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Preliminary phytochemical screening of ethanolic leaf extract of two *Tephrosia* species

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ABSTRACT: Genus *Tephrosia* is an important genus of family Fabaceae possess various medicinal properties. Two species of this genus namely- *Tephrosia purpurea* and *Tephrosia villosa* were investigated for the presence of their phytochemical compounds. Preliminary phytochemical analysis was performed as per standard methods. Phytochemical screening was carried out on ethanolic leaf extract. Phytochemical analysis revealed the presence of primary and secondary metabolites in leaf extract. Reported phytoconstituents in ethanolic extract were carbohydrates, proteins, amino acid, alkaloids, phenols, flavonoids, phytosterols, terpenoids, saponin, glycosides, gum and mucilage. Secondary metabolites are responsible for the medicinal properties of plants.

KEYWORDS: Fabaceae, ethanolic extract, primary phytochemical, secondary phytoconstituents

I. INTRODUCTION

Primary and secondary phytochemical compounds are naturally occurring chemical compounds which are important for vital and non-vital functions of the plant. Carbohydrates, proteins and amino acid etc. are primary phytochemicals which are the main source of energy for plants. Secondary products are derived from nitrogen metabolism called secondary modification (Czapek,1921). Secondary metabolites mainly formed by plants as part of defence mechanism against pathogen and to face several environmental stresses. These products also significant for human as medicine to protect them from various diseases. Secondary phytoconstituents includes phenols, flavonoids, alkaloids, steroids, terpenoids, saponin, glycosides, etc. play a significant role in plant defence. These phytochemical compounds contain various bioactivity and their therapeutic potential can be used in drug preparation to cure various disease. The chemical structure derived from these phytochemicals can utilized as model for the formation of new synthetic drugs (Raaman, 2006). The potential pharmacological activities of any plant can be predicted through identification of its phytochemical compounds (Emraan et al,2006).

Every phytochemical compound belongs to specific chemical class which is determined by applying the phytochemical tests to plant extract. Extraction of some phytochemical dependent on the solvent used because few phytochemicals are extracted only in specific solvent like alkaloids are extracted in alcohol not in distilled water. More than one test can accurately determine the presence or absence of any phytoconstituents. Phytochemical screening of ethanolic leaf extract of two *Tephrosia* species namely *T. purpurea* (L.) Pers. and *T. villosa* (L.) Pers. was carried out to find out the phytochemical compounds present in leaves.

II. MATERIALS AND METHODS

Preliminary phytochemical analysis was performed in ethanolic extracts of dry leaf powder of *T. purpurea* and *T. villosa*. For phytochemical analysis, mature leaves were washed in running tap water followed by shade drying for 15 days. Dry leaves were powdered by the mechanical grinder, sieved and stored in a dry place in airtight containers for phytochemical tests.

For preparation of ethanolic leaf extract, 5 gm leaf powder was dissolved in 100 ml of 70% of ethanol in a beaker and covered with aluminium foil. This alcoholic solution was left for 72 hours with occasional stirring. After 72 hours, the leaf fragments removed by filtering the extract with muslin cloth and again filtered by Whatman paper 1. After centrifugation at 2500 rpm for 20 minutes, the supernatant was poured into a porcelain bowl and evaporate on a water bath at 40°C till brownish-black crude extract obtained (**Photoplate 1**). The crude extract was re-dissolved in distilled water to perform phytochemical analysis. Preliminary phytochemical tests were carried out by standard methods of Sofowora (1993). Harborne (1998), Trease and Evans (2002).

Following tests were done to find out the presence of primary as well as secondary phytochemicals.

Phytochemical tests:

Test for Protein and Amino acid:

Ninhydrin test: 5% ninhydrin solution + 2 ml of leaf extract+ heated for 2-3 min. development of purple colour confirm presence of Protein and Amino acid.

Xanthoproteic Test: 2 ml of extract + 2 ml of conc. nitric acid; appearance of yellow colour.

Test for carbohydrates:

Fehling's test: 2 ml of extract + 1ml dilute HCL and neutralized with alkali + heat with Fehling's solution (A+B) Formation of red precipitate confirmed the presence of carbohydrates.

Molisch's test: 2 drops of Molisch's reagent + 2 ml of extract + shake+ conc H_2SO_4 , formation of the violet ring at the junction of two liquid indicated the presence of carbohydrates.

Test for Alkaloids

Dragendorff's test: 2 ml extract + 2 ml of 1% HCL + 1 ml Dragendorff's reagent, the appearance of orange-red precipitate confirms presence of alkaloids.

Wagner's test: 2 ml of acidified leaf extract + Wagner's reagent was added in it, the formation of orange-brown precipitate confirms alkaloids.

Test for Phenols

Ferric chloride test: 3-4 drops of $FeCl_3$ + 2 ml extract; formation of bluish-black colour confirm presence of phenols.

Lead acetate test: 2ml extract + 2-3 drops of 10% lead acetate solution,the appearance of yellow precipitate.

Test for Flavonoids

Alkaline reagent test: Few drops of 2 % NaOH+ 2 ml leaf extract, solution colour turned into intense yellow + disappeared after addition of dilute acid shows presence of flavonoids.

Shinoda test: 5 ml of 95 % ethanol +2 ml leaf extract + addition of conc. HCl drop wise+ few fragments of Magnesium ribbons; appearance of pink scarlet colour.

Test for Terpenoids

Salkowski test: 2 ml of extract + 3 ml chloroform +conc. H_2SO_4 ; Formation of the reddish-brown ring at the junction of two liquids confirms presence of terpenoid compounds.

Libermann Burchard's test: 3 ml chloroform + 2 ml of extract + acetic anhydride+ solution heated +cooled down + 2 ml conc. H_2SO_4 slowly added along the side of a test tube, a violet ring appears at the junction.

Test for Glycosides

Glycoside test: Few drops of aqueous NaOH + leaf extract, the formation of yellow colour confirm the presence of glycosides.

Keller- Killani test: 2 ml of glacial acetic acid +leaf extract + 1-2 drops 2% solution of $FeCl_3$ poured into a test tube containing 2 ml conc. H_2SO_4 , the appearance of the brown ring at the interphase indicated the presence of cardiac glycosides, the appearance of a violet ring below the brown ring confirmed the result.

Test for Saponin

Froth test: 2 ml of extract + 20 ml distilled water+ shaken for 10 minutes; formation of 1 cm layer of foam shows presences of saponins.

Olive oil test: Few drops of olive oil + leaf extract; the formation of emulsion.

Test for gum and mucilage

Alcohol test: 5 ml ethanol +2 ml extract, extract become turbid shows presence of gum and mucilage.

Ruthenium red test: Ruthenium red solution +2 ml of extract, the formation of pink colour proved presence of gum and mucilage.



III. RESULTS

The preliminary phytochemical screening of leaf extract of two *Tephrosia* species was carried out in ethanolic extract. Primary compounds – protein, amino acids and carbohydrate and secondary phytochemicals like phenols, alkaloids, flavonoids, terpenes, glycosides, phytosterols etc. were reported in phytochemical analysis of ethanolic leaf extract *T.purpurea* and *T.villosa*, results of phytochemical tests are presented in **table 1 and photoplate 2 and 3.**

Discussion:

Genus *Tephrosia* possesses various pharmacological properties therefore extensively used in traditional therapeutic systems in many countries. Present work was carried out to determine the phytochemical constituents of two *Tephrosia* species.

Preliminary phytochemical analysis of ethanolic leaf extract of *Tephrosia* species confirms presence of various phytoconstituents. The intensity of the chemical group was varying accordingly the solvent used, due to the difference in the polarity of extraction solvent. Results of preliminary analysis is in line with previous studies on phytochemical screening on *Tephrosia* species (Muthukrishnan *et al.*, 2014; Priyadarshan *et al.*, 2016; Nigam and Arnold, 2018).

Alkaloids produced by many flowering plants and it contain many properties like anti-inflammatory, anti-microbial, anti-fungal anti-cancer, anti-ulcer, analgesic, diuretic, anti-tumour, neuro pharmacologic and local anaesthetic (Kurek, 2019; Badri, 2019).

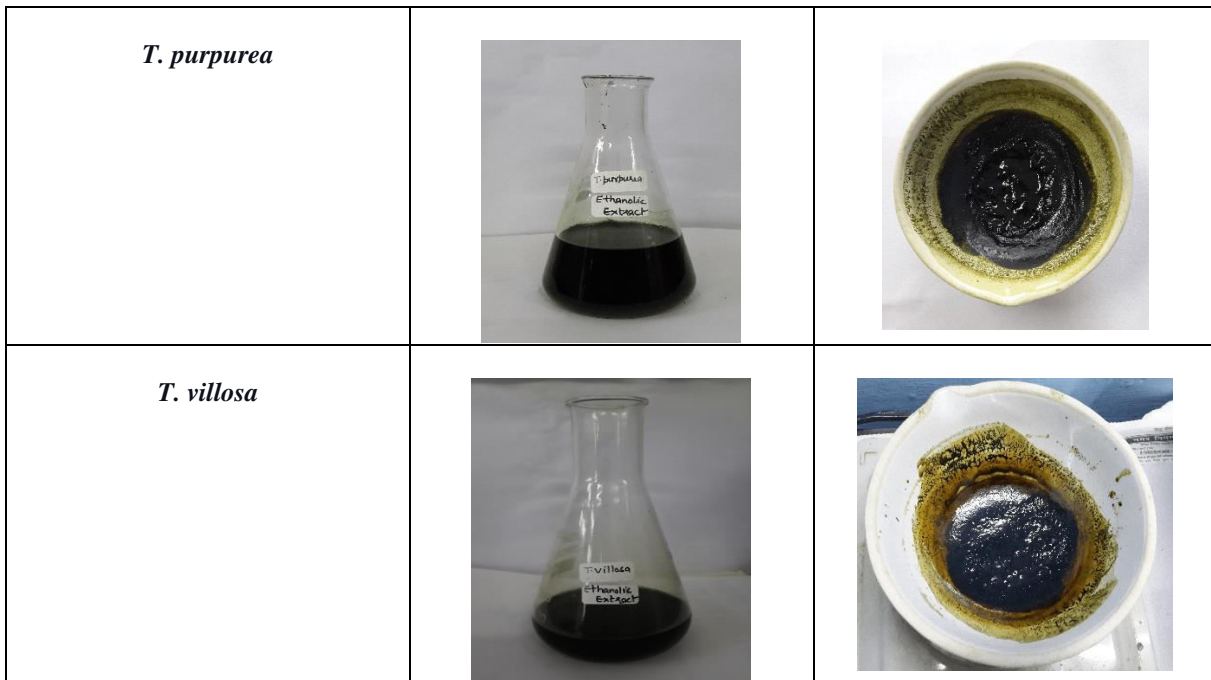
The phenolic compounds have beneficial health effects and disease-preventing properties like cardioprotective, neuro-protective, cancer chemo-preventive, anti-inflammatory properties and antioxidant properties (Castillo-Pichardo *et al.*, 2009; Huyut *et al.*, 2017; Brezani *et al.*, 2018;).

Flavonoids compounds are large group of polyphenol compounds possess anti-inflammatory, antibacterial, anti-oxidant, antiviral activity and anti-cancer (Kumar and Pandey, 2013). Terpenoids are isoprene molecules used in pharmaceutical preparations, they possess antimicrobial antioxidant, anti-inflammatory, antiparasitic, antimalarial activity (Duraipandiyan *et al.*, 2012). Glycosides contain anti-tumour activity (Dostkotch *et al.*, 1972). Cardiac glycosides show inhibitory activity against rhinovirus (Kamano *et al.*, 1988). Saponins are chemically varied group of compounds have anti-inflammatory, antimicrobial, anti-oxidant, anti-carcinogenic, anti-protozoan properties (Moses *et al.*, 2014).

Phytochemical screening of leaf extract revealed the presence of various phytochemical compounds in leaf extract which confirms the therapeutical potential and medicinal of these two *Tephrosia* species.

Phytoconstituents	Phytochemical tests	<i>T. purpurea</i>	<i>T. villosa</i>
Carbohydrate	Fehling’s test Molisch’s test	++	++
Protein & Amino acid	Ninhydrin test Xanthoproteic test	++	++
Alkaloids	Dragendorff’s test Wagner’s test	++	++
Phenols	Ferric chloride test Lead Acetate test	+++	+++
Flavonoids	Shinoda test and Alkaline reagent test	++	+++
Terpenoids	Salkowaski test Liebermann burchard’s test	+++	+++
Glycosides	Glycosides test Keller Killani test	++	+++
Saponin	Olive oil test Foam test	++	+++
Gum and mucilage	Ruthenium Red test Alcohol test	++	++

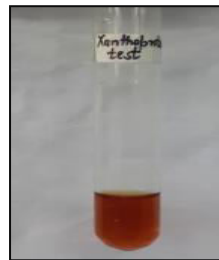
Table 1: phytochemical test of ethanolic leaf extract of Two *Tephrosia* species



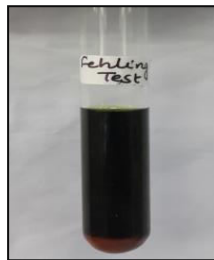
Photoplate : 1 Ethanolic and crude leaf extract of *T. purpurea* and *T. villosa*



Ninhydrin Test



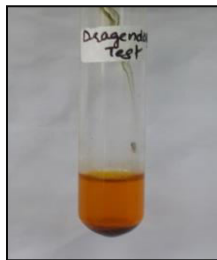
Xanthoproteic Test



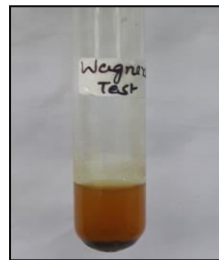
Fehling Test



Molisch



Dragendorff's Test



Wagner's Test



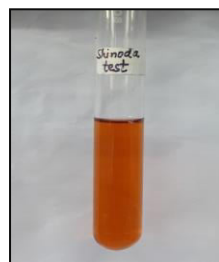
Ferric Chloride Test



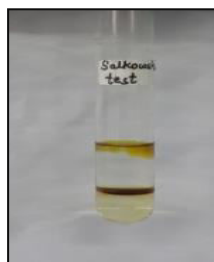
Lead Acetate Test



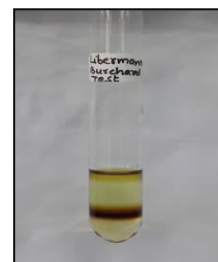
Alkaline Reagent Test



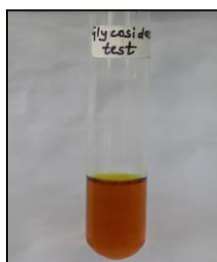
Shinoda Test



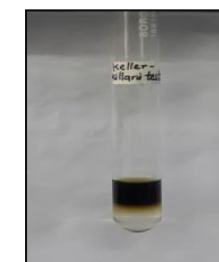
Salkowski Test



Liebermann Burchard



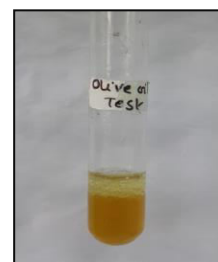
Glycosides test



Keller Killani Test



Froth Test



Olive oil



Alcohol Test



Ruthenium Red Test

Photoplate 2 : Preliminary phytochemical test of ethanolic leaf extract of *T. purpurea*



Ninhydrin Test



Xanthoproteic Test



Fehling Test



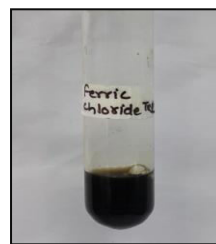
Molisch Test



Dragendorff's Test



Wagners Test



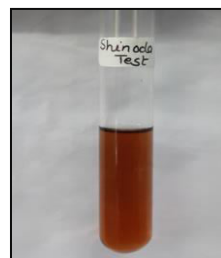
Ferric Chloride Test



Lead Acetate Test



Alkaline Reagent Test



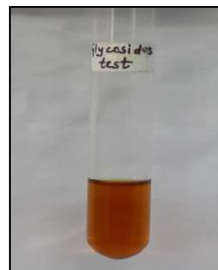
Shinoda Test



Salkowski Test



Liebermann Burchard Test



Glycosides test



Keller Killani Test



Froth Test



Olive oil Test



Alcohol Test



Ruthenium Red Test

Photoplate 3 : Preliminary phytochemical tests of ethanolic leaf *T.villosa*

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