

Content available at: <https://www.ipinnovative.com/open-access-journals>

International Journal of Clinical Biochemistry and Research

Journal homepage: <https://www.ijcbr.in/>

Original Research Article

Radical scavenging activity of fruit extracts Tribulus Terrestris with natural antioxidant Ascorbic acid: A comparative study

R M Madhumitha Sri¹, S Dhandayuthapani², A Arifa², S Ravichandran^{3,*}¹Dept. of Pharmaceutical Technology, Alagappa College of Technology, Anna University, Chennai, Tamil Nadu, India²Dept. of Chemistry, St. Peter's University, Chennai, Tamil Nadu, India³Dept. of Chemistry, Lovely Professional University, Phagwara, Punjab, India

ARTICLE INFO

Article history:

Received 18-11-2021

Accepted 10-12-2021

Available online 05-01-2022

Keywords:

Radical scavenging activity

Fruit extracts

Menopausal symptoms

Biological activities

Antioxidant

ABSTRACT

The main aim of this work was to analyze through a systematic review the ability of Tribulusterrestris to promote the increase of hormonal levels as a mechanism for the relief of menopausal symptoms. The criteria used for the selection were: studies involving menopausal women and use of Tribulusterrestris as a treatment for sexual dysfunction and / or other menopausal symptoms. The survey of literature shows that women who used Tribulusterrestris had drastic improvement in symptoms such as vaginal lubrication, sensation in the genitals during intercourse and other constructive activities. In this study, we have found a significant increase in bioavailable testosterone after the use of the plant extracts which has great potential in the treatment of sexual intercourse and unique symptoms of menopause.

This is an Open Access (OA) journal, and articles are distributed under the terms of the [Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License](#), which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprint@ipinnovative.com

1. Introduction

In recent times, an extensive research work^{1–10} has been carried out to prove biological activities and the pharmacology of fruit extracts. The purpose of this work is to generate a database for further analysis of the identified phytochemical and pharma-cological properties of this plant to facilitate future research. This will promote in confirmation of its conventional use alongwith its utility resulting to higher revenues from the plant. Saurabh Chhatre Tribulus terrestris, commonly called as Gokshur or puncture vine, has been used in the Indian and Chinese systems of medical for treatment and cure of various diseases for mankind.

The plant parts consists of a variety of chemical constituents which are medicinally important such as flavonoids, flavonol glycosides, steroidal saponins and



Fig. 1: Plant material having scavenging activity

alkaloids. It possess diuretic, antidiabetic, absorption enhancing central nervous system, hepatoprotective, anti-inflammatory, analgesic, anticancer and antibacterial

* Corresponding author.

E-mail address: ravichandran.23324@lpu.co.in (S. Ravichandran).



Fig. 2: Tribulus terrestris

activities. The plant diversity is dominated by dicotyledons. The pharmacological uses of the plants are commonly attributed to their essential oils having more pharmacological activities such as prevention and cure of cancer and cardiovascular diseases. This kind of effects are may be due to its high structural diversity of the essential oil constituents.

At the present study, fruits of the plant, Tribulus terrestris is collected, dried and powdered. The constituents present in the powder is to be identified by chemical tests the compounds were identified by their UV and IR spectra The anti oxidant property towards nitric oxide free radical scavenging was also studied.

2. Experimental Analysis

2.1. Collection of plant material

The plant material collected from Kadambathur in Thiruvallur District of Chennai and the specimen was kept in laboratory of Sri Sai Ram Siddha Medical College for further process. Then the collected plant species were allowed to dry in sunshade for a period of one week and after that it was crushed into fine powdered.



Fig. 3: Collection of plant material

About 200 gm of powder was weighed and soaked in methanol for one day (24 hrs) then it was poured in to soxhlet apparatus and allowed to boil for 1 hr.

The solvent is heated to reflux. The chamber containing solid material slowly fills with warm solvent. Some of compound dissolves in the warm solvent. When the Soxhlet chamber is nearly full, the chamber is emptied by the siphon. The solvent is returned to the distillation flask. After extraction the solvent is removed, by using a rotary evaporator. The non-soluble portion of the extracted solid remains in the thimble and is removed. This extract is refereed as chloroform extract.

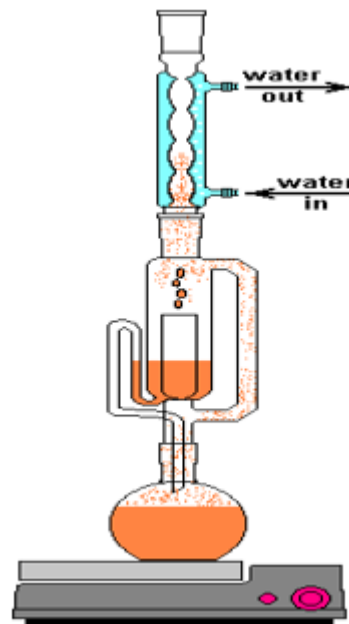


Fig. 4: Soxhlet chamber used for plant extraction

2.2. Chromatography separation

A simple straight glass tube tapered at the bottom was used as column in our study. The adsorbent was supported on a plug of cotton. The cotton piece was kept in position in the tube. The tube was then clamped vertically and a slurry of adsorbent is prepared with the extract was added in portions, so that the tube was packed uniformly with the adsorbent. The adsorbent added was pressed from above with the flattened glass rod, before the next portion was added. This was continued till nearly two third of it was filled. The column was covered by a plug of cotton wood. 10 ml Petroleum ether was added through the mouth [eluent-1]. The developed chromatogram was treated with succession of solvents having increasingly powerful eluent action. The various portions of the column were thus washed out one by one and collected in different receivers. Gradual elution using 100 ml of each solvent and solvent mixture was carried out for the collection of various fractions. The name of the solvents used and the series of fractions collected in this method are given Table 1.

Table 1: The name of the solvents used

S.No.	Eluent system	Bottle number
1.	10 ml Petroleum ether[100%]	1
2.	10 ml Petroleum ether[100%]	
3.	5 ml Petroleum ether +5 ml chloroform [50% Petroleum ether]	2
4.	5 ml Petroleum ether +5 ml chloroform [50% Petroleum ether]	
5.	10 ml chloroform [100%]	3
6.	10 ml chloroform [100%]	

2.3. Phytochemical screening

The prepared extract was subjected to phyto chemical screening. The following tests were carried out.

2.4. Tests for alkaloids- Morquies test

For detecting the alkaloids 2 – 3 ml of the extract was added with Ammonical chloroform and the mixture was stirred for one minute. Extraction of alkaloids from the extract was accomplished by shaking the solution with 0.5 ml of 2 N-H₂SO₄ and separation of the acid layer by means of a dropper. A few drops of solution were tested with the following alkaloidal reagents. A small quantity of the solution was placed in a glass plate and allowed to evaporate to dryness. A drop of Morquies reagent (HgCl₂ + KCN) was added and the colour was observed. Appearance of Reddish colour which turns blue indicates the presence of alkaloids.

2.5. Test for Terpenoids (Leibermann Buchard test)

2 ml of extract was dissolved in chloroform and to this 2 drops of acetic anhydride was added and concentrated sulphuric acid was added along the sides of the test tube and the colour was observed. Appearance of red colour indicates the presence of terpenoids.

2.6. Test for Flavanoids (Shinoda's test)

2 ml of extract was warmed and to the warmed solution a piece of Magnesium ribbon was added followed by 2 drops of concentrated HCl drop by drop. Absence of orange or yellow colour indicates the presence of flavanoids.

2.7. Phytochemical screening

5 ml of extracts were dissolved in 100 ml of ethanol and tested for phytochemical components like alkaloids, Terpenoids, Flavanoids and Phenols. The following is the results obtained (Table 2).

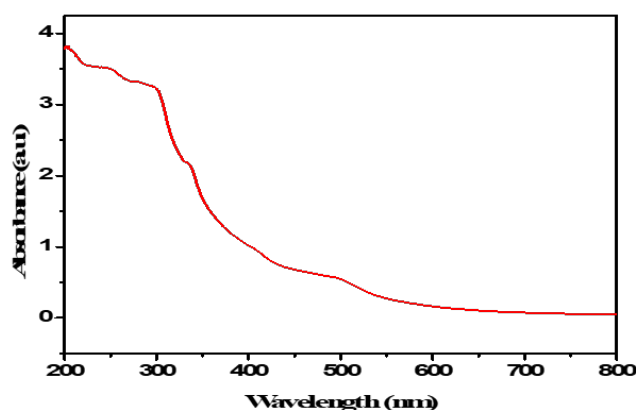
The Table 2 shows that the extract contains alkaloids and flavonoids

Table 2: Phyto chemical screening results

	Name of the tests	Extract	Inference
1	Morquies test:	+	Presence of alkaloids
2	Leibermann Buchard test	-	Absence of Terpenoids
3	Shinoda's test	+	Presence of Flavanoids

2.8. UV spectra

The UV-Visible spectrum of the product was recorded using Labtronics UV Visible Spectrophotometer (Range 200-1000 nm) using carbinol as solvent. The absorption maxima found out in various compounds are summarized below.



Graph 1: UV spectra

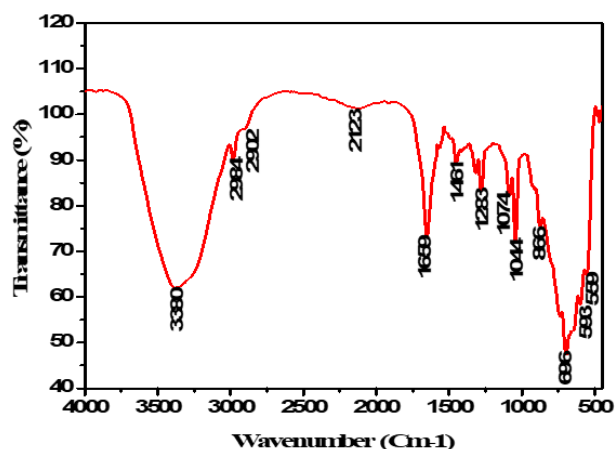
The ultraviolet-visible electronic spectra of the prepared compound showed absorption bands that could be attributed to $\pi \rightarrow \pi^*$ electronic transitions. These transitions are assigned in relevance to the structures of the compound.

2.9. IR spectra

Infrared spectroscopy is one of the most powerful analytical techniques which offers the possibility of chemical identification. The IR spectrum of the product was recorded in solid state (KBr pellet) using Shimadzu FTIR spectrophotometer. IR absorption frequencies (cm⁻¹) of the synthesized compound are given below:

Table 3: The characteristics of IR spectra

S.No	Wavelength in cm ⁻¹	Characters
1.	3266cm ⁻¹	O-H stretching
2.	1656cm ⁻¹	C-H stretching
3.	1561 cm ⁻¹	C=O stretching
4.	1238cm ⁻¹	C-O-H bending
5.	1157 cm ⁻¹	C-O stretching



Graph 2: IR spectra

2.10. Radical Scavenging evaluation

A scavenger is a chemical substance which removes unwanted reaction products. This can be detected by ESR spectra. It is formed by heterolytic cleavage of a bond. Compounds disposing off radical species, scavenging them, suppressing their formation and opposing their action, are called anti oxidants. Examples for such free radicals formed in our body are, Nitric oxide free radical, Hydroxide free radical, Super oxide free radical and Peroxy free radical.

2.11. Procedure

The antioxidant activity test can be conducted with a reaction mixture containing 2 ml of sodium nitroprusside with 0.5 ml of phosphate buffer saline and changing the volumes of aqueous extract (10,20,30,40,50 μ g) and incubated at 25°C for a period of 15 minutes. After the incubation, 0.5 ml of reaction mixture added to 1 ml of sulphuric acid and allowed to stand for complete diazotization. To this 1 ml of Naphthylethylene dimine dihydrochloride (NEDD) added, the absorbance of which was recorded at 540 nm and compared with standard ascorbic acid. IC₅₀ value is the concentration of sample needed to inhibit 50% of NO radical production. The percentage of NO• radical scavenging can be calculated as per the formula given below:

$$\begin{aligned} \text{\% of scavenging [NO•]} &= \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100 \\ &= \frac{\text{Optical density of control} - \text{optical density of test}}{\text{optical density of control}} \times 100 \\ &= \frac{A_0 - A_1}{A_0} \times 100 \end{aligned}$$

Where, A₀ was the absorbance of the control and A₁ was the absorbance in the presence of the sample of extract or ascorbic acid. The results obtained are tabulated below.

Table 3 shows the optical density values of different concentrations of —t with the reagents. The values indicated that when the concentration increases, the optical density

Table 4: Optical density of sample and standard

S.No	Concentration of sample (ml)	Optical density	
		Sample	Ascorbic acid (standard)
1	0	0.21	0.18
2	2	0.22	0.24
3	4	0.24	0.25
4	6	0.26	0.25
5	8	0.30	0.29
6.	10	0.52	0.66

Table 5: Calculation of inhibition sample

S.No	Concentration ml of solution	A ₀	A ₁	A ₀ -A ₁	Inhibition $\frac{A_0 - A_1}{A_0} \times 100$
1	2	0.46	0.44	0.04	8.69
2	4	0.46	0.38	0.08	17.39
3	6	0.46	0.37	0.09	19.57
4	8	0.46	0.35	0.11	23.91
5	10	0.46	0.32	0.14	30.43

Table 6: Calculation of inhibition standard

S.No	Concentration ml of drug solution	A ₀	A ₁	A ₀ -A ₁	Inhibition $\frac{A_0 - A_1}{A_0} \times 100$
1	2	0.18	0.14	0.04	22.2
2	4	0.18	0.10	0.08	44.4
3	6	0.18	0.09	0.09	50
4	8	0.18	0.07	0.11	61.1
5	10	0.18	0.06	0.12	66.7

decreases which indirectly shows the absorption increases. That is the free radicals formed will be more scavenged at higher concentration. Thus, we can conclude that the NO free radical scavenging property is a linear dependence of extract concentration. This is clearly shown in the plot of concentration versus OD.(Graph 1)

Table 4 illustrates the percentage inhibition of the sample. Analysis of the observed value made us to conclude that the inhibitory property of the sample increases with concentration. This is also indicated from Graph 2 which is the plot of concentration versus percent inhibition.

Table 5 illustrates the percentage inhibition of control (ascorbic acid). Analysis of the observed value made us to conclude that the inhibitory property of control (ascorbic acid) increases with extract concentration. This is also indicated from graph 4 which is the plot of concentration versus percent inhibition.

3. Conclusions

In the present study, Tribulus Terrestris plant was collected, dried and powdered. The powder was subjected to Soxhlet extraction using water as solvent. The extract was concentrated and the constituents were separated by

column chromatography using 100% Petroleum ether, 50% Chloroform +50% Petroleum ether, and 100% Chloroform as eluent. The various fractions were collected and TLC was run with chloroform as developing solvent. The fraction which gave only one spot in TLC test was used to identify the specific constituent. Test for alkaloid, Terpenoid and flavonoids, were carried out. The phyto chemical analysis shows that it contains alkaloid and flavonoids. Antioxidant activity found to be low. It is essential. To conduct clinical trial study, to support its antioxidant property for therapeutic condition. Thus efforts need to be focus the pharmacological potential of this plant.

4. Source of Funding

None.

5. Conflict of Interest

The authors declare no conflict of interest.

References

1. Mohammed MS, Alajmi MF, Alam P, Khalid HS, Mahmoud AM, Ahmed WJ. Chromatographic finger print analysis of anti-inflammatory active extract fractions of aerial parts of Tribulus terrestris by HPTLC technique. *Asian Pac J Trop Biomed*. 2014;4(3):203–8.
2. Gama CR, Lasmar R, Gama GF, Abreu CS, Nunes CP, Geller M, et al. Clinical Assessment of Tribulusterrestris Extract in the Treatment of Female Sexual Dysfunction. *Clin Med Insights Womens Health*. 2014;7:45–50.
3. Khaleghi S, Bakhtiari M, Asadmobini A, Esmaeili F. Tribulus terrestris Extract Improves Human Sperm Parameters In Vitro. *J Evid Based Complementary Altern Med*. 2017;22(3):407–12.
4. Elhamakhtari. Tribulusterrestris for treatment of sexual dysfunction in women: randomized double-blind placebo - controlled study. *DARU Journal of Pharmaceutical Sciences*. 2014;22:40–53.
5. Adaay MH, Mosa A. Evaluation of the effect of aqueous extract of Tribulusterrestris on some reproductive parameters in female mice. *J Mater Environ Sci*. 2012;3(6):1153–62.
6. Pandey R, Bhavani S, Krishna B. Tribulus terrestris Fruit Extract Protects Against Oxidative Stress-Induced Apoptosis. *Pharma Biol*. 2007;45(8):619–25.
7. Shantanu U, Dighe RS, Jadhav DN. Biological Potential of Tribulusterrestris. *J Drug Deliv Ther*. 2020;10(3):262–4.
8. Dakshayini PN, Basha PM. Tribulusterrestris fruit extract improves antioxidant defense in female reproductive tract: A comprehensive study in diabetic rats. *J Innov Pharm Biol Sci*. 2018;5(2):101–7.
9. El-Sheikh TMY, Al-Fifi Z, Alabboud MA. Larvicidal and repellent effect of some Tribulusterrestris L., (Zygophyllaceae) extracts against the dengue fever mosquito. *J Saudi Chem Soc*. 2016;20(1):13–9.
10. Taavoni S, Ekbatani NN, Haghani H. Effect of Tribulusterrestris, ginger, saffron, and Cinnamomum on menopausal symptoms: a randomised and controlled clinical trial. *Menopause Rev*. 2017;16(1):19–22.

Author biography

R M Madhumitha Sri, Student

S Dhandayuthapani, Assistant Professor

A Arifa, Student

S Ravichandran, Associate Professor

Cite this article: Sri RMM, Dhandayuthapani S, Arifa A, Ravichandran S. Radical scavenging activity of fruit extracts Tribulus Terrestris with natural antioxidant Ascorbic acid: A comparative study. *Int J Clin Biochem Res* 2021;8(4):289-293.