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PHYTOCHEMICAL INVESTIGATION AND PHARMACOLOGICAL ACTIVITIES OF *TEPHROSIA PURPUREA* (L.) PERS. BARK

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ABSTRACT

Present study was performed to evaluate the Phytochemistry, antioxidant, antifungal and cytotoxic potential of *Tephrosia purpurea* methanolic extract. The plant of *Tephrosia purpurea* was purchased from a herbal shop at local market of district Bannu, dried, grounded to powder form and was then saturated with methanol to prepare methanolic crude extract. The phytochemical screening of *Tephrosia purpurea* showed the presence of saponins, phlobatannins, tannins and terpenoids while anthraquinones and flavonoids are absent. The plant showed 90% death rate of brine shrimps after 24 hrs and 100% after 48 hrs at 3mg/mL concentration. It also showed inhibition against fungus *Aspergillus flavus, Aspergillus niger and Aspergillus fumigatus* which are 89, 90 and 91% respectively at 3mg/mL concentration. Free radical scavenging activity of *Tephrosia purpurea* at 3mg/mL concentration was also very significant which are 89, 90, 8%, 74, 78 and 85% against DPPH, Phosphomolybdate, Hydroxyl radicals, Hydrogen peroxide, ABTS and Beta carotene, respectively.

Keywords: *Tephrosia purpurea***,** Antioxidant activity, Phytochemistry, Antifungal activity, Cytotoxicity.

INTRODUCTION

From ancient times, the medicinal plants play a basic role in the health care facilities of human beings. Ayurveda, medicinal plants contain many components of therapeutic value, therefore plant based drugs, medicines or formulations are used for the treatment of different human disorders (WHO, 1993). As compared to modern medicines plant based drugs are most valuable and beneficial becausethey contain therapeutic agents of easily access, relatively low prices and have no side effects (Agbor and Ngogang, 2005). Plants produce antioxidant compounds which secure the cell from the toxic effect of reactive oxygen species. Reactive Oxygen Species (ROS) such as hydroxyl radical, hydrogen peroxide and superoxide anion produce endogenously (mitochondrial electron transport chain and beta-oxidation of fat), do attack on the macromolecules of cell e.g. lipids, DNA and proteins resulted cell/tissue damage thus causes various diseases such as aging, cataract, asthma, carcinoma, arthritis, dementia, Parkinson's disease and mongolism (Halliwell and Gutteridge *et al.,* 1990). The compounds which scavenge these free radicals or oxidants or ROS are called antioxidants, now when the level of oxidant is increased or amount of antioxidant decreased than this going toward "Pro-oxidants" and a state called oxidative stress (Rimbach *et al.,* 1990). To resolve this problem we need to use an ideal amount of antioxidants in our foods (Gupta *et al.,* 2004). Various parts of plant e.g. roots, stem, leaves, bark, and fruits have the capacity to produce natural antioxidant (Rababah *et al.,* 2004).The medicinal plants contain different nutrients and non-nutrients molecules which shows significant role against microbes (antimicrobial activity) which defend us from various pathogens (Bajpai *et al.,* 2005; Sun *et al.,* 2002). In the treatment of cancer medicinal plants play a pivotal role (Crabbe, 1979; Mitscher *et al.,* 1987). The Ethanol and methanol extracts of aerial parts have anti-cancer agents against a human nasopharyngeal epidermis tumor cell line (KB) while here in present study the *Tephrosia purpurea* (L.) Pers. (TP) bark shows 100% anticancer activity (Santram *et al.,* 2006; Zafar *et al.,* 2004). Different phytochemicals such as flavonoids, alkaloids, tannins, steroids, phenols, carbohydrates and terpenoids are present in different parts of TP while in present study the bark of TP shows the presence of saponines, phlobatannins, tannins and terpinoids (Gupta *et al.,* 1980; Pelter *et al.,* 1981). TP, family (Fabaceae) is a perennial herb which is known as wild indigo in English, kolinji in Tamil, Sharpunkha in Sanskrit and pila in Sinhala. It is common in Sri Lanka, India, Pakistan, China and Hawaii. In both dry and wet areas its growth is perfect (Jayaweera *et al.,* 1982). This plant is broadly considered as a medicinal plant. In the Ayurveda system, TP is denoted as Sarwa wran vishapaha which means that it can heel every type of wound. The diseases such as liver and spleen enlargement and inflammation is effectively treated through this plant, therefore, it is also called plihāri or plihaśathru (pliha = spleen) (Sivarajan and Balachandran, 1993). The bark as well as the whole plant is used in drugs (Joy *et al.,* 1998). A decoction of roots is used to treat cough,

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dyspepsia, ulcer, asthma, skin diseases, and fever and is also used as a blood purifier (Lodhi and Singhai *et al.*, 2006).

MATERIALS AND METHODS

Plant collection

Mature and fully dried bark of TP was purchased from a herbal shop at local market of district Bannu and was properly identified by Dr. Faizan Ahmad a botanist at Department of Botany, UST Bannu. Collected plant sample was ground mechanically up to mash size 0.1 mm.

Extract preparation

About 60 g fine powder of TP was socked in 20 mL of the 80% methanol with gentle shaking and then placed it at room temperature for 7 days, thus after the seven days the plant is extracted and filtered by using Whatman No. 1 filter paper and then filtrate was concentrated on rotary evaporator (Panchun Scientific Co., Kaohsiung, Taiwan) under reduced pressure at 40 °C, after the concentration the extra methanol was evaporated at 37°C to obtain a pure crude extract of sample which then stored at 4 °C for further *in vitro* investigation.

Each sample was dissolved in 95% methanol at a concentration 1 mg/mL and then diluted to prepare the series concentrations for antioxidant assays. Reference chemicals were used for comparison in all assays. The DPPH (2,2-diphenyl-2-pierylhydrazyl) activity was proceeds by following the method of (Duraipandiyan and Ignacimuthu, 2009) with some slight modifications. The phosphomolybdenum method was performed according to the procedure of (Umamaheswari and Chatterjee, 2008). Hydroxyl radical scavenging activity of extracts was assayed by the method of (Halliwell and Gutteridge, 1999). H_2O_2 free radical scavenging activity was assayed as by Ruch *et al.* (1989). The ABTS (2,2-azinobis (3-ethlbenzthiazoline-6-sulphonicacid) scavenging assay was done with a slight modification of (Re *et al.*, 1999). The β-Carotene bleaching assay proceeds according to (Elzaawely *et al.*, 2007). The Cytotoxic brine shrimp lethality test was done by following the procedure of (Meyer-Albert *et al.*, 1992). Method of (Duraipandiyan and Ignacimuthu, 2009) was followed to assess Antifungal activity of *Tephrosia purpurea* methanolic extract (TPME). The phytochemical screening of plant extract was proceeds by following the standard protocol (Khan *et al.*, 2012; Pochapski *et al.*, 2011; Evan *et al.*, 2009) with some slight modification.

RESULTS AND DISCUSSION

Antioxidant activities

Antioxidant are natural products that scavenge free radicals or Oxidant eliminated due to oxidative stress and secure us from there serious effect, medicinal plants produce antioxidants abundantly, for this purpose we also investigate the TPME (*Tephrosea purpurea* methanol extract) in present work and it's find that our results are mostly similar to the investigation of (Hogerman *et al.*, 1998) reported that the medicinal plants have high free radicals scavenging property. The TPME are highly reactive against DPPH, ABTS, H_2O_2, hydroxyl radicals, Beta carotene and Phosphomolybdate free radicals, our results also support by Kilani *et al.*, (2008). The ability of TPME to scavenge the above mentioned free radicals is quite significant but not as much as ascorbic acid as mentioned in below Tables (1, 2, 3, 4, 5 and 6) respectively.

Table 1. Scavenging activities of Ascorbic acid and TPME for DPPH free radicals.

Samples	% Ascorbic acid scavenging	% TPME scavenging
3mg/mL	94.59±0.1	89.1±0.1
1.5	91.37±0.0	86.35±0.0
0.75	89±0.2	82.05±0.2
0.37	87.35±0.3	79.33±0.1

Data are mean ± SD; TPME = *Tephrosia purpurea* methanol extract; DPPH = 2,2-diphenyl-2-pierylhydrazyl.

Table 2. Scavenging activities of Ascorbic acid and TPME against Phosphomolybdate free radicals.

Samples	% Ascorbic acid scavenging	% TPME scavenging
3mg/mL	97.05±0.3	90.58±0.2
1.5	96.2±0.0	85.88±0.1
0.75	94±0.2	77.05±0.3
0.37	92±0.1	70.58±0.0
Data are mean ± SD		

Table 3. Scavenging activities of Ascorbic acid and TPME against OH free radicals.

Samples	% Ascorbic acid scavenging	% TPME scavenging
3mg/mL	90.1±0.2	82.33±0.2
1.5	87.23±0.3	80.12±0.1
0.75	84.98±0.1	76.23±0.3
0.37	81.03±0.2	70.65±0.2
Data are mean ± SD		

Table 4. Scavenging activities of Ascorbic acid and TPME against H_2O_2 free radicals.

Samples	% Ascorbic acid scavenging	% TPME scavenging
3mg/mL	90.1±0.2	82.33±0.2
1.5	87.23±0.3	80.12±0.1
0.75	84.98±0.1	76.23±0.3
0.37	81.03±0.2	70.65±0.2
Data are mean ± SD		

Table 5. Scavenging activities of Ascorbic acid and TPME against ABTS free radicals.

Samples	%Ascorbic acid scavenging	%TPME scavenging
3mg/mL	85.24 ±0.0	78.88 ±0.1
1.5	74.73 ±0.1	67.25 ±0.1
0.75	68.1 ±0.2	62.56 ±0.2
0.37	61.03 ±0.1	58.35 ±0.0
Data are mean ± SD; ABTS = 2,2-azinobis (3-ethlbenzthiazoline-6-sulphonicacid		

Table 6. Scavenging activities of Ascorbic acid and TPME against beta carotene free radicals.

Samples	% Ascorbic acid scavenging	% TPME scavenging
3mg/mL	93.12±0.3	85.03±0.3
1.5	87.93±0.0	81.12±0.4
0.75	84.17±0.1	76.34±0.2
0.37	76.46±0.2	70.12±0.1
Data are mean ± SD		

Antifungal activity

Medicinal plants contain phenolic compounds which shows antimicrobial activity or antifungal activity (Baydar *et al.*, 2004).The antifungal activity is also due to bioactive compounds saponins (Mothan *et al.*, 2007). TPME contains saponins that's why it have significant antifungal property as show here in present study against fungal strains *Aspergillus niger*, *Aspergillus flavus and Aspergillus fumigatus*, the inhibition activity against these strains is 89, 90 and 91% respectively at high 3mg/mL concentration and terbinofine is positive control which shows 100% inhibition against these fungal strains, while the DMSO is -ive control which shows no inhibition. The data is given in (Table 7).

Table 7. Antifungal assessment of TPME (% Inhibition).

Fungal Strain	Samples	TPME	Terbinofine
Aspergillus flavus	3mg/mL	89%	100%
	1.5	82%	98%
	0.75	78%	91%
	0.37	72%	85%
Aspergillus niger	3mg/mL	90%	100%
	1.5	83%	96%
	0.75	79%	87%
	0.37	75%	88%
Aspergillus fumegatus	3mg/mL	91%	100%
	1.5	86%	97%
	0.75	79%	89%
	0.37	77%	85%

Cytotoxic activity

Those plants which show effective anticancer activity are of great importance, because today cancer is one of the dangerous disease. For this purpose the anticancer activity (Cytotoxicity) of TPME was determined against brine shrimps growth. The survival and death of brine shrimps against TPME is noted in Table 8. From which it is clearly examine that at 0.37 mg/mL, 30% survival and 70% death were noted, similarly at 0.75, 1.5 and 3mg/mL, 20%, 10% and 0% survival and 80%, 90% and 100% death occurred respectively, which supported by 100% cytotoxic activity showed by *Arceuthobium oxycedri* methanolic fraction against brine shrimps. It means that this plant contains bioactive element against cancer causing pathogen..

Table 8. Survival and death rate against TPME of brine shrimps.

Samples	Total no of Brine shrimps	After 24hrs		After 48hrs		After 72 h	% Survival			% Death		
		Live	Dead	Live	Dead		After 24h	After 48h	After 72 h	After 24h	After 48hrs	After 72h
3mg/mL	10.0±0	1.0±0	9.0±0	0.0±0	10.0±0	All Dead	10%	0%	0.0%	90%	100%	100%
1.5	10.0±0	2.0±0	8.0±0	0.0±0	10.0±0		20%	0%		80%	100%	
0.75	10.0±0	2.0±0	8.0±0	1.0±0	9.0±0		20%	10%		80%	90%	
0.37	10.0±0	3.0±0	7.0±0	2.0±0	8.0±0		30%	20%		70%	80%	100%

Data are mean ± SD

Phytochemical screening

The phytochemical screening was also done for the purpose to provide information about the presence of various phytochemicals in the TPME. This study was performed according to the standard protocol (Khan *et al.*, 2012; Pochapski *et al.*, 2011; Evan *et al.*, 2009) with some slight modification. The data are given in Table 9 which shows the presence of phlobatannins, tannins, saponins and terpenoids.

Table 9. Phytochemical screening of TPME.

Phytochemicals	Phlobatannins	Anthraquinone	Tannins	Flavonoids	Terpenoids	Saponins
TPME	+	–	+	–	+	+

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