Ann. For. Res. 66(2): 173-184, 2023
ISSN: 18448135, 20652445

ANNALS OF FOREST RESEARCH
www.e-afr.org

COMPARATIVE ASSESSMENT OF PHARMACOLOGICAL POTENTIAL AND ANTIOXIDANT EFFICACY OF BIOACTIVE ISOLATED FROM TRIBULUS TERRESTRIS: IN VITRO EVIDENCES

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Abstract:

Tribulus terrestris is an angiosperm weed that grow attach to the ground, It utilized in Ayurveda as an aphrodisiac and other reproductive ailments. Different solvent extractions of *Tribulus* plant parts have been shown to possess significant biological activities. Though, fruits are traditionally used in the herbal preparations for treatment. Also leaves, stems and roots have been reported to contain pivotal bioactives and therapeutic efficiency. Hence, here we set out the presence of bioactive and their in vitro antioxidant efficacy in a comparative evaluation among different part like stem, root, leaves and fruits. A variety of approaches were employed to determine the anti-oxidative potential for the extraction. Results and data presented in this work showing that fruits of *Tribulus terrestris* plant possess strong antioxidant activity in comparison to other parts which can give focus in using in herbal preparation treatment and medication.

Key words: *Tribulus terrestris*, *antioxidant, aphrodisiac, bioactive, herbal medication.*

Introduction:

Tribulus terrestris is an angiosperm annual weed herb *(phymally; Zygophyllaceae)* that grows in arid environments all over the world. It used as an aphrodisiac action and for other reproductive disorders, this plant come in many name in different part of the words, in Ayurveda

came as *(Puncture vine, caltrop) mean yellow vine ,also goathead, abrojo de Tierra Caliente (in Spanish), gokshura (Sanskrit), Burzeldorn (in German), croix-de-malte (in French) also in Europe, North America, Africa, Australia, and South Asia. Tribulus terrestris* plant can be found in temperate and tropical climates. It gets its name from the extremely sharp thorns that sprout from the seed. The fruits of plant are the most widely utilized portion, but young stem, leaves, and roots have all been used in herbal medicine. Tribulus ripe and dried fruits in general are collected and aqueous decoction is used for therapy (Qureshi *et al*., 2014 , Zhu *et al*., 2017) .

Tribulus extracts are said to increase the levels of luteinizing hormone in the body hormone and also that send a signal through the body for testosterone production; hence it has become popular herbal remedy to increase libido and as a natural remedy for treatment infertility. Its ability to relax smooth muscles and by away allowing greater blood flow to the genitals, that makes the Tribulus an excellent herbal remedy for erectile dysfunction. It appear that there is another attribution for increasing body mass via the testosterone generation; hence Tribulus is being used by body building enthusiasts (Reshma *et al.,* 2019).

Tribulus has been used to treat a wide range of illnesses in the past of Europe, China and India, herbal medicine practitioners have suggest to using Tribulus as a natural treatment for lowering cholesterol and hypertension. In addition, it has also been used traditionally against constipation.

Interestingly, Tribulus is used to treat many types of headaches and to stimulate the central nervous system. This plant is reported to contain a whole battery of bioactive principles. According to reports, kaempferol, kaempferol 3-glucoside, kaempferol 3-rutinoside, tribulosin, harmine, and steroidal Saponins are Tribulus' primary bioactive components. These chemicals were discovered in various Tribulus plant parts (Saurabh *et al*., 2014)

The chemical constituents identified from Tribulus belong to class of carbohydrates, amino acids, peptides, glycosides, tannins, terpenoids, phenols, aponins, alkaloids, steroids and flavonoids (Alamgir, 2014) These components individually and synergy are reported to possess a variety of biological activities viz.. antioxidant, anti-inflammatory, hypocholesterolemic, anti-microbial and anti-tumor properties. It has been demonstrated that Bulgarian Tribulus terrestris lowers lipid peroxidation levels. Additionally, it has been demonstrated to boost the activity of superoxide-dismutases (SOD), a group of enzymes crucial to the defense against free radicals. Additionally, this plant has been shown to improve the liver's ability to reduce its glutathione content (Jiang et al., 2014).

Different solvent extractions of Tribulus plant parts have been shown to possess significant biological activities like diuretic activity (in vivo), aphrodisiac (ex vivo), anti-urolithic (in vivo), cardiac disorders (in vitro), antibacterial (in vitro), radioprotection action (in vivo), larvicidal (in vivo), anticariogenic (in vivo), immunomodulatory (in vivo), anti-hypertensive (in vivo),

spasmolytic (in vitro), anti-inflammatory (in vitro), hepatoprotective (in vitro), anti-hypertensive activity (in vitro) and antispasmodic activity (in vitro) (Qureshi *et al*., 2014 , Zhu *et al*., 2017, Jiang *et al*., 2014) .

Figure. 1. Photograph of *Tribulus terrestris* (A) and Fruits (B).

It has been discovered that using Bulgarian Tribulus terrestris supplements resulted in lower levels of lipid peroxidation, which is unmistakably a sign of its effectiveness. Additionally, this herb has some antioxidant advantages, including elevated glutathione levels and SOD activity, which may be the cause of elevated antioxidant activity. B. Tribulus terrestris is not frequently used for these antioxidant advantages because stronger antioxidant supplements are available, but it is nonetheless an added advantage linked to the use of Bulgarian Tribulus terrestris.

One of the most common chemical compounds discovered in *T. terrestris* is protodioscin (PTN). In sated of, it has been proven to have potent COX-2 inhibitory action (Santos *et al.,* 2019)

Though, fruits are traditionally used in the herbal preparations for medical treatment, also leaves, stems and roots have been reported to contain pivotal bioactives and therapeutic efficiency. Hence, here we set out the presence of bioactive and their action in vitro antioxidant efficacy in a comparative evaluation among leaves, stem, root and fruits.

Materials and Methods

Chemicals: All fine chemicals were analytical grade and obtained from Sigma Aldrich, USA and other chemicals were locally procured.

Preparation of extracts of *Tribulus terrestris* extract

Tribulus terrestris plant was identified and collected from the local area, which was growing as weed. Plant parts were separated as leaves, stem, roots and fruits. They were separately dried in shade and powdered. The powder of plant parts were separately soaked in 70% methanol (70methanol+30Water) and macerated at room temperature for 6 hours, filtered and the filtrate

collected. The filtrate was vacuum evaporated to obtain dry extract powders. The dry powders were separately dissolved in dimethyl sulphoxide (DMSO) for the uses in assays.

Estimation of Phenols

Total phenols were estimated by (Chandra and Avula , 2014) incubating an aliquot of the extract with 0.2 ml of Folin Ciocalteu reagent followed by 2% Sodium carbonate and the optical density was measured at 660nm. The amount of phenols in the extracts were expressed as mg gallic acid equivalents/ g (mg GAE/g).

Estimation of flavonoids

Total flavonoids in the extracts were assessed by (Chandra and Avula , 2014) incubating an aliquot of the extract with 2% aluminum chloride for 30 minute and the color measured at 560nm. The flavonoid content was expressed as milli gram equivalent of Catechin/ g (mg CE/g).

Estimation of tannins

The tannins in the extracts were estimated using Folin-Denis reagent and measured at 725 nm (7). Tannins were calculated by using slandered tannic acid and expressed as mg Tannin equivalents/g (mgTE/g (Ogawa and Yazaki 2018) .

DPPH scavenging assay

The DPPH free radical scavenging assay (Campos and Lissi ,1997) was performed by mixing aliquots (100µl) of different concentrations of the samples with 100µl DPPH (0.1mM, in 95% Ethanol) and incubating for 30min. An ELISA plate reader was used to measure the absorbance at 517nm.(Molecular devices E750). The scavenging property was compared to butylated hydroxy toluene (bHT) and data expressed as IC50 value.

ABTS scavenging assay

ABTS scavenging efficacy was estimated according to Briefly (Marcocci *et al.,* 1994), 1.0 mM AAPH 2.5 mM ABTS in PBS (pH 7.4) was heated in a water bath at 70ºC for 2 min.With additional PBS, the resulting blue–green ABTS radical solution was adjusted to an absorbance of 0.650 at 734 nm.20µl of the samples were added to 980 ml of the ABTS radical solution. The mixture was incubated at 37ºC in dark for 10min, and it measured at optical density 734 nm. The color was measured and the result was expressed as IC50 values in comparison to the standards Vitamin C and Trolox .

***Nitric oxide* scavenging**

As previously stated, the potential of S. delicatula extracts to scavenge nitric oxide was found. (Aruoma ,1994). Aliquots of different concentrations of the test samples were incubated with Sodium nitroprusside (5mM) in a 96 well plate for 90min at room temperature. Griess reagent in an equivalent volume (1% Sulphanilamide and 0.1% N–ethylene diamide in 5% orthophosphoric acid) was added and absorbance read at 540nm using an *ELISA* plate reader. Ascorbic acid was used as standard and expressed as IC50 value.

Preformed hypochlorous acid radical scavenging assay

Hypochlorous acid radicals (HOCl) were prepared by (Cotelle *et al* ., 1996)adjusting the pH of 1% (v/v) solution of Sodium hypochlorite (NaOCl) to 6.2 with (0.1M) Sulphuric acid. The working stocks were prepared by assessing the concentration using molar extinction co-efficient of $100M^{-1}cm^{-1}$ (235nm). The reaction mixture contained in mM: taurine-10; HOCl-1; PBS-100 (pH 7.4) and test samples (variable concentrations) in a final volume of 1ml. 10 ml of potassium iodide was added to each tube, and yellow coloration developed was read at 350nm. The results were expressed as IC_{50} value.

Hydrogen peroxide scavenging assay

Test samples were incubated with 10mM hydrogen peroxide for 30 min and the absorbance measured at 240nm. The ability was expressed as percent inhibition.

Inhibition of lipid peroxidation in vitro

Test samples were incubated with 10% oil (in SDS reagent) at 37◦C for 1 hour. It was followed by addition of thiobarbituric acid reagent and kept in boiling water bath for 20min. The absorbance measured at 535 nm. The percent inhibition was calculated in comparison to standard BHT (Ohkawa *et al.,* 1979)

Statistical analysis: Data are presented as mean, percentage ± and Standard error. Data from three different experiments with six replicates per concentration for each. The data analyzed by one way ANOVA followed by post hoc Tukey's test using Graphpad Prism 5.0

Results

Tribulus terrestris was identified for plant parts and were separated as leaves, stem, roots and fruits, they were separately dried in shade and powdered and other chemical analyses were carried out, data are presented as mean percentage ± Standard error and data from three different experiments each with six replicates per concentration were statically analyses. The total phenols, total flavonoid and total tannins were estimated for different plant part are shown in figure (2,3and4) The data analyzed by one way ANOVA followed by post hoc Tukey's test using Graphpad Prism 5.0**.** The DPPH, Hydrogen peroxides ABTS , Nitric acid , Hypochlrous acid and Lipid peroxides scavenging effects of the *Tribulus terrestris* plant parts are shown in figure (5,6,7,8,9, and 10)

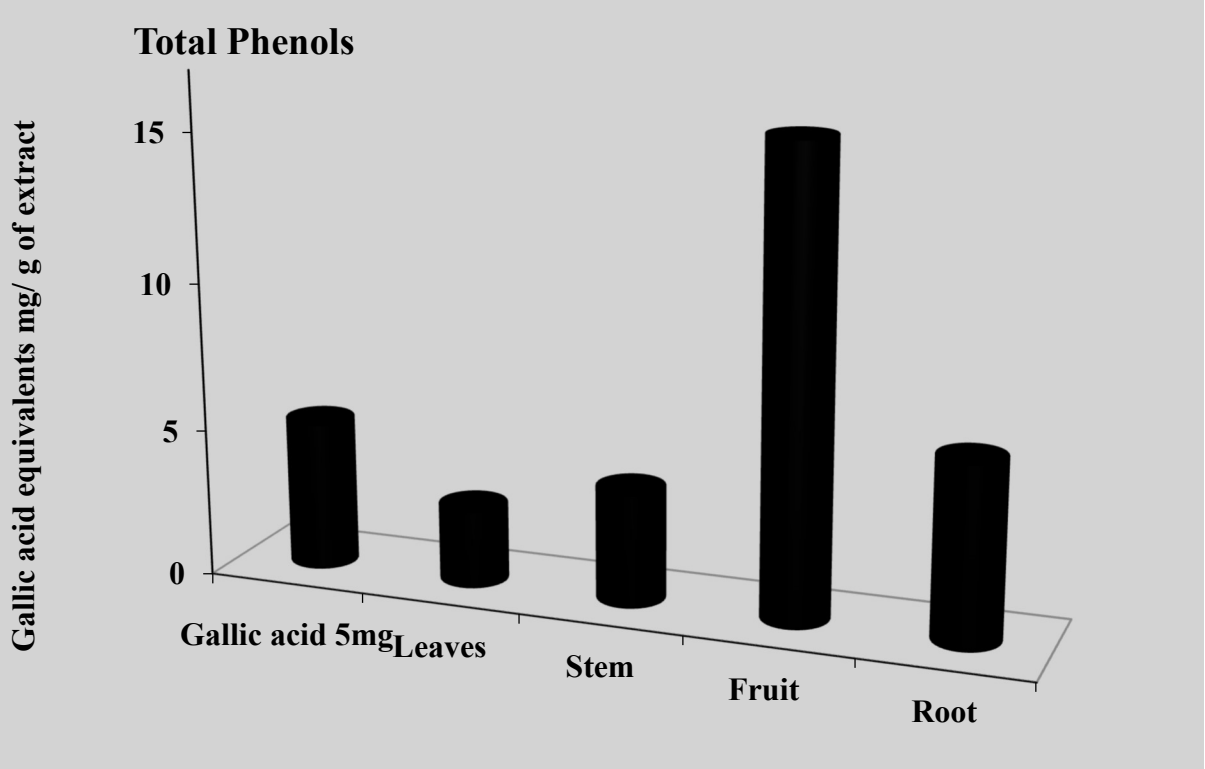

Figure 2: Phenol content of extracts from *Tribulus terrestris* plant parts.

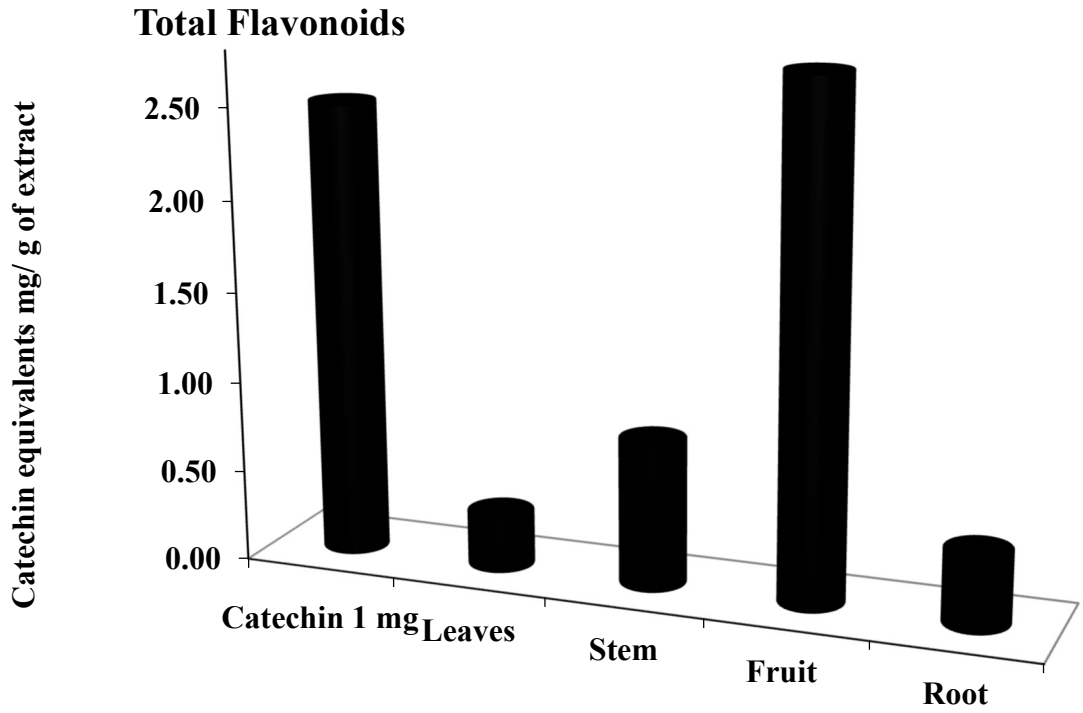

Figure 3: Flavonoid content of extracts from *Tribulus terrestris* plant parts.

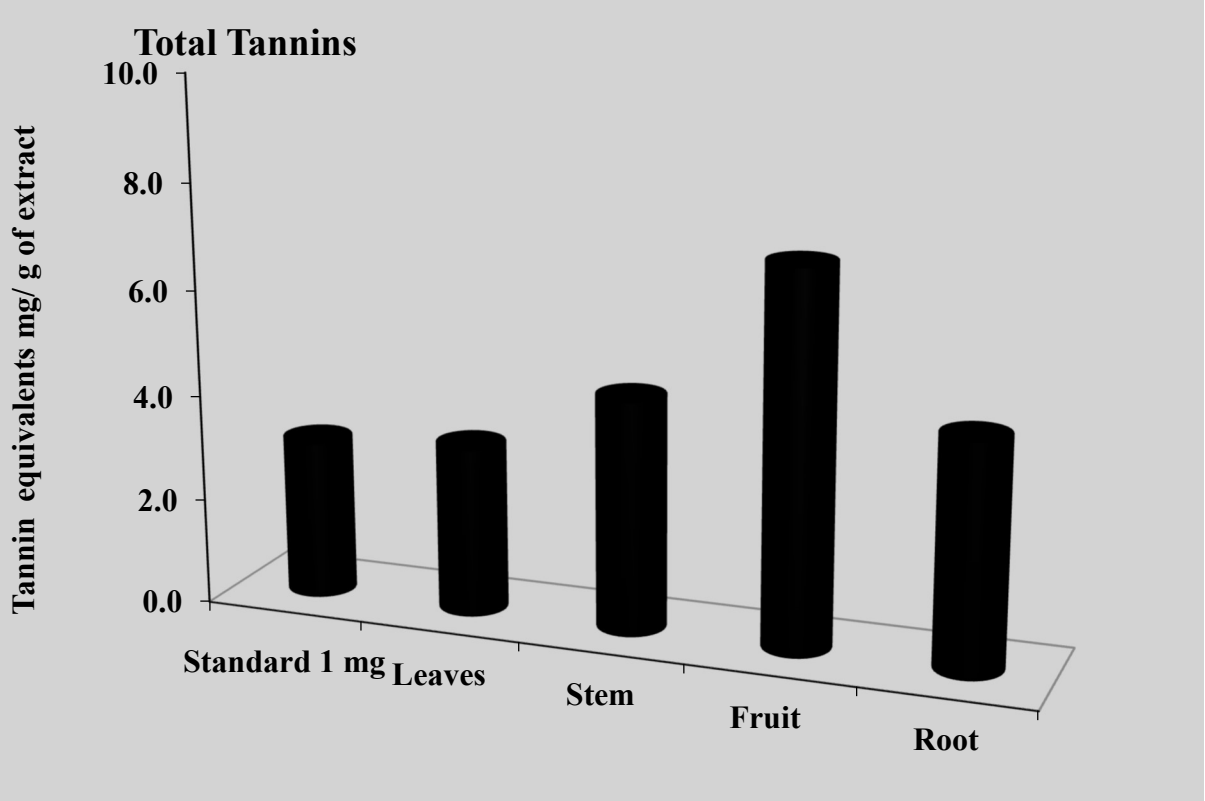

Figure 4: Tannin content of extracts from *Tribulus terrestris* plant parts.

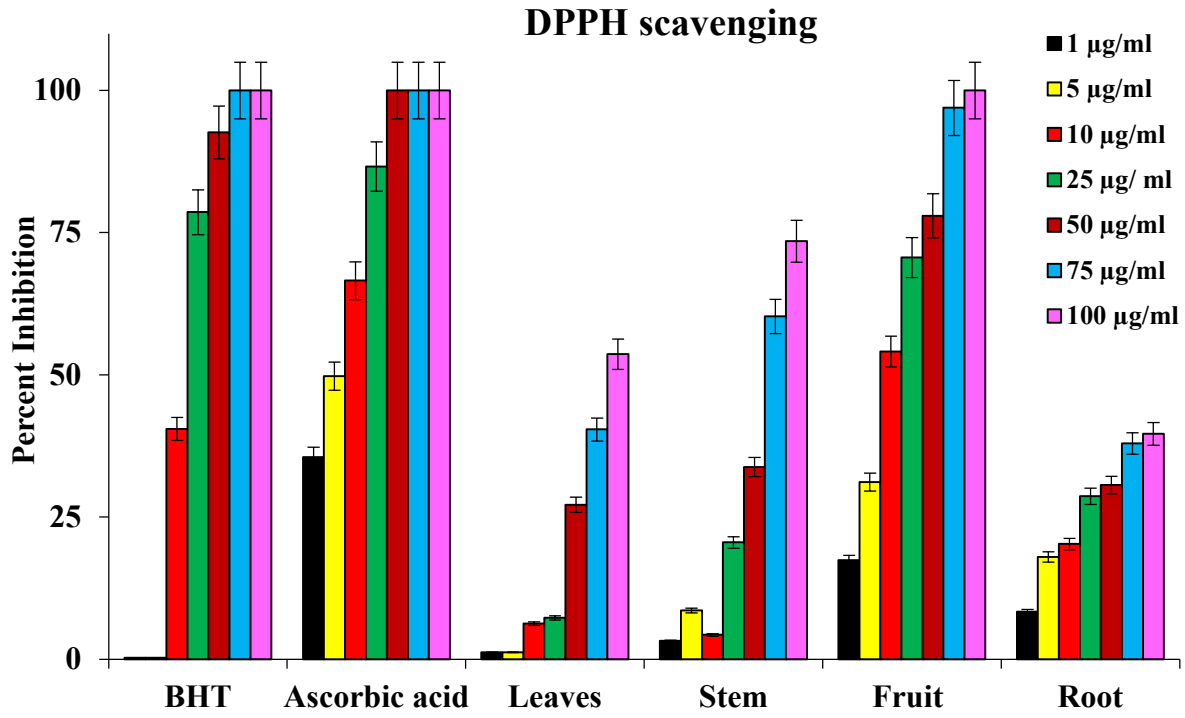

Figure 5: Showing DPPH scavenging effects of the *Tribulus terrestris* bioactives parts.

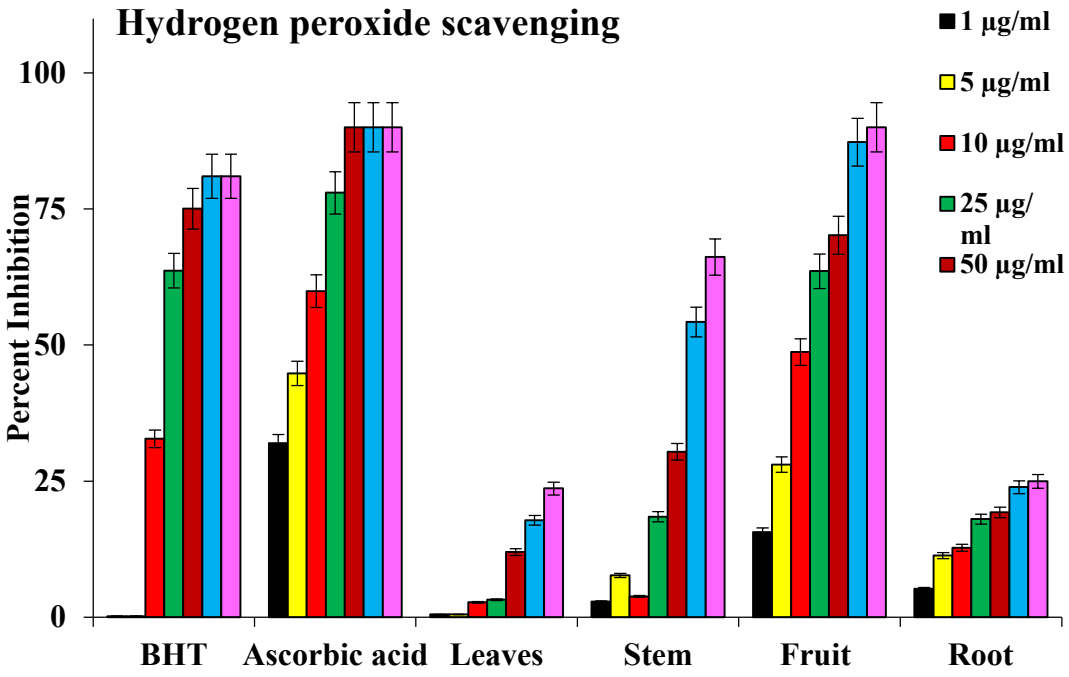

Figure 6 : Showing Hydrogen peroxide scavenging effects of the *Tribulus terrestris* bioactives parts.

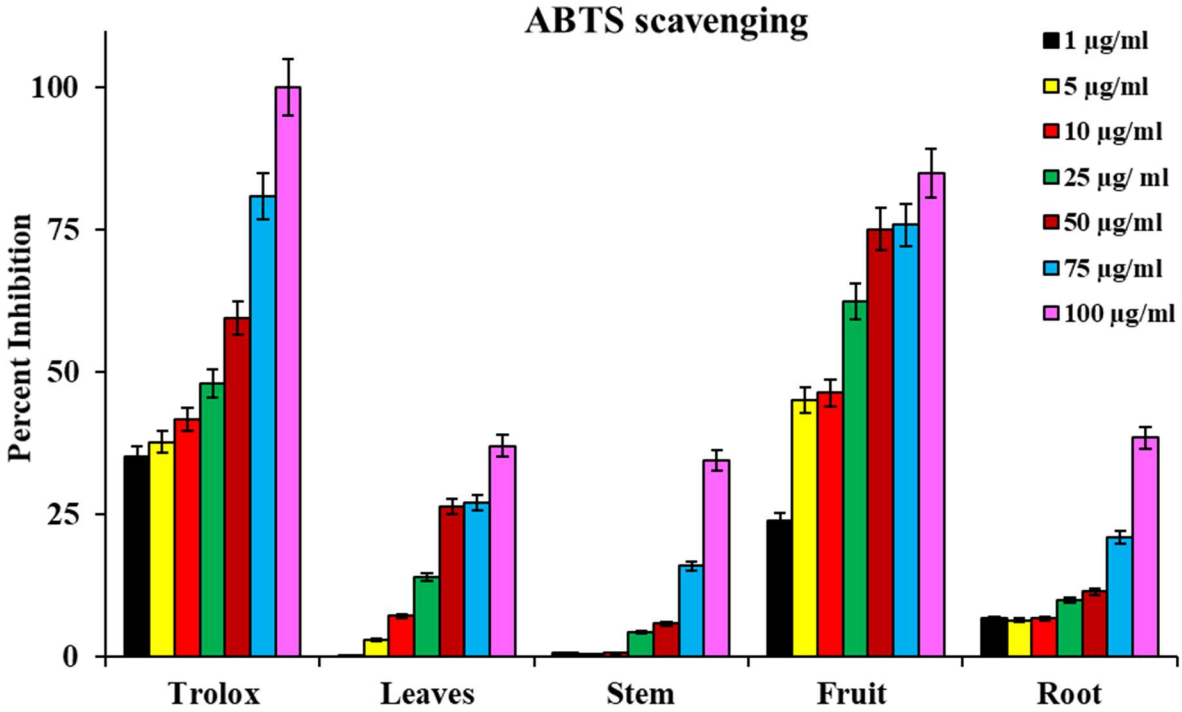

Figure 7: Showing ABTS scavenging effects of the *Tribulus terrestris* bioactives parts.

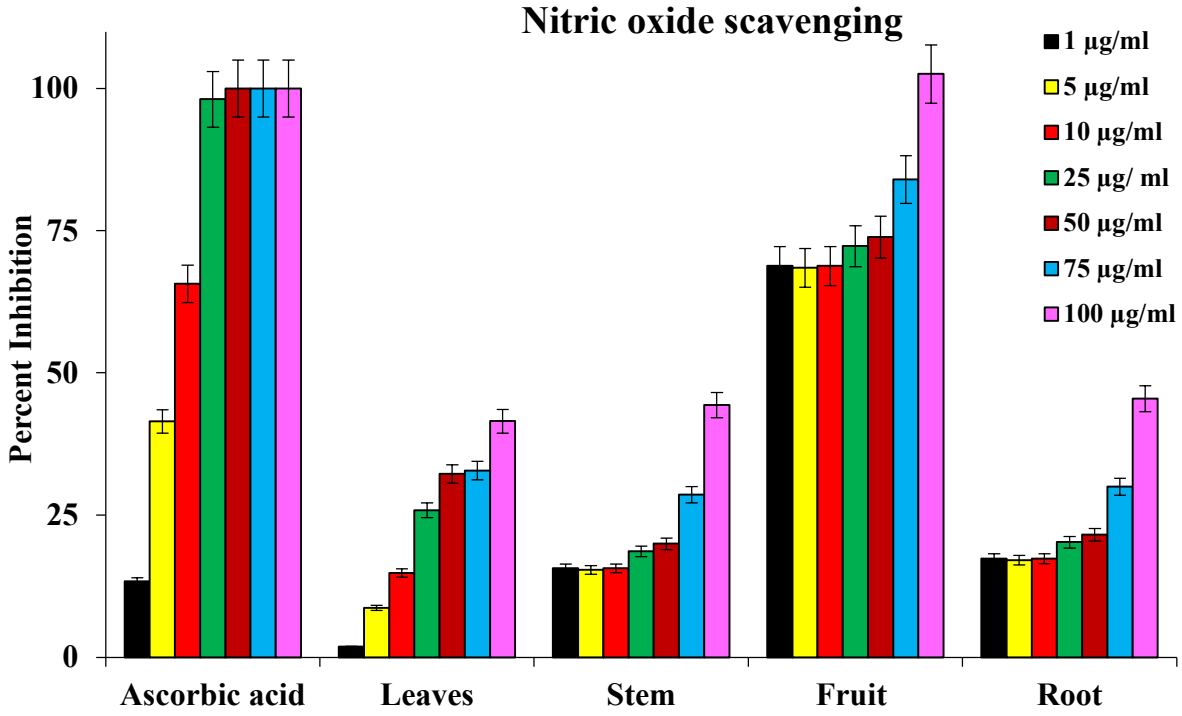

Figure 8: Showing Nitric oxide scavenging effects of the *Tribulus terrestris* bioactives.

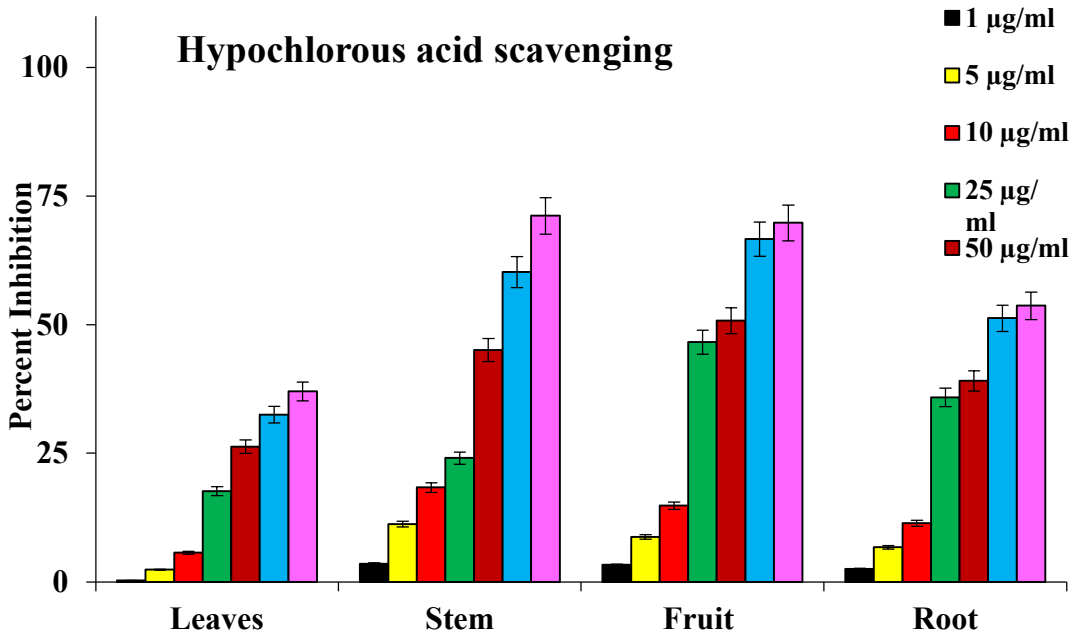

Figure 9: Showing Hypochlorous acid scavenging effects of the *Tribulus terrestris* bioactives.

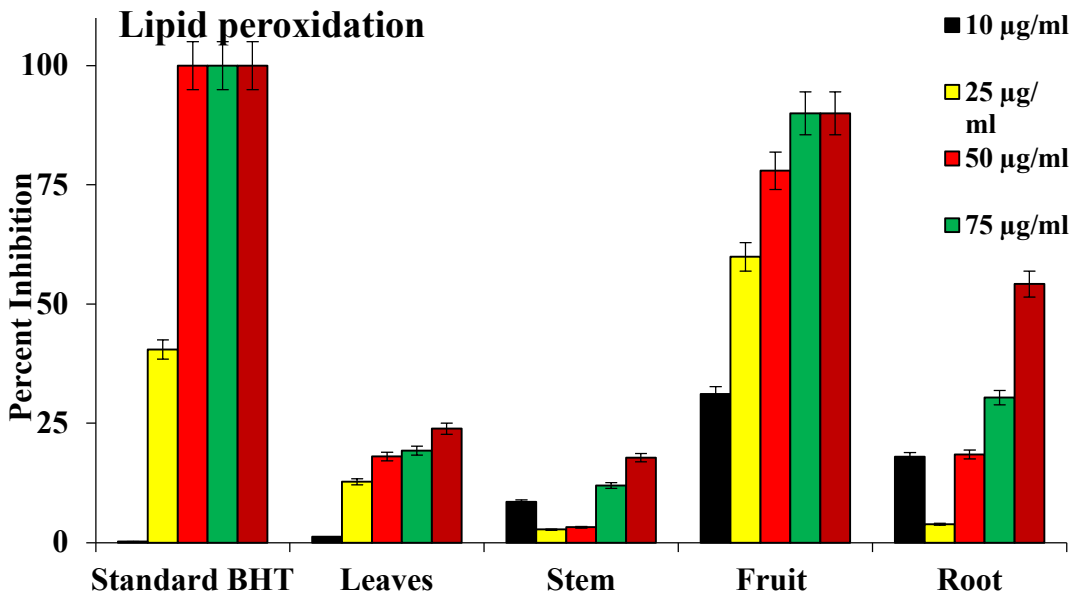

Figure 10: Showing Xanthine oxidase inhibition properties of the *Tribulus terrestris* bioactive.

Results for different part of *Tribulus terrestris* extracts for ABTS and DPPH free radical assay systems were used since they are the most popular methods to assess the anti-oxidative property of the test compounds (figure 5and 7). These two stable radical chromogens (the violet DPPH radicals and the blue-green ABTS radical anions) are easy to use, because it gives a high level of sensitivity and allow for analysis large number of samples in the same time. In the DPPH scavenging assay, the standards demonstrated an excellent antioxidant activity with IC50 values at 14.5 (BHT) and 13.3µg/ml (ascorbic acid). While the IC50 of fruit extract was at 18.0µg/ml which prove it to be an efficient antioxidant. (figure 5 and 7), also the ABTS assay fruit extract demonstrated there is a significantly excellent antioxidant having that an IC50 of 25.8µg/ml (figure. 7).

An additional array for assays was employed to assess the ability of the samples to inhibit the generation of other types of free radicals. Hypochlorous radicals and nitric oxide radicals are major reactive species formed in metabolic reactions *in vivo* a variety of compounds (figure 8 and 9). It's have been demonstrated scavenge reactive species like superoxide, hydroxyl ions, hydrogen peroxides etc... In this study the leaves and fruit extract provide to be excellent scavengers of Nitric oxide radicals (figure 8). However only fruit extract efficiently scavenged as hypochlorous acid radicals. (figure 9). It was also found that the fruit extract also proved to be excellent inhibitors of lipid peroxidation in vitro as estimated by thiobarbituric acid method (figure10).

Discussion

Normal physiological actions such as breathing and some cell-mediated immunological processes generate free radicals on a constant basis.. Indeed, ROS participate directly in defence against infection and also are important coordinators of the inflammatory response and various signal

cascades. The generation of large amounts of ROS results in deleterious effects on intracellular antioxidant defence causing activation of lipid peroxidation, protein modification, and oxidative DNA damage. All the samples demonstrated significant antioxidant properties as tested via the battery of assays. Fruit extract found out to be most efficient antioxidant in comparison to other parts of the plant which its evident come from the lower IC50 values. (Reshma *et al.,* 2019*,* Saurabh *et al.,* 2014, Ogawa and Yazaki , 2018).

Conclusion

The data presented in this study clearly demonstrates that among the plant parts assessed and analysis, fruits of *Tribulus terrestris* possess stronger antioxidant activity.

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