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PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY SCREENING OF CHLOROFORM LEAF AND AERIAL PART EXTRACTS OF TEPHROSIA VILLOSA

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ABSTRACT

The present research work mainly focuses on phytochemical and antioxidant screening of chloroform leaf and aerial part extract of *Tephrosia villosa*. The phytochemical screening is done by preparing chloroform extract of leaves and aerial parts of the plant. Antioxidant activity is examined by *DPPH* assay method using free radical scavenging activity of the extract. The secondary metabolites like phenols, glycosides, tannins, reducing sugars, terpenoids, flavonoids present in the extract may be responsible for the attributed activity. The following secondary metabolites are identified by general phytochemical tests. From the above research work carried out in the view to note that the flora should be explored for their versatile biological activity and should serve as an alternate remedy for conventional dosage forms. The natural system of medicine is gaining passion due to their fewer side effects than that of traditional medicines. Different species of *Tephrosia villosa*. should be explored for conceivable for antioxidant activity.

KEYWORDS: *Tephrosia villosa*, Phytochemical Screening, DPPH, Antioxidant activity.

INTRODUCTION

The plant of the taxonomical nomenclature *Tephrosia villosa* belongs to the family Fabaceae / Leguminosae.[1] Which is commonly known as Pea. Family. The common Synonyms of the plant are: *Tephrosia hirta*, *Tephrosia incana.* Tephrosia is an annual or perennial bushy herb[2] it grows to a normal height of 0.3-1.3 m. *Tephrosia villosa* mainly a widespread species found in southern and eastern Africa, southern Asia and the Arabian Peninsula.[3] The genus *Tephrosia* comprises between 300 to 400 species of annual and perennial woody herb, distributed in tropical and subtropical regions of the world.[4]

Tephrosia species has been reported for many versatile activities such as Anticancer activity,[5] anticonvulsant activity,[6] Antibacterial Efficacy,[7,8] Anti hyperlipidemic activity,[9] Antimicrobial activity,[10] Anti inflammatory activity,[11] *In vitro* antioxidant[12] and cytotoxic potential,[13] Estrogenic Activity,[14] antileishmanial activity,[15] antihistamine activity.[16]

The figures of the plant are gven below in fig I and fig II.

Fig I: Illustrates Pods of the plant.

Fig II: Illustrates Aerial parts of the plant.

Uses of *Tephrosia species (Leguminosae)*

- To Control erosion,
- For Reclamation,
- Soil improver,
- High nitrogen-fixing potential.
- It controls pests and diseases
- The seeds are emollient and had beneficial effect in ascariasis;
- Used in the treatment of diabetes and dropsy
- Used as antipyretic, antioxident, antihyperlipidimics.

Recently, increases in the prevalence rates of chronic diseases along with rapid increases in aging populations have led to great demands for foods with health-improving functionalities. A considerable body of literature supports the role of oxidative stress in the pathogenesis of age-related human diseases such as cancer, diabetes, immune system decline and brain dysfunction. There have been various types of phytonutrients with multiple biological effects including anti-inflammatory, anticancer, antiallergic, antiviral and antiaging activities, therefore much attention has been focused on the biological properties of natural foods and herbs.

PLAN AND OBJECTIVE

The design and objective of the present research work concentrates on the identification of the phytochemical constituents of leaf and aerial part (twigs and stem part) extracts of *Tephrosia villosa*. and to screen for antioxidant activity of the following extracts and to focus on alternate remedy for antioxidant activity .

MATERIALS AND METHODS

Collection and authentication of plant material

The leaves of the plant, *Tephrosia villosa*. growing in the local areas of vizianagaram and Visakhapatnam of Andhra Pradesh state were collected during the month of September-October. It was identified and authenticated by Dr. S.B.Padal, Dept. of Botany, Andhra University and Sample specimen was kept in our laboratory for future reference. Plant material was garbled at first to remove all the dust particles and unwanted material then it was washed thoroughly, initially with tap water and then with distilled water and then allowed to dry in shade. The dried plant material was pulverized to fine powder and stored at room temperature in air tight container until used further.

Preparation of Plant extracts

To 1Kg of *Tephrosia villosa*. Leaf and aerial part powder, 2litres of solvent, viz. Chloroform, was added consequently for preparing the extract. (flow chart-1).Extraction with the solvent was done for one day at 27^0C, after maceration the supernatant of each solvent was recovered by filtering through whatmann filter paper. This process was repeated thrice and the respective solvent from the supernatant was evaporated in a Rota vapor to obtain crude extracts which are to be stored at 4^0c until used for evaluation.

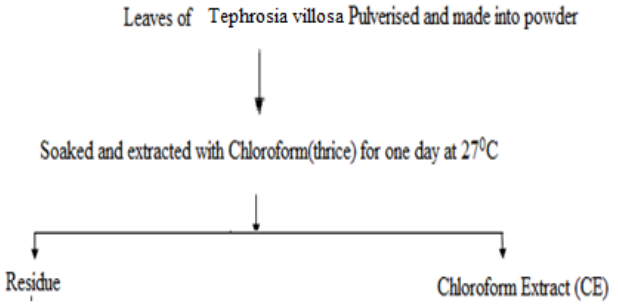

Fig III: Schematic representation of showing extraction procedure from leaves of *Tephrosia villosa.*

Table I: Tests carried out for Preliminary Phytochemical Screening of the extracts.

S. No.	Name of the test	Procedure
1	Mayer's test (for Alkaloids)	2ml of plant extract was taken and to it 2ml of concentrated HCl and Mayer's reagent were added. Green color or white precipitate indicates presence of Alkaloids.
2	Keller-killiani test (for Cardiac glycosides)	0.5 g of extract was added with 5 ml of water, 2 ml of glacial acetic acid containing one drop of ferric chloride solution was added. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides.
3	Ferric chloride test (for Flavonoids)	About 0.5g of each extract was boiled with 5 ml of distilled water and then filtered. To 2 ml of this filtrate, a few drops of 10% ferric chloride solution was added. A green-blue or violet coloration indicated the presence of a phenolic hydroxyl group.
4	Xanthoproteic test (for Proteins)	The extract (few mg) was dissolved in 2 ml water and then 0.5 ml of conc. HNO3 was added in it. Yellow color indicated the presence of proteins.
5	Ferric chloride reagent test (for Tannins)	The test sample of each extract was taken separately in water, warmed and filtered. To a small volume of this filtrate, a few drops of 5 % w/v solution of ferric chloride prepared in 90 % alcohol were added. Appearance of a dark green or deep blue color indicated the presence of tannins.
6	Salkowaski test (for Sterols and Phenols)	A few milligrams of the plant extract was dissolved in 2 ml chloroform and then 2 ml of conc. H_2SO_4 was added from the sides of the test tube. The test tube was shaken for a few minutes. Red colour development in the chloroform layer indicated the presence of sterols.
7	Foam test (for Saponins)	0.5 gram of each extract was boiled with 5 ml of distilled water and filtered. To the filtrate, about 3 ml of distilled water was further added and shaken vigorously for about 5 minutes. Frothing which persisted on warming was taken as an evidence for the presence of saponins.
8	Salkowaski test (for terpenoids)	To 0.5 g of each extract, 2 ml of chloroform was added, followed by a further addition of 3ml of concentrated H_2SO_4 to form a layer. A reddish brown coloration of the interface indicated the presence of terpenoids.

9	Fehling's solution test (for Reducing sugars)	About 0.5 g of each extract was dissolved in distilled water and filtered. The filtrate was heated with 5 ml of equal volumes of Fehling's solution A and B. Formation of a red precipitate of cuprous oxide was an indication of the presence of reducing sugars.
10	Anthraquinone	An aliquot of 0.5 g of the extract was boiled with 10 ml of H_2SO_4 and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for color changes.

Phytochemical Investigation on T.Villosa

Table II: Results of Preliminary Phytochemical analysis of leaf extract of Tephrosia villosa.

S.No	Phytochemical constituent	Name of the test	Chloroform	
			Leaf extract	Aerial part extract
1	Alkaloids	Mayer's test	-	-
2	Cardiac glycosides	Keller-killiani test	+	+
3	Flavonoids	Ferric chloride test	+	+
4	Proteins	Xanthoproteic test	-	-
5	Tannins	Ferric chloride reagent test	+	+
6	Terpenoids	Salkowaski test	+	+
7	Saponins	Foam test	-	-
8	Sterols	Salkowaski test	-	+
9	Sugars	Fehling's solution test	+	+
10	Anthraquinones		-	-

+ =Presence

- = Absence

ANTIOXIDANT ACTIVITY

DPPH free radical scavenging activity: The 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity ofthe extracts were determined by the method of Kilani *et al*.9 with some modifications. Aliquots of varying concentration of the extract were mixed with freshly prepared DPPH in methanol (final concentration 100 µM) and the absorbance at 514 nm was measured after incubation for 0.5 h in the dark at room temperature. Methanol was used as control and ascorbic acid as reference compound. Inhibition (IC50) was calculated from the graph of DPPH scavenging activity.

Table III: Results of antioxidant activity of leaf and aerial part extract of Tephrosia villosa.

S.No	Concentration (µg/ml)	% Inhibition of *T.villosa extracts*		
		leaf extract	aerial part extract	STD
1	10	31.32	34.65	42.5
2	20	37.42	39.23	48.58
3	30	40.83	46.31	59.03
4	40	49.09	51.94	65.71
5	50	54.25	55.78	71.82

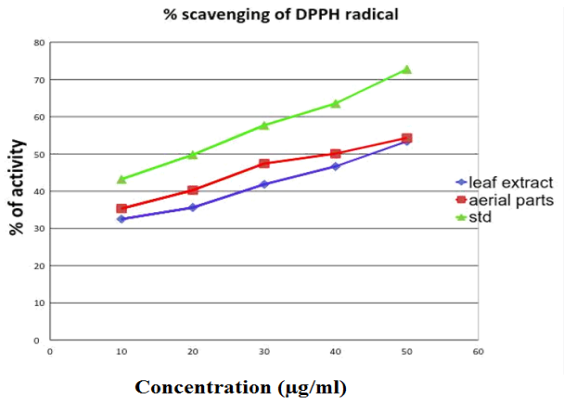

FIG III: Illustrates the diagrammatic representation of antioxidant activity.

RESULTS AND DISCUSSIONS

From the results we can implicate that the chloroform extract of leaf and aerial parts of tephrosia villosa found active as an antioxidant alternative. Different secondary metabolites are identified from the extract which acts as a root for antioxidant activity. The chloroform extracts of leaf and aerial parts found to posses increase in activity in increase in concentration of the extract but finally they were found to show less potent than that of the standard. The leaf extract showed high potency at 50 µg/ml with %inhibition of 54.25 whereas the aerial part extract showed high potency at 50 µg/ml with %inhibition of 55.78. both the extracts showed less potency than the standard used which gave 71.82 % of inhibition at 50 µg.ml.

The secondary metabolites mainly the phenols, sterols, flavonoids, reducing sugars which are identified in the phytochemical investigation may be responsible for the free radical scavenging activity. The potency may be attributed to the penetration coefficient of the secondary metabolites and the possible mechanism may be the cell wall disruption of the cell.

CONCLUSIONS

From the present study it can be implicated that the use of flokflore propperties of this plant exhibit antioxidant activity. Thus it supports phytochemical investigation of other terrestrial sources which are responsible for pharmacological activity. Further the extract should be studied for their active constituents and isolation process should be carried out and formulated into a potent dosage form.

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