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SHORT COMMUNICATION

Antioxidant Activity of *Tribulus terrestris*: A Comparative Study Between Extract and Homoeopathic Mother Tincture in Male Infertility

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Abstract

Background: Phytochemical constituents and its therapeutic potential of *Tribulus terrestris*, has been extensively studied. This study evaluated the phytochemical composition of *T. terrestris*, extensively examining both in its extract form and homoeopathic mother tincture. **Aim:** The objective is to identify, quantify, compares and assess the phytochemical screening and antioxidant activity of both in *T. terrestris* ethanolic extract and homoeopathic mother tincture. **Methods:** Phytochemical screenings were performed to identify and quantify the compounds in the ethanolic extract and homoeopathic mother tincture. Antioxidant activities were assessed using DPPH, nitric oxide scavenging, and FRAP assays, with IC_{50} values calculated to compare efficacy**. Results:** Phytochemical screenings confirmed the presence of various compounds like alkaloids, tannins, flavonoids, phenols, terpenoids, and glycosides, though saponins were found only in the ethanol extract, not in the mother tincture. The ethanol extract showed the lowest IC_{50} values of 3.957 µg/ml in the DPPH assay, while the homoeopathic mother tincture had an IC_{50} value of 35.343 µg/ml, indicating competitive antioxidant activity. Similarly, in the nitric oxide scavenging assay, the ethanol extract exhibited IC_{50} values of 11.069 µg/ml and the mother tincture showed 30.737 µg/ml, highlighting potent nitric oxide scavenging activity. Additionally, the ethanol extract demonstrated competitive FRAP values with an IC_{50} of 30.216 µg/ml, and the mother tincture had 31.557 µg/ml, showcasing antioxidant potential. The presence of diverse phytochemical constituents in both its extract and homoeopathic mother tincture is noted. The comparison of antioxidant activity between the two samples consistently favors the mother tincture, indicating its efficacy in scavenging free radicals and mitigating oxidative damage. **Conclusion:** *T. terrestris* is a promising homoeopathic remedy for male infertility, especially in improving sperm parameters, supported by its rich phytochemical components and potential antioxidant effects. The comprehensive analysis, including the comparison between extract and homoeopathic mother tincture, provides valuable insights for future research.

Major Findings: The study found that the homoeopathic mother tincture of *Tribulus terrestris* contains key phytochemicals with significant antioxidant activity. It effectively scavenges free radicals, reducing oxidative stress. These findings suggest its potential role in improving sperm health and treating male infertility, supporting its therapeutic use in homoeopathic medicine.

Keywords: Free Radical Scavenging, Herbal Remedies, Natural Antioxidants, Oxidative Damage, Sperm Parameter

1. Introduction

Tribulus terrestris, also known colloquially as "stoneroot" or "goat's head", belongs to the plant kingdom with a rich heritage of therapeutic effects[1]. For centuries it has been used as folklore medicine in Chinese and *Ayurveda* which predominantly treated various conditions like infertility, sexual dysfunction,

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heart ailments, renal calculi and urinary tract infections, *etc*[2]. The plant has been extensively studied for its phytochemical components and pharmacological activities. The therapeutic effects of *T. terrestris* is due to the presence of various secondary metabolites like alkaloids, saponins, flavanoids and tannins[3,4]. Secondary metabolites in plants serve important roles in defence mechanisms against pathogens and predators, as well as in attracting beneficial organisms for pollination and seed dispersal[5]. These compounds have also been identified as potential sources of dietary supplements and medicinal components for the human body.

Preliminary phytochemical screenings have confirmed the existence of saponins, flavonoids and tannins in *T. terrestris* extracts only[3,6]. These compounds have been found to have an aphrodisiac effect and are probably the main cause of this property. However, further analysis is essential to determine the exact concentrations and vital synergistic effect of these compounds in relation to other medicinal properties attributed to *T. terrestris*[7]. The study aims to identify the various phytochemical components in its extract and homoeopathic mother tincture. There has been growing interest in complementary therapies for treating male infertility in recent years. These traditional herbal medicines have been found to reduce oxidative stress which poses a major threat to male fertility[8].

From various research studies it has been observed that *T. terrestris*, a medicinal plant native to South Africa, exhibits significant antioxidant activity[1,3]. This antioxidant activity is thought to be beneficial in improving sexual functions and sperm production in both aspects of quality and quantity[9]. *T. terrestris* contains various constituents such as steroids, flavonoids, alkaloids, and vitamins, which contribute to its antioxidant properties[3]. Furthermore, one specific compound found in *T. terrestris*, protodioscin, has been clinically tested and shown to be particularly effective in treating oligozoospermic infertility, a condition characterized by low sperm count[10].

This is supported by studies that have shown a decrease in free radicals and malondialdehyde levels with the use of *T. terrestris*. As a result, *T. terrestris* has shown promising results as a potential treatment option for male infertility[11]. Additionally, *T. terrestris* has been found to possess antimicrobial and antibacterial properties, which may further aid in protecting the male reproductive system from infections or inflammation that could contribute to infertility[11]. Furthermore, *T. terrestris* has been found to have a therapeutic effect on germination and physiological functions of the testes. This stimulation leads to the precocious development of the testes and improved reproductive function[12]. In a study conducted on oligozoospermic patients, *T. terrestris* was found to be more effective in managing oligozoospermia compared to a placebo[13]. Additionally, *T. terrestris* extract has shown the potential to improve the morphology and number of spermatozoa in rats[14].

Overall, the antioxidant activity of *T. terrestris*, along with its potential effects on spermatogenesis and testosterone levels, suggest that it may have beneficial effects to treat male infertility. The increased testosterone levels observed *in-vivo* studies further support the potential benefits of *T. terrestris* for male infertility[15,16]. Moreover, the *T. terrestris* may increase the Leydig cell numbers which directly enhances androgen hormone potentially improving male infertility[14]. *T. terrestris* helps in the management of male infertility due to its various properties like antioxidant, anti-inflammatory, and antimicrobial effects. Furthermore, *T. terrestris* has shown potential in improving spermatogenesis, increasing testosterone levels, and enhancing sperm concentration and motility[14]. Hence the aim of this article is to explore the phytochemical constituents of *T. terrestris* and their antioxidant activity, particularly concerning male infertility[11]. In addition, the article aims to comparison between the ethanolic extract and homoeopathic mother tincture of *T. terrestris* by identifying the various metabolites and quantifying the antioxidant activity.

2. Materials and Methods

2.1 Identification and Collection of Drugs

Tribulus terrestris is a small flowering plant with yellow flowers and distinctive spiky fruits that resemble goat heads.This plant is a low-growing herb with stems and branches covered in dense hair. Its leaves are arranged opposite each other, with each pair typically having one smaller leaf. The leaves are divided into 3 to 6 pairs of oblong leaflets, each about 6 to 12 mm long, with fine hairs on both surfaces. The flowers are solitary, pale yellow, and about 0.8 to 1.5 cm wide, appearing either

in the axils of the leaves or opposite them. The fruit is round and consists of usually 5 hairy or nearly hairless segments, each containing several seeds separated by partitions. This entire plant, including its roots, is utilized in the preparation of homoeopathic remedies[17]. *T. terrestris,* a plant from the *Zygophyllaceae* family, has small yellow flowers and prickly fruits that look like goat heads. Its phytochemical makeup and antioxidant properties have garnered significant attention in the realm of male infertility research, prompting numerous studies[9].

According to the Homoeopathic Pharmacopoeia of India, the *T. terrestris* plant was identified as said above and collected from the herbal garden of Venkateswara Homoeopathic Medical College and Hospital, Chennai for this study. The whole plant including roots of *T. terrestris* was dried in the shade. Drying was ensured until all moisture content was completely evaporated and the whole plant became completely dried which was suitable for grinding. After drying, the entire plant was crushed using an iron mortar and pestle, then stored in a labelled airtight container. The dried and powdered *T. terrestris* was then extracted one after another with ethanol, chloroform, and water using a Soxhlet apparatus.

The standardized homoeopathic mother tincture *T. terrestris* was purchased from Dr. Willmar Schwabe, India, GMP certified drug manufacturing unit.

2.2 Determination of Phytochemical Constituents

2.2.1 Qualitative Analysis of Phytochemicals

Chemical tests were carried out with the homoeopathic mother tincture and ethanolic extract of *T. teresteris* using standard methods to identify the phytochemical components which include alkaloid, falvonoid, tanin, phenol, terpenoid, glycoside, sapoinin, steroid, carbohydrate, protein, coumarins, anthocyanins, leucoanthocyanins, anthraquinones, cholesterol, xanthoproteins, quinones, triterpenoids, gums and mucilage and fats fixed oils[17-28].

2.3 Determination of Antioxidant Activity

2.3 1 DPPH Radical Scavenging Activity

The fractions were tested for their ability to scavenge free radicals using a DPPH assay. A solution of DPPH was made by dissolving 24 mg in 100 mL of ethanol and stored at -20°C. To create the working solution, the DPPH solution was diluted with ethanol, and 3 mL portions were mixed with 1 mL of sample at different concentrations (20, 30, and 40 g/mL). After shaking and incubating the mixture in darkness at room temperature for 15 minutes, absorbance was measured at 517 nm. A control without sample was also prepared to calculate the radical scavenging activity as a percentage of DPPH radical scavengers using a specific equation[29].

2.3.2 Nitric Oxide Radical Scavenging Activity

This activity was assessed using the Griess-Ilosvay reaction with sodium nitroprusside. In this experiment, a mixture containing sodium nitroprusside (10 mM) and phosphate buffer (pH 7.4) was combined with either a sample or vitamin C, then incubated at 25°C for 150 minutes. After incubation, nitrite was extracted, and sulfanilic acid reagent was added and left for 5 minutes. Following this, 1% Naphthyl Ethylene Diamine Dihydrochloride (NEDD) was introduced and left to stand for 30 minutes at 25°C, after which the absorbance of the resulting pink solution was measured at 540 nm[30].

The percentage of nitric oxide inhibition was calculated using the following equation:

A0 was the absorbance of control, and A1 was the absorbance of the treated sample.

2.3.3 Ferric Reducing Antioxidant Potential (FRAP) Assay

The iron-reducing effect of plant extracts was tested using a modified FRAP assay. This method relies on the ability of antioxidants to convert a colorless iron complex (Fe^{3+}- tripyridyltriazine) to a blue-colored complex (Fe^{2+}- tripyridyltriazine) at low pH. The reduction process is tracked by measuring absorbance changes at 593 nm. To conduct the assay, a fresh FRAP reagent was prepared daily by combining acetate buffer, TPTZ, and ferric chloride. Standard $FeSO_4 \times 7H_2O$ solutions were used to create a calibration curve. Sample solutions were mixed with the FRAP reagent and incubated at 37°C for 30 minutes. After incubation, absorbance was measured at 593 nm, and the difference from the blank sample was used to calculate the FRAP value, representing the reducing capacity in mmol Fe^{2+}

per gram of sample. Each measurement was performed three times for accuracy[31].

3. Results

3.1 Phytochemical Constituents

Table 1 revealed the determination of the phytochemical constituents between *T. terrestris* extract and its homoeopathic mother tincture. In this study, 20 bioactive compounds were evaluated, out of which 17 phytochemical constituents were identified in the *T. terrestris* extract, and 16 phytochemical constituents were determined in the *T. terrestris* mother tincture. The bioactive constituents such as alkaloids, flavonoids, tannins, glycosides, phenols, terpenoids, steroids, carbohydrates, proteins, coumarins, cholesterol, xanthoproteins, quinones, gums, and mucilage, were identified in both ethanol extract and mother tincture. Whereas the following constituents, such as anthocyanins, leucoanthocyanins, anthraquinones, triterpenoids, fats, and fixed oils, were not identified in both extract and homoeopathic mother tincture. Saponin compound was identified only in the ethanol extract, not in the mother tincture.

3.2 Antioxidant Activity

In the assessment of antioxidant properties through various assays, the results reveal intriguing insights into the effectiveness of different samples.

3.2.1 DPPH Radical Scavenging Activity

The TT-ethanol sample exhibited a concentration dependent response, the activity being 57.679 µg/ml, 61.889 µg/ml, and 67.054 µg/ml for

concentrations of 20 µg/ml, 200 µg/ml, and 40 µg/ml, respectively (Figure 1). Notably, mother tincture demonstrated significant activity exhibited at the values of 6.794 µg/ml at 20 µg/ml concentration, surpassing both TT-ethanol and the standard (Vitamin E) in this regard. The lowest IC_{50} values of 3.957 µg/ml exhibited by ethanol extract and IC_{50} value of 35.343 exhibited by the homoeopathic mother tincture indicating a competitive antioxidant activity.

3.3.2 Nitric Oxide Scavenging Activity

In this assay, TT-ethanol showed concentration dependent efficacy, the activity exhibited at the values of 62.953 µg/ml, 78.628 µg/ml, and 92.597 µg/ml for concentrations of 20 µg/ml, 200 µg/ml, and 40 µg/

Table 1. Qualitative phytochemical analysis of *T. terrestris* ethanolic extract and mother tincture

Si. No.	Phytochemical Constituent	Ethanolic Extract	Mother Tincture
1	Alkaloid	+	+
2	Flavonoid	+	+
3	Tannin	+	+
4	Phenol	+	+
5	Terpenoid	+	+
6	Glycoside	+	+
7	Saponin	+	-
8	Steroid	+	+
9	Carbohydrate	+	+
10	Protein	+	+
11	Coumarins	+	+
12	Anthocyanins	-	-
13	Leucoanthocyanins	-	-
14	Anthraquinones	-	-
15	Cholesterol	+	+
16	Xanthoproteins	+	+
17	Quinones	+	+
18	Triterpenoids	-	-
19	Gums and mucilage	+	+
20	Fats and fixed oils	-	-

'+' indicates presence of compounds; '-' indicatesabsence of compounds

ml (Table 2). The mother tincture displayed notable scavenging potential, outperforming the TT-ethanol and standard (Vitamin E) at lower concentrations. Notably, the standard exhibited the lowest IC_{50} values of 11.069 µg/ml by ethanol extract and 30.737 µg/ml by mother tincture, emphasizing its potent nitric oxide scavenging activity.

3.3.3 Ferric Reducing Antioxidant Power (FRAP)

The TT-ethanol sample demonstrated varying efficacy across concentrations, the activity exhibited at the values of 77.948 µg/ml, 61.723 µg/ml, and 12.457 µg/ml for concentrations of 20 µg/ml, 200 µg/ml, and 40 µg/ml, respectively (Figure 2). The mother tincture consistently exhibited high FRAP, with IC_{50} values of

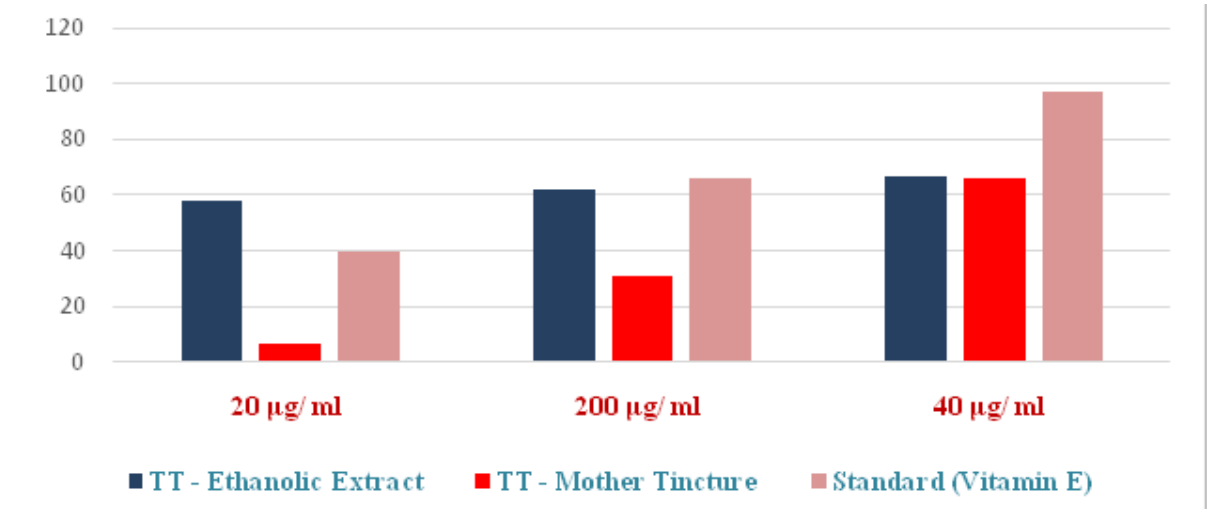


Figure 1. DPPH radical scavenging activity.

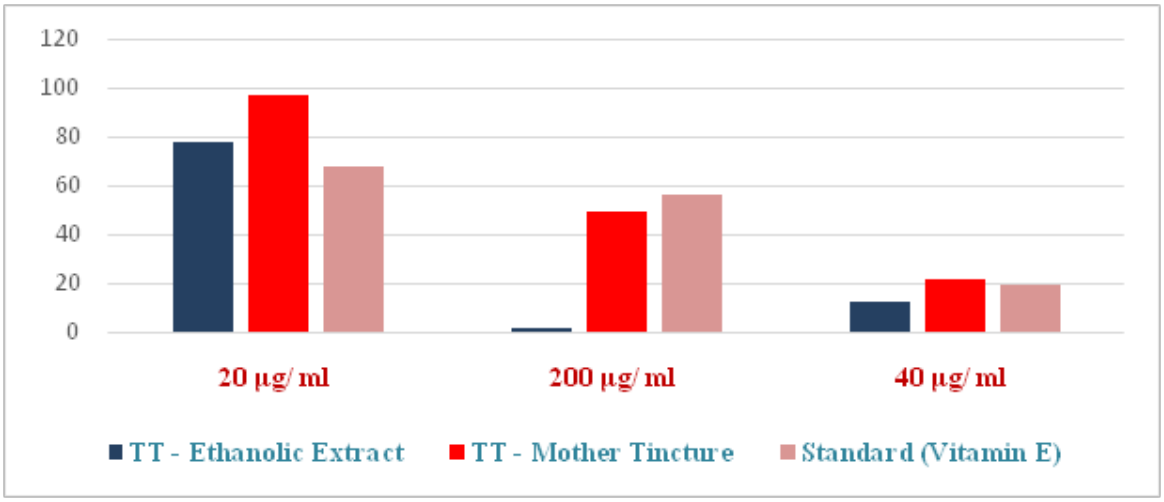


Figure 2. Ferric reducing antioxidant power.

Table 2. Nitric oxide scavenging activity

Sample	Concentration			IC_{50} Value
	Concentration 20 µg/ ml	Concentration 200 µg/ ml	Concentration 40 µg/ ml	
TT-ethanol	62.953 ± 0.1	78.628 ± 0.034	92.597 ± 0.017	11.069
Mother tincture	34.84 ± 0.006	48.753 ± 0.006	63.263 ± 0.125	30.737
standard (Vitamin E)	72.437 ± 0.027	86.484 ± 0.006	98.216 ± 0.006	2.33

96.585 µg/ml, 48.934 µg/ml, and 21.922 µg/ml for the corresponding concentrations. The lowest IC_{50} values of 30.216 µg/ml by ethanol extract and 31.557 µg/ml by mother tincture showed competitive FRAP values, further emphasizing its antioxidant potential.

4. Discussion

The examination of *T. terrestris* extract and homoeopathic mother tincture showed the existence of different plant chemicals such as alkaloids, saponins, tannins, flavonoids, phenols, terpenoids, and glycosides. The presence of alkaloids in *T. terrestris* suggests potential activities such as analgesic and anti-inflammatory effects[32]. Flavonoids are known for their ability to prevent oxidation and reduce inflammation, while phenols possess antibacterial and antifungal activities[33]. Terpenoids exhibit anticancer and anti-inflammatory effects, while saponins have hepatoprotective and immunomodulatory activities[34]. Tannins are known for their astringent and antioxidant properties, and glycosides have diverse physiological activities including cardio-protective and anticancer effects[33]. The concentrations of these phytochemical constituents can differ based on the extraction technique employed and the particular plant part used for extraction.

Further study is required to determine the exact concentrations of these phytochemical constituents in *T. terrestris* extracts and homoeopathic mother tinctures and to explore the possible synergistic effects of these compounds in relation to other medicinal properties attributed to *T. terrestris*.

The potential antioxidant activity of *T. terrestris* has also been explored. The results suggest that the mother tincture consistently demonstrated potent antioxidant activity across all three assays. The TT-ethanol and standard (Vitamin E) also exhibited notable antioxidant properties, with variations in efficacy depending on the specific assay and concentration. This research suggests that *T. terrestris* may be able to clear away free radicals and decrease oxidative harm, which could help protect male reproductive factors from damage caused by factors like Malathion[9]. Additionally, *T. terrestris* has been linked with an increase in sperm production, sexual stamina, and sperm parameters. These findings indicate that *T. terrestris* may have potential as a natural treatment for male infertility, and further research is warranted to explore its full therapeutic potential in this area. In conclusion, *T. terrestris* has been found to have phytochemical constituents that contribute to its antioxidant activity[35]. These antioxidant properties, which have a promising role in improving the sperm parameters both qualitatively and quantitatively along with its effects on spermatogenesis, make *T. terrestris* a promising remedy in the treatment of male infertility[9].

5. Conclusion

Present study also concludes the *T. terrestris* in its both ethanolic extract and homoeopathic mother tincture has the antioxidant activity which helps in improving male fertility. Previous studies have demonstrated that *T. terrestris* has the potential to enhance spermatogenesis, increase testosterone levels, and improve sperm quality and count[36]. Additionally, *T. terrestris* exhibits antioxidant properties that can protect against oxidative damage to sperm cells and improve overall reproductive function[9]. By inhibiting free radicals, stabilizing the blood-testis barrier, and protecting sperm DNA from oxidative stress, *T. terrestris* extract can contribute to the alleviation of male infertility[14,37,38].

Overall, the antioxidant activity of *T. terrestris* holds great potential as a natural treatment option for male infertility. This knowledge can then be used to guide the appropriate use of *T. terrestris* in homoeopathic treatments, ensuring that the remedies are tailored to specific health conditions and maximizing their efficacy. Further research and clinical exploration are necessary to elicit the mechanisms of *T. terrestris* in treating male infertility.

6. Acknowledgement

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7. Author Contribution

Dr. S. Sandhya contributed significantly to the study's design, data acquisition, literature review, and homoeopathic case studies, particularly on *Tribulus terrestris* and its role in antioxidant activity and male

infertility. Dr. G. Senthil Kumaran provided valuable expertise in homoeopathy, ensuring the study's alignment with its principles. Dr. Kirtida Desai, as the primary mentor, guided the research methodology, supervised the study's critical analysis, and oversaw the contributions of both co-authors. All authors have read and agreed to the published version of the manuscript.

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