. niger, A. fumigatus,* and *Fusarium* species. No activity was found against *K. pneumoniae* and *S. typhi* at any of the tested concentrations. The ZOI was >5 mm for all other cultured species with a maximum ZOI of 10.5 mm against *Fusarium* species with 40 mg/ml concentration.[27] Parekh and Chanda demonstrated the activity of the aqueous and ethanolic extracts of *T. indica* leaves against *Pseudomonas aerogenes*, *E. coli*, *K. pneumoniae*, *Proteus mirabilis*, *P. vulgaris,* and *Salmonella typhimurium*. ZOI was found to be 3 mm which was only observed against *K. pneumoniae*. No efficacy was found in the cultured plates of the other species with both the extracts.[28] In another study conducted by Parekh and Chanda Sumitra, the aqueous and ethanolic leave extracts of *T. indica* were found not to be effective against *S. aureus, Staphylococcus epidermidis,* and *Stictonaclia subflava*, with no ZOI observed.[29]

Table 1: Reported pharmacological activities of *Tylophora indica*

Activity determined	Model	Reference
Anti-inflammatory[8]	Mouse macrophage cell line	Yang *et al.*, 2006
Antiasthmatic[9-13]	Humans	Shivpuri *et al.* (1968, 1969, 1972)
		Thiruvengadam *et al.*, 1978
		Umamaheswari *et al.*, 2017
Antiallergic[14]	Rats	Nayampalli and Sheth 1979
Hepatoprotective[15,16]	Rats	Vipul *et al.*, 2007
		Mujeeb *et al.*, 2009
Antitumor[17-19]	Human cancer cell lines	Gao *et al.*, 2004
		Wu *et al.*, 2009
		Rao *et al.*, 2000
Immunomodulatory[20]	Rats	Ganguly *et al.*, 2001
Diuretic[21]	Rats	Meera *et al.*, 2009
Antidiarrheal[22]	Rodents	Patel *et al.*, 2006

Experiments conducted by Balasubramanian *et al.* of the ethyl acetate leaf extracts at different concentrations (1 mg/ml, 10 mg/ml, and 50 mg/ml) against *K. pneumoniae, E coli, S. aureus, S. typhi,* and *P. aeruginosa* isolated from HIV patients were studied for their antimicrobial activity. ZOI found was between 11 and 19 mm at all concentrations while resistance was observed with 10 µl of 1 mg/ml concentration against *K. pneumoniae* and *E coli*. The extract was most effective against *S. aureus* and *K. pneumoniae* (ZOI 19 mm).[24] Noor *et al.* also conducted antimicrobial assays with ethanolic extracts against *S. aureus, S. epidermidis, Streptococcus pyogenes, Enterococcus faecalis, B. subtilis, E. coli, K. pneumoniae, P. aeruginosa, P. vulgaris, S. typhi, S. typhimurium, Shigella dysenteriae* type 1, *Vibrio cholerae, S. aureus, E. coli, and P. aeruginosa*. A comparison was observed among the activity of the leaf extracts of the cultivated parent plant, *in vitro* raised plant, and the leaf callus. No activity was found for the leave extracts of the parent plants *S. aureus, S. epidermidis, S. pyogenes, E. faecalis, B. subtilis, S. aureus, P. vulgaris, S. typhi, S. typhimurium, S. dysenteriae* type 1, and *V. cholerae* at concentration 100 mg/ml. Increased activity was found for the *in vitro* raised plants and the leaf callus.[30] Sangeeta *et al.* in their study with *T. indica* leaf extracts (methanolic, petroleum ether, and aqueous) concluded that the methanolic and aqueous extracts could inhibit the growth of *E. coli*. No significant activity was observed against *M. roseus* and *P. flavescens*.[25]

A study has also been conducted on normal strains of *E. coli* and *P. aeruginosa* as well as multidrug-resistant (MDR) strains of *E. coli, P. aeruginosa,* and *S. aureus*. Deepika *et al.* conducted this study with aqueous (cold, warm, hot, and boiling water) and organic solvent (methanol, ethanol, petroleum ether, and chloroform) extracts of *T. indica* stem and leaves. The MDR strains were resistant to all the extracts. Antimicrobial activity was detected against *E. coli* by the methanolic extract and against *P. aeruginosa* by the petroleum ether and chloroform extracts. Higher ZOI against the normal strains were observed when they were tested synergistically with the antibiotic (tetracycline). No tests were conducted against the MDR strains with the combination of the plant extract and the antibiotic.[31] Another comparative study was performed by Gami and Parmar on the extracts (aqueous, methanol, petroleum ether, and chloroform) of *in vivo* and *in vitro* developed *T. indica* leaves.

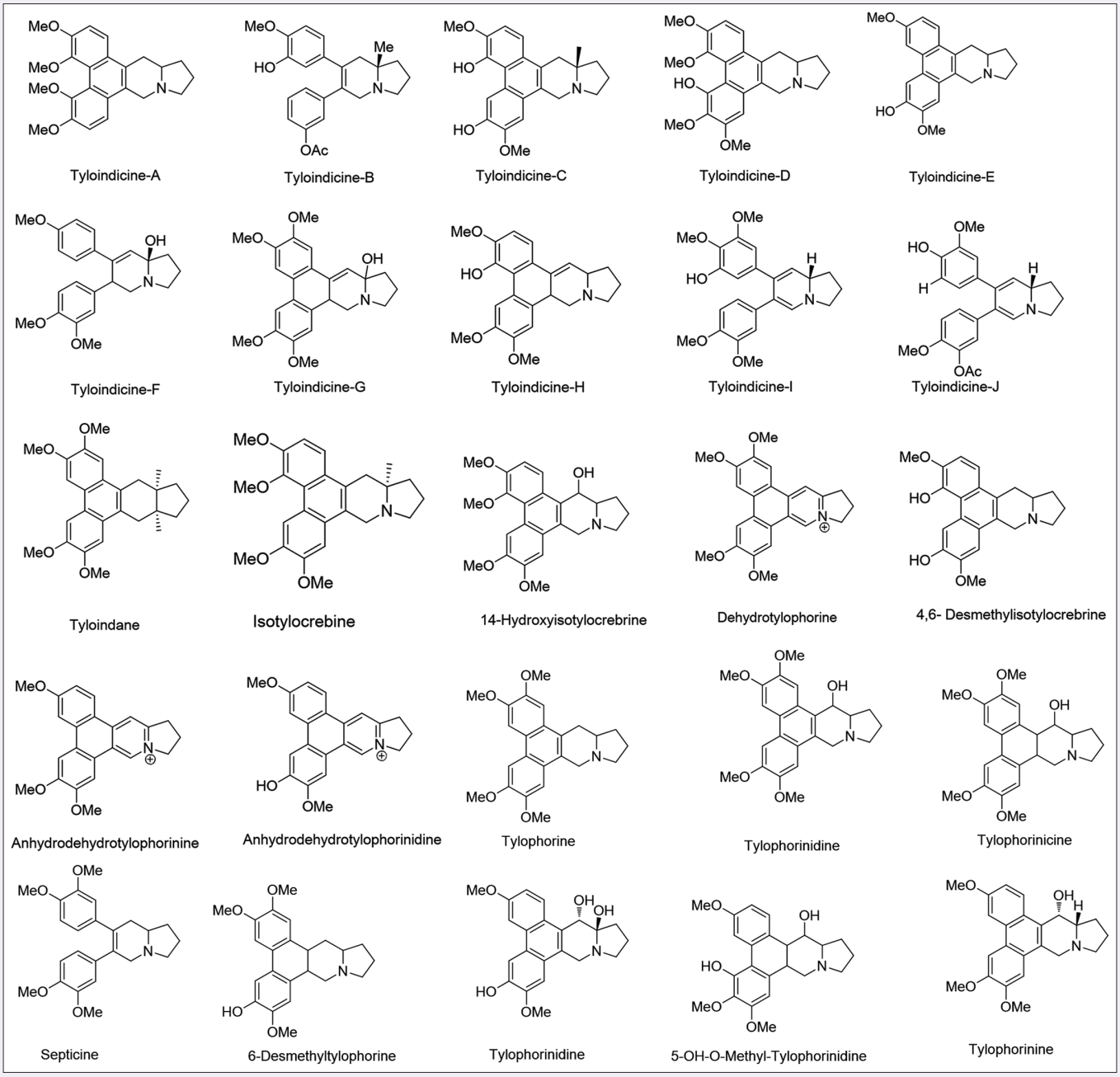

Figure 6: Figures illustrate chemical structures of various alkaloids found in *Tylophora indica*[5]

The results showed that the ZOI was larger for the *in vivo* extracts as compared to the *in vitro* developed extracts. It has also been concluded that the methanolic and chloroform extracts have more potential to be developed as antimicrobial agents.[26] Sellathurai *et al*. investigated the antimicrobial activity of crude leaf and callus extracts (aqueous, methanol, ethanol, petroleum ether, and chloroform) of *T. indica* against *S. aureus, Streptococcus faecalis, M. luteus, B. subtilis* (Gram-positive bacteria)*; E. coli, S. typhi, P. vulgaris, P. aeruginosa, K. pneumoniae, Enterobacter aerogenes* (Gram-negative bacteria), and a fungus*, Candida albicans*. The extracts were ineffective against *S. aureus*. On analysis of the diameter of ZOI, it was determined that the leaf extracts showed more promising antimicrobial and antifungal effects as compared to the callus extracts.[32]

Noor *et al*., analyzed the antimicrobial activity of the ethanolic and aqueous extracts using fresh leaves of *T. indica* against *S. aureus*, *E. faecalis*, *E. coli*, *K. pneumoniae*, *P. aeruginosa,* and *P. vulgaris*. In this study, some beta-lactamase (*bla*) possessing species were also tested (*E. coli* [*bla* ampC], *Klebsiella* spp. [*bla* CTX-M], and *Klebsiella* spp. [*bla* SHV]). The ethanolic leaf extracts were observed to be more efficient as compared to the aqueous extracts. The aqueous extracts were potent only against *E. coli* and *K. pneumoniae* with ZOI 12.33 and 11.00 mm, respectively, while the ethanolic extracts were active against *E. coli*, *K. pneumoniae, P. aeruginosa,* and *E. coli* (*bla* ampC) with ZOI 12.67, 11.67, 11.33, and 11.67 mm, respectively.[33] Another study was conducted on the antimicrobial efficiency of the leaves of *T. indica* in the parent plant and *in vitro* grown plant by Deshwal and Siddiqui. The potency of the aqueous and ethanolic extracts was observed against

different microbes (*C. albicans*, *Candida parapsilosis*, *Candida krusei*, *Aspergillus flavus, A. niger*, *A. fumigatus*, *Rhizopus* species, *Alternaria* species, *Penicillium* species, *Fusarium* species, and *Mucor* species). The alcoholic leaf extracts of parent plant were found to be most effective than other extracts against *C. krusei, A. flavus, A. niger,* and *A. fumigatus*.[34]

TYLOPHORA INDICA STEM

Sangeeta *et al*. studied the activity of *T. indica* stem extracts (methanolic, petroleum ether, and aqueous) against three bacterial strains (*E. coli, M. roseus,* and *P. flavescens)*. ZOI was observed against *E. coli* by all the extracts while the petroleum ether extract also showed ZOI (2 mm) against *P. flavescens*. No ZOI was observed against any of the other solvents or bacterial species.[25] As per Deshwal and Siddiqui, the alcoholic stem extracts of the parent plant of *T. indica* were able to inhibit the growth of *A. niger* only while more effective inhibitions were observed in the alcoholic extracts of the *in vitro* raised plant stem against *C. parapsilosis*, *C. krusei*, *A. flavus,* and *A. fumigatus*.[34]

TYLOPHORA INDICA SHOOT

The efficacy of an aqueous and hydroalcoholic crude shoot extract of *T. indica* was studied by Linz-Buoy *et al*. against 3D7 and RKL-9 strains of *Plasmodium falciparum*. The extracts exhibited significant results. For the drug-sensitive *P. falciparum* 3D7, the EC_{50} value was found to be 2.542 μg/ml and 2.364 μg/ml for the aqueous and hydro-alcoholic extracts respectively. For the drug-resistant strain *P. falciparum,* RKL-9, the EC_{50} value was determined to be 15.240 μg/ml and 9.717 μg/ml, respectively, for both the extracts. These values depict that the extracts are effective in killing *P. falciparum* which is one of the causes of malaria.[35]

ALKALOID COMPOUND ISOLATED FROM *TYLOPHORA INDICA*

Sathyabama and Jayasurya used the crude alkaloid extract from *T. indica* leaves and conducted antimicrobial assay against *S. aureus, E. coli,* and *P. aeruginosa* by the disc diffusion method and turbidity analysis. The ZOI at 20 μg/ml was found to be 0.341, 0.326, and 0.371 mm, respectively. The alkaloid compound was isolated and found to be tylophorinidine alkaloid cation. Its mode of action was also analyzed and it has been reported to inhibit bacterial protein synthesis. Treatment with the alkaloid also resulted in the leakage of protein and sugars from the bacterial membranes.[36] Chaturvedi and Chowdhary analyzed the secondary metabolites (tylophorine, tylophorinine, and kaempferol) present in methanolic extracts of *T. indica* for their effects against the influenza virus. It was concluded that *T. indica* possesses anti-influenza activity at 20 mg/ml concentration.[37]

FUTURE SCOPE AND BOTTLE NECKS OF APPLICATIONS OF *TYLOPHORA INDICA*

T. indica has been found to be useful in the treatment of a number of diseases such as asthma. Its potential as a medicament thus cannot be ignored. *T. indica* has been found to be effective not just against microbes but also against both fungal and viral species such as *Aspergillus*, *Candida*, and influenza virus. *T. indica*'s possible role in controlling the growth and spread of malarial parasites such as *P. falciparum* cannot be denied. Extensive research needs to be carried out to evaluate the antimicrobial activity of *T. indica* against microbial and foodborne pathogens such as *Listeria monocytogenes, Campylobacter jejuni,* and *Giardia lamblia* can be potential targets. More studies can be focused on observing the effects of *T. indica* on disease-causing parasites such as *Taenia* and *Enterobius*. Not only this but also the effects of this plant on many pathogenic viruses are still the area of exploration such as TMV and HIV, and its antimicrobial activity in synergism with various natural antimicrobial agents, such as bacteriocins, is also an attractive area under current investigation.

Another unexplored area is analysis of the efficacy of *T. indica* extracts as a preservative alone, in combination with other plant extracts or with other natural substances such as bacteriocins which are already incorporated in various food packaging materials. Such studies can give major insights to improving packaging techniques of food materials, with improved shelf life, which can go a long way of reducing incidents of foodborne illnesses and intoxications.

In spite of the fact that *T. indica* has a wide scope of applications in infectious diseases, its use in dosage form may be limited as some phytochemicals such as alkaloids are normally toxic in nature. Thus, toxicity studies must be assured and the dose must be finalized before its application as the human body may possess some natural microflora such as *E. coli,* which may be affected by higher doses. Some antimicrobial studies have shown contraindicating results which need to be investigated before its application in the treatment of infectious diseases in both human and animals.

CONCLUSION

One of the major challenges being faced is the provision of basic health facilities, especially in developing and underdeveloped nations of the world. This challenge has been made more challenging with the evolution of drug-resistant microbial species. In the past few decades, scientists have started exploring the usage of herbs as a source of medicine which is already being used traditionally. Approaches need to be investigated to observe the growth of the plants by both *in vitro* techniques and to conserve the vulnerable species.

Studies report that the different parts of *T. indica* possess a wide spectrum of antimicrobial activities against Gram-positive and Gram-negative bacteria which can be explored for their applications in the health and food industry. The antimicrobial assays of the extracts in *in vitro* grown plants have also presented applicable results. This may be due to the increased production of phytochemicals in the provided, suitable environmental, and growth conditions. More investigations are needed into the dosage determination, and if found applicable, large-scale cultivation of the plant can be promoted as India provides the suitable environmental conditions for its growth. This can also help contribute to the growth of the economy of the country and improve the quality status of health of all infected people and the shelf life of food.

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Conflicts of interest

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