

**PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACTS OF *TEPHROSIA PURPUREA* (L) PERS AND *PHYLLANTHUS NIRURI***Nigam<sup>1\*</sup> R, Arnold<sup>2</sup> R<sup>1\*</sup>Awadesh Pratap Singh University, Rewa (M.P), India<sup>2</sup> Departments of Botany, Govt. Girls P.G. College, A.P.S University, Rewa (M.P), India[reninigam83@gmail.com](mailto:reninigam83@gmail.com)**Abstract**

In recent times much attention has been paid to biologically active plants because of their low production cost and fewer adverse effects compared with allopathic drugs and produce a definite physiological action on the human body. *Tephrosia purpurea* (L) Pers and *Phyllanthus niruri* were used as the traditional medicines in India and other parts of world. Both plants were very useful for various pharmacological and biological activities. However, the use of the herbs is not based on any evidence of their contents. In the present study the effects of 2 types of solvents, water and ethanol were investigated to determine the presence of various phytochemical constituent and *in vitro* antioxidant activities from various parts of *Tephrosia purpurea* and *Phyllanthus niruri*. The reason for selecting *in vitro* method was to minimize the usage of experimental animals. The therapeutic effects of tannins, phenols and flavonoids can be largely recognized to their antioxidant properties. The antioxidant property was determined using DPPH scavenging activity. The study revealed that *T. purpurea* and *P. niruri* extract contained proteins, diterpines saponin, phenol, and flavonoid based on phytochemical screening. In DPPH assays ethanolic leave extract of *P. niruri* possessed highest antioxidant activity with IC<sub>50</sub> value of 36.68 µg/ml as compared to other extract. Meanwhile, *P. niruri* aqueous leave extract showed the activity which was 50.89 µg/ml. Aqueous leave extract of *T. purpurea* possessed the lowest antioxidant activity 57.49 µg/ml. This study confirmed that *P. niruri* extract have great potential as a natural antioxidant source as compared to *T. purpurea* extract.

**Keywords:** *Tephrosia purpurea* (L) Pers, *Phyllanthus niruri*, Total phenolic content, Total flavonoid content, *In vitro* antioxidant activity, DPPH scavenging activity.

## Introduction

India has a very extended, old, secure and diverse cultural living conditions associated with the use of many botanical plants in the officially recognized systems of health such as Homeopathy, Ayurveda, Naturopathy, Siddha, Yoga and Unani. Medicinal plants contain chemical constituent that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs [1]. In present time herbal medicine or drugs from medicinal plants are used in the treatment and cure of sicknesses and diseased conditions. In the recent years, traditional drugs have gained immense popularity as considerable advances have been made in our understanding of the biosynthesis of natural products [2]. Although much progress in the field of herbal medicines, laws regulating these drugs are still in their infant stage. General awareness of natural being safe is not only false but also misleading, as herbal remedies contain pharmacologically active molecules that may show undesirable or adverse reactions [3]. So proper authentication of plant to be used as a drug along with its safety and efficacy studies is necessary to generate data for the acceptability of herbal medicines in the global market [4]. And knowledge of the chemical constituents of plant is helpful in the discovery of therapeutic agents. Reactive oxygen species (ROS) and free radicals (FR) are small molecules naturally generated as by-product of cellular metabolism. Exposure to environmental hazards such as chemicals, radiations and gases increases their production in the body to toxicity levels [5]. ROS leads to damaging the lipids in the cell membranes, proteins in tissues as well as enzymes, carbohydrates and DNA to induce oxidation. This oxidative damage may play a causative role in aging and several diseases which are cancer, cataracts, cognitive dysfunction like myocardial infarction, heart failure, hypertension, atherosclerosis, parkinson's disease, alzheimer's disease, muscular dystrophy, multiple sclerosis, diabetes, rheumatoid arthritis, chronic inflammatory diseases, sickle cell anaemia, and acute renal failure [6-14]. Endogenous antioxidants in the body, such as glutathione and  $\alpha$ -tocopherol can maintain and counteract the produced FR and ROS when they are within the physiological limit [15]. Improper balance

between the oxidants and antioxidants in favour of the oxidants, is potentially leading to oxidative stress state [16]. Therefore, the body requires exogenous antioxidant supply to prevent the oxidative stress [17]. The dietary antioxidant can be obtained naturally from plants or manufactured synthetically, but most of the natural antioxidants have better antioxidant activity compared with the synthetic one such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are considerably safer [18-19]. Various compounds which have antioxidant activity have been isolated from plants, many of them are polyphenols including phenols, phenolic acids, flavonoids, tannins, and lignans [13].

*Tephrosia purpurea* Linn family Leguminosae is commonly known as Sarpankh. Synonyms of *T. purpurea* Linn are Thila (Gujarati), Sarponkh (Hindi), Vempali (Telugu), Sharapunkha (Sanskrit). This drug is also not official in Ayurvedic Pharmacopoeia [20]. It is one of the excellent plants gifted by the nature for human beings and is composed of all the essential constituents that are required for normal and good human health. Leaves of *T. purpurea* are taken as emetic in the form of leaf juice or decoction and with sugar is also used in jaundice. It is a copiously branched, suberect, herbaceous perennial herb which occurs throughout the Indian [21]. According to Ayurveda literature, this plant has also been given the name of "wranvishapaka" which means that it has the property of healing all types of wounds [22]. It is an important component of some preparations such as Tephroli and Yakrifit used for liver disorders [23]. Whole plant has been used to cure tumors, ulcers, leprosy, allergic, and inflammatory conditions such as rheumatism, asthma, and bronchitis [24]. The aqueous extract of *Tephrosia purpurea* seeds has shown significant *in vivo* hypoglycemic activity in diabetic rabbits [25] and the ethanolic extracts possess potential antibacterial activity. The flavonoid isolated from the plant has been reported to have antimicrobial activity [26]. It has also been reported to mast cell stabilizing; acquire hepatoprotective and erythrocyte membrane integrity enhancing effect in various animal models [27, 28]. Phytochemical investigations on *T. purpurea* have revealed the presence of various phytoactive constituents such as glycosides, rotenoids, isoflavones, flavanones,

chalcones, flavanols, flavones and sterols [29]. *Phyllanthus niruri* (*P. niruri*) is a tropical shrub belonging to family Phyllanthaceae and grows commonly during winter as a weed in India, Brazil, Malaysia and Indonesia [30, 31]. Synonyms of *P. niruri* are Chanca Piedra (Brazil), Bhumyamalaki (South India) Dukong Anak (Malay) and zhu zi cao (Chinese) [32]. *P. niruri* is used in Unani, Ayurvedic and Chinese traditional medicine that have a broad range of properties which are antiviral activities against hepatitis B, antimicrobial, hepatoprotective, anticancer, hypocalcemic agent, asthma, gonorrhoea, bronchitis and syphilis [33-35]. This plant has also shown antibacterial, hypoglycaemic, analgesic, anti-inflammatory, cardioprotective, and anti-hyperuricemic properties [32]. Several active phytochemicals have been discovered in *P. niruri*, such as flavonoids, alkaloids, terpenoids, lignans, polyphenols, tannins, coumarins, and saponins [36]. A number of flavonoids had been successfully identified from *P. niruri*, including rutin and quercetin, which are well known to have significant antioxidants and chelating properties [37-39]. In the present study, antioxidant potential of *P. niruri* and *T. purpurea* was investigated through 2,2-diphenyl-1-picrylhydrazil (DPPH) activity assays and also qualitative phytochemical screening assay with two solvent for comparison. Protective antioxidant by plant products properties such as *P. niruri* may contribute as therapeutic drugs for free radical induced pathologies.

## Materials and Methods

### Chemicals and reagents

All chemicals and solvents used in this study were of analytical grade obtained from Himedia lab. Pvt. Limited.

### Plant collection

Plant material of *Tephrosia purpurea* L. and *Phyllanthus niruri* were collected from vindhya region. The plant material was authenticated at the Department of Botany, Govt. Girls College, Rewa, (M.P.), A voucher specimen as a herbarium has been kept for future reference.

### Preparation of aqueous and ethanolic extract

Powdered material of *Tephrosia purpurea* L. and *Phyllanthus niruri* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with

petroleum ether (60-80°C) by maceration process. The extraction was continued till the defatting of the material had taken place. 100 g. of *Tephrosia purpurea* L. and *Phyllanthus niruri* dried material were exhaustively extracted with ethanol and aqueous using maceration process. The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extracts and stored at 4 °C in labeled sterile bottles until further use [40].

### Qualitative phytochemical analysis

The powdered plant extracts were subjected to qualitative phytochemical tests using standard procedure [41-42].

### Antioxidant activity

#### DPPH free radical scavenging assay

For DPPH assay, the method of Olajuyigbe OO et al. 2011 [43] was adopted. A solution of 0.135 mM DPPH in methanol was prepared and 1 ml of this solution was mixed with 1 ml of different concentrations of the different extracts. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. Ascorbic acid was used as reference standard while methanol was used as control. Reduction of the stable DPPH radical was used as a marker of antioxidant capacity of *Tephrosia purpurea* L. and *Phyllanthus niruri* extracts. The change in colour was measured at 517 nm wavelength using methanolic solution as a reference solution. This was related to the absorbance of the control without the plant extracts. The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%. All the tests were carried out in triplicates. Though the activity is expressed as 50% inhibitory concentration (IC<sub>50</sub>), IC<sub>50</sub> was calculated based on the percentage of DPPH radicals scavenged.

The lower the IC<sub>50</sub> value, the higher is the antioxidant activity.

## Results and Discussion

### Result of percentage yield of different extract

The crude extracts so obtained after the maceration process, extracts was further concentrated on water bath for evaporate the solvents completely to obtain the actual yield of extraction. The yield of

extracts obtained from both plants using water and ethanol as solvents are depicted in the Table 1 and 2.

#### Result of phytochemical screening

Phytochemical analysis of aqueous and ethanolic extracts of leaf, stem and root sample of *Tephrosia purpurea* L. showed the presence of flavonoids, phenolics, amino acids, proteins, saponins, diterpenes table 3. The ethanolic and aqueous extract of leaf, stem and root sample of *Phyllanthus niruri* showed the presence of glycosides, flavonoids, phenolics, amino acids, proteins, saponins, diterpenes table 4.

#### DPPH Scavenging Activity

The scavenging activity was increased along with the increase of the concentration of sample used. It can be seen at the highest concentration among samples, ethanolic leaf extract of *P. niruri* exhibited the highest DPPH scavenging activity, followed by aqueous leaf extract of *P. niruri* and the lowest was aqueous leaf extract of *T. purpurea* Fig 1. The low IC<sub>50</sub> suggested a high antioxidant activity; therefore ethanolic leaf extract of *P. niruri* has the most effective antioxidant activity in this DPPH assay.

#### Conclusion

The present study showed the antioxidant activity of ethanolic and aqueous plant extract of *Phyllanthus niruri* and *Tephrosia purpurea*. The antioxidant activity of the plants extract may be due to the presence of different phytochemicals such as phenol, flavonoid, terpenoid, and saponin. The study supports the use of *P. Niruri* and *T. purpurea* in ayurveda and traditional medicine throughout the world. It can be used as a potential source of antioxidant which can be used to cure various ailments. There is still a lot of scope for further research, especially towards the mechanism of biological activity of phytochemicals and antioxidant activity from this plant.

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#### Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper

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**Table 1:** Result of percentage Yield (%) of *Tephrosia purpurea* L.

S. No.	Parameter	Ethanollic			Aqueous		
		Leaves	Stem	Root	Leaves	Stem	Root
1.	(%) Yield	4.6	2.3	3.1	3.2	2.5	3.3

**Table 2:** Result of percentage Yield (%) of *Phyllanthus niruri*

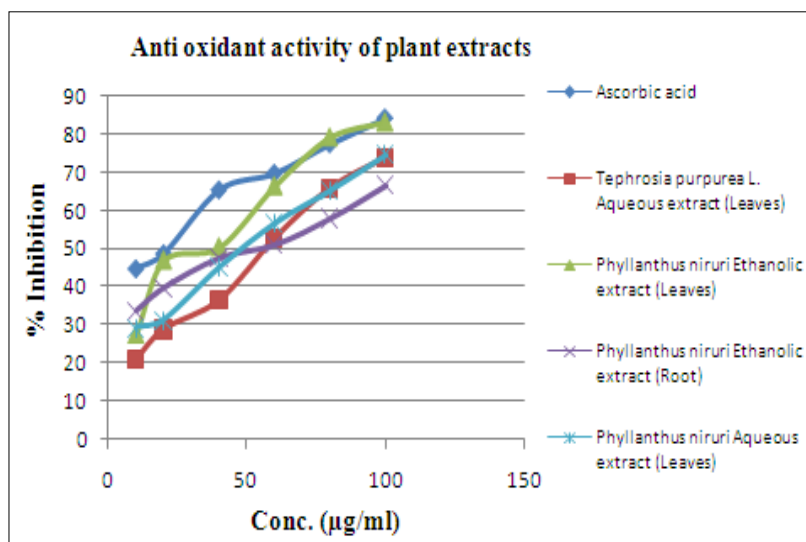
S. No.	Parameter	Ethanollic			Aqueous		
		Leaves	Stem	Root	Leaves	Stem	Root
1.	(%) Yield	5.2	4.8	2.8	5.8	4.6	2.7

**Table 3:** Result of phytochemical screening of *tephrosia purpurea* l.

S. No.	Constituents	Ethanollic			Aqueous		
		Leaves	Stem	Root	Leaves	Stem	Root
1.	Alkaloids	-	-	-	-	-	-
2.	Glycosides	-	-	-	-	-	-
3.	Flavonoids	+	+	+	+	+	+
4.	Phenolics	-	-	-	+	-	-
5.	Amino Acids	-	-	-	+	+	+
6.	Carbohydrate	+	-	-	-	-	-
7.	Proteins	-	-	-	+	+	+
8.	Saponins	-	-	-	+	+	+
9.	Diterpines	+	+	-	+	+	+

**Table 4:** Result of phytochemical screening of *phyllanthus niruri*

S. No.	Constituents	Ethanollic			Aqueous		
		Leaves	Stem	Root	Leaves	Stem	Root
1.	Alkaloids	-	-	-	-	-	-
2.	Glycosides	-	-	-	+	-	-
3.	Flavonoids	+	-	+	+	+	+
4.	Steroids	-	-	-	-	-	-
5.	Phenolics	+	-	+	+	-	-
6.	Amino Acids	+	+	-	+	+	+
7.	Carbohydrate	-	-	-	+	+	-
8.	Proteins	+	+	-	+	+	+
9.	Saponins	-	+	+	-	+	+
10.	Diterpines	-	+	-	-	-	+



**Figure 1:** Graph of *in vitro* free radical scavenging activity