

Phytochemical and Antimicrobial Activity of Fumes and Powder Extracts of *Tinospora cordifolia*

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Abstract. Herbal medicines have a long therapeutic history serving large population of the world. However, the quality control and quality assurance still remains a challenge because of the high variability of chemical components. *Tinospora cordifolia* is commonly referred to as *Amrita* or *Giloy* or *Guduchi*. All the parts of the plant are immensely useful due to the presence of different secondary metabolites. We aimed to investigate the qualitative and quantitative (carbohydrate and poly-phenolic compound) analysis of secondary metabolites present in extracts of *T. cordifolia* powder and its herbal fume (generated from Yagya), followed by the study of its antimicrobial activity against *E. coli* and *P. aeruginosa*. The present study's powder and fume cocktail (hexane, ethyl-ether and methanol) extracts (10 mg/ml) showed, respectively 30 mm and 21.67 mm Zone of inhibition against *E.coli* indicating equivalent efficiency of herbal fumes. Similarly, fumes contained all secondary metabolites (qualitative testing) similar to that of powder extract. In addition, carbohydrate and poly-phenolic compound present in the fume extracts (0.277 mg/g and 0.29mg/g), were equivalent or higher than powder extracts (0.131 mg/g and 0.26mg/g) indicating holistic utility of fumes of *T. cordifolia*.

Keywords. *Tinospora cordifolia*, Herbal fumes, Secondary metabolites, Antimicrobial activity.

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Introduction

Herbal medicines have a long therapeutic history and are still serving many of the health needs of a large population of the world. However, quality control and quality assurance still remain a challenge because of the high variability of chemical components involved. The genus *Tinospora* (Menispermaceae), is a perennial, deciduous, large, glabrous, climbing shrub with a weak and fleshy stem, found throughout Asia, Africa, and Australia. The prominent member of the *Tinospora* genus, out of 34 different species, is *T. cordifolia*, which is a well-recognized and widely distributed traditional plant that is used successfully in Indian Ayurveda medicine and is found throughout the tropical Indian subcontinent and China [1]. It has numerous ethnomedicinal applications in Ayurvedic pharmacology. The plant *T. cordifolia* is a stem and in Ayurvedic practice, generally a decoction of Amrita is given to the patient.

All the parts of the plant are immensely useful due to the presence of different secondary metabolites of pharmaceutical importance belonging to various groups such as steroids, diterpenoid lactones, sesquiterpenoids, glycosides, alkaloids, and phenolics [2]. It was reported that Guduchi also possesses adaptogenic potential. The decoction is prepared alone or in combination with other herbs [3]. According to the Bower Manuscript a medical treatise, recorded in the 6th century A.D. in the Sanskrit language on the birch bark leaves, *T. cordifolia* is used in the recipe for the preparation for 'Amrita-Prâsa clarified butter' and 'Amrita Oil', which is considered as soma (food for immortality) [4]. *T. cordifolia* has shown many promising biological activities, such as anti-oxidative, antimicrobial, anti-hyperglycemic, anti-inflammatory, osteoprotective, hepatoprotective, antidiarrheal, and anti-stress effects. *T. cordifolia* is a rich source of protein as well as micro-nutrients such as iron, zinc, copper, calcium, phosphorus, and manganese. Terpenes, alkaloids, flavonoids, steroids, and glycosides are among the secondary plant metabolites found in it [5]. The genus *Tinospora* is reported to contain over two hundred different phytochemicals from diverse chemical classes

in phytochemical characterization, with diterpenoids representing the most abundant chemical class [6]. We aimed to investigate the differences between the qualitative and few quantitative analysis of secondary metabolites present in extracts of *T. cordifolia* powder and its herbal fume (generated from Yagya), followed by the study of its antimicrobial activity against *E. coli* and *P. aeruginosa*.

Methodology

Sample collection

Dried Sample of *T. cordifolia* was collected from the Shantikunj Pharmacy, Dev Sanskriti University, Shantikunj-Haridwar, India.

Herbal fume preparation via the process of Yagya

The Yagya was performed using mango wood (400gm), ghee (clarified butter) (40 gm) and an herbal mixture of dried *T. cordifolia* stem powder in a Yagya-chamber (12.5 X 12.5 feet room) with closed controlled conditions. The herbal fumes were collected with the help of an air sampler (APM 433) from Envirotech Instruments Pvt. Ltd, Delhi. The fumes generated by Yagya were subjected to an air sampler present inside the Yagya chamber. The herbal fumes were absorbed in air sampler impingers containing solvent system of hexane, ethyle-ether and methanol in a 1:1:1 ratio. The resultant solution of impinger was subjected to a rotary evaporator at 60°C and when about 1 ml of sample was been left, it was further subjected to a freeze drying at -40°C. The resultant power of herbal fumes was prepared using DMSO (Dimethyl Sulfoxide) and methanol in various dose concentrations. The herbal fumes preserved in DMSO were subjected to antimicrobial analysis while in methanol were used in phytochemical analysis.

Herbal powder extraction of *T. cordifolia*

Powdered stem materials (5 gram) were extracted in soxhlet with 20 ml of a 1:1:1 solvent system of hexane, ether, and methanol. The extract was filtered and then the filtrate was subjected to a rotary evaporator at 60°C and when

about 5 ml of sample was left, it was further subjected to a lyophilizer. These extracts were re-suspended in DMSO for further analysis.

Chemical characterization of herbal fumes and herbal powder

Qualitative and quantitative chemical characterization of the herbal fumes and herbal powder was performed [8, 9]. The secondary metabolites analyzed for qualitative analysis were carbohydrate, poly-phenol, flavonoid, alkaloids, tannins, and for quantitative analysis were poly-phenol [10] and carbohydrate [8, 9].

Antimicrobial action of herbal fumes

The antimicrobial activity of herbal fumes and herbal powder was analysed by the kirby-bauer agar well diffusion method [11] against *E. coli* and *P. aeruginosa*. Inoculums were prepared by direct colony suspension method. A small volume of sterile water was poured inside a test tube to which 50 µl of 24 hr broth culture was added until it matched the 0.5 McFarland standard turbidity. The dose concentration used for antimicrobial analysis was 10 mg/ml.

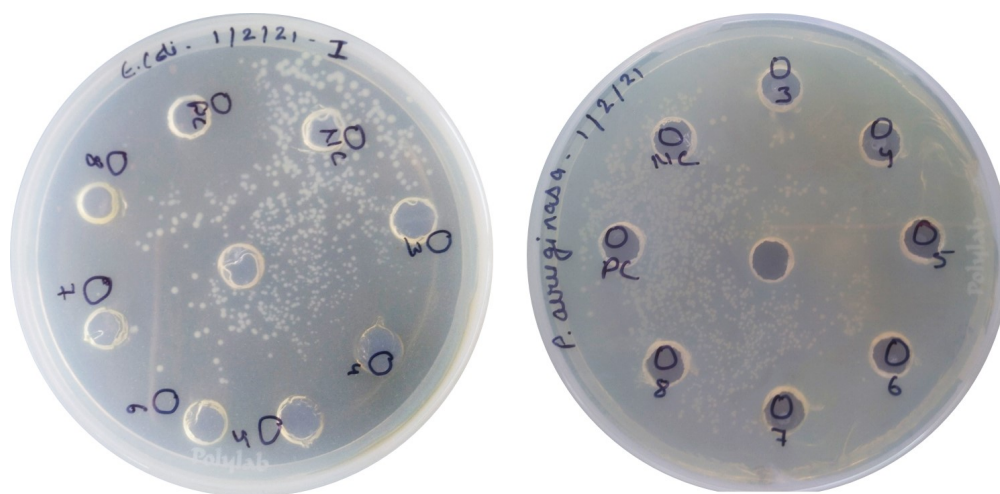


Figure 1: Zone of inhibition by *T. cordifolia* herbal fume and herbal powder in *P. aeruginosa*, Yagya herbal fumes (3,4,5), herbal powder (6,7,8). PC = positive control, NC = the negative control.

Name of bacteria	Zone of inhibition (in mm)			
	Herbal fumes	herbal powder	Positive control	Negative control
<i>E. coli</i>	21.67 ± 2.36	30 ± 4.08	25	0
<i>P. aeruginosa</i>	25 ± 0	23.5 ± 1.5	30	0

Table 1: Zone of inhibition of herbal fumes and herbal powder extract of *T. cordifolia*.

Results and Discussion

There are ample descriptions of Yagya in Vedic literature that refer to its multiple applications such as social development, spiritual development, ecological balance, and preventing and curing diseases. Among them, one of the most important aspect is health applications.

The therapeutic application of Yagya is known as Yagya Therapy or Yagyopathy. It has been observed that Vedic literature describes the treatment of a wide range of diseases (both infectious and non-infectious) and ailments through Yagya, along with the precautions and procedures [12]. Joshi et al. reported that Yagya can be alter-

Name of secondary metabolites	Observation	
	Herbal fumes	herbal powder
Flavonoids	Present	Present
Alkaloid	Presents	Absent
Saponins	absent	Absent
Tannins	Present	Absent
Protiens	Present	Present
Cardiac glycosides	Present	Absent
Carbohydrate	Present	Present
Terpinoids	Present	Absent

Table 2: Qualitative phytochemical analysis of herbal fumes and herbal powder of *Tinospora cordifolia*

nate therapy for the treatment of lung disease [13]. *T. cordifolia* calms the mucous membrane of the respiratory system and hence its herbal fume can be used to treat inflammation due to its anti-inflammatory and antioxidant properties [14].

The Yagya is a holistic treatment. In Ayurveda, the application of medicinal-fume for therapeutic purposes has been discussed in detail. Charak Samhita 5/25-61 describes the use of herbal fume inhalation as a technique to cure or prevent diseases, which is similar to Yagya fume inhalation for treatment [15]. In the present study, dried stems of *T. cordifolia* is used for phytochemical analysis and antimicrobial activity against bacteria *E. coli* and *P. aeruginosa* responsible for multiple diseases like lung disorder, skin disease, colon infection, and pneumonia, and it has been found that 10 mg/ml *T. cordifolia* of have antimicrobial activity against *E.coli* and *P. aeruginosa* with the zone of inhibition of 21.67 mm and 25 mm in case of herbal fumes obtained by Yagya, while in herbal powder of *T. cordifolia* the zone of inhibition observed is 30 mm in *E. coli* and 23.5 mm in *P. aeruginosa* (Table 1). The herbal powder had more antimicrobial activity against *E.coli* while in the case of *P. aeruginosa* the results were nearly the same (Table 1). Further study quantifying the comparison between fume and powder is required.

Phytochemical Analysis of *Tinospora cordifolia*

The ethnomedicine literature, which includes traditional ancient Indian scriptures and Ayurvedic medicine, is rich in pre-clinical and clinical substantiation data that supports the immunomodulatory-related structures and functions of *T. cordifolia* herbal preparations. The present study speaks about *T. cordifolia* as a rich source of antioxidant components both in powder and fume forms.

Phenolic compounds act as antioxidant agents by scavenging the free radicals due to the presence of hydroxyl groups in them [16, 17], and they also act as reducing agents, hydrogen donors, metal chelators and singlet oxygen quenchers due to their redox properties [18]. Phenolic acids and flavonoids are considered typical phenolic compounds which possess antioxidant activity and they are widely distributed in the plant kingdom [19]. The use of synthetic antioxidants has been restricted due to their health risks and toxicity. The present study, it was found that the poly phenolic compound present in herbal powder of the *T. cordifolia* is 0.26 mg/g and in herbal fumes generated by Yagya is 0.29 mg/g (Supplemental Figure 1). The total carbohydrate content in the herbal fumes of Yagya is 0.277 mg/g, which is 53% more than the carbohydrate content found in herbal powder, which is 0.131 mg/g (Supplemental Figure 1), which might be due to mango wood fume along with

herbal mixture fume. Also the herbal fume activity compared to its powder extract was less against *E. coli*, which is gut bacteria, suggesting holistic benefits of herbal fume of *T. cordifolia* and Mango. This result reflected the ancient practice of Yagya for building strength and energy in the body.

The qualitative analysis of phytochemicals of herbal fumes and herbal powder was also studied (Table 2). In the fumes, flavonoids, alkaloids, tannins, proteins, cardiac glycosides and carbohydrates were present, while in herbal powder cardiac glycosides, tannins, and alkaloids were absent, while others were present (Table 2). The study conducted by Garg et al. suggested that cardiac-glycosides, alkaloid, and carbohydrates were absent in the herbal powder of *T. cordifolia* (stems) [20], which were similar to the current findings; the only difference was that carbohydrates were present in our findings. The extract of herbal fumes generated by Yagya contained all of these secondary metabolites indicating its holistic utility.

In another study performed by Jeyachandran et al. it was found that *T. cordifolia* dried stems, aqueous, ethanol and chloroform extract of 100 mg/ml had antimicrobial activity against *E. coli* with 4 mm, 9 mm and 8 mm zones of inhibition, respectively, while in the present study, the cocktail extract (hexane, ethyle-ether and methanol in 1:1:1 ratio) showed a 30 mm zone of inhibition against *E. coli* [21], which was very higher than ethanolic extract alone; the fume also had higher zone of inhibition indicating efficient extract of all secondary metabolites and hence its holistic utility. The herbal fume extract also had similar extraction efficiency to that of powder extract in terms of poly-phenolic content. Prasad and Chouhan studied the antimicrobial and antioxidant activity of *T. cordifolia*. The authors reported that the highest total poly-phenolic content was 0.31 mg/g of methanol, which was close to the present findings, which was 0.29 mg/g in herbal fumes and 0.26 mg/g in herbal powder of fumes [22].

As the incidence and susceptibility to acute and chronic diseases continuously increase, many health-conscious individuals have shifted their

mindset to a holistic treatment that incorporates measures and routines that promote disease prevention and resilience. The process of Yagya, which is offering medicated herbal mixture to fire, facilitates the aim of holistic treatment of the person. The ancient Indian practices were well aware of the nutrient level, phytochemical utility, and antimicrobial application of Guduchi (*T. cordifolia*) and used this herb in Yagya.

Compliance with ethical standards Not required.

Conflict of interest The authors declare that they have no conflict of interest.

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