Phyto-Pharmacological Investigation and Antimicrobial study of Tephrosia purpurea Extract

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Abstract:

The ethno-pharmacological review claimed Tephrosia purpurea is used to cure several types of external wounds and gastro-duodenal disorders. The plant has also been claimed to cure kidney, liver spleen, heart and blood related disorders. The present study has been undertaken to investigate and evaluate the Anti-microbial activity, and Antioxidant activity of the Tephrosia purpurea aerial part. The results of extractive values showed, the alcohol extract have higher quantity of extract (6.3%) in comparison to other solvent extracts. Qualitative phytochemical studies of Carbohydrate & Glycoside showed a good characteristic colour and precipitate in all five tested reagents. Protein and amino acid were found slightly. Phenolic compounds and Flavonoids were abundantly present in all the extracts. The result showed the plant Tephrosia purpurea has significant antioxidant activity ethanolic extract (71.60) as dose dependent manner as compare to standard (92.96). The zone of inhibition of ethanolic extract of Tephrosia purpurea was effective at 200 mg/ml.

KEYWORDS: ANTIOXIDANT ACTIVITY ,FLAVONOIDS, ZONE OF INHIBITION, GLYCOSIDE

INTRODUCTION

Herbal products, botanical products, or phytomedicines are plant-based items that are used to treat illnesses or preserve health. Herbal supplements are products derived only from plant sources and intended for internal use. The active compounds in herbal medications are derived from plant parts, such as leaves, roots, seeds, or flowers of plants are used as herbal remedies or supplements. When used as supplemental medication, they might have therapeutic advantages. Herbal medicines have gained recognition as complementary and alternative medicine in recent years, as well as dietary supplements for illness prevention. Herbal medicine is defined by the World Health Organization (WHO) as the use of herbs, herbal materials, herbal preparations, and completed herbal products that have plant parts, other plant materials, or combinations of plant materials as

active agents. The leaves, stems, blossoms, roots, and seeds of plants are the source of these herbs.In most parts of the world, the development and mass manufacturing of chemically manufactured medications throughout the past century has changed health care.[1]

The healthcare system provides preventive, promotive, curative, and rehabilitative care and is holistic in nature. Chronic disorders, psychotic disorders, non-communicable and metabolic diseases, immune modulation, etc., such as rheumatoid arthritis, jaundice, nervous debility, skin diseases like vitiligo and eczema, sinusitis, and bronchial asthma, are the main focus areas of unani medicine. The six elements of the Unani system are:[2]

(a) pure air;

(b) food and drinking water;

(c) physical movement and rest;

(d) psychic movement and rest;

(e) sleep and wakefulness; and

f) retention of useful materials and evacuation of waste materials from the body.

These elements are crucial for the prevention of disease and the promotion of health. In Unani medicine, there are four types of treatment: Regimental therapy, surgery, pharmacotherapy, and diet therapy.

Antioxidants are substances that prevent oxidation, which is a chemical process that might result in the production of free radicals (autoxidation). Degradation of organic molecules, including living things, is caused by autoxidation. To increase the useable life of industrial products including polymers, fuels, and lubricants, antioxidants are commonly added. Chemicals known as antioxidants appear to lessen oxidative stress. Antioxidants are categorized into two main groups based on their solubility in lipids (lipophilic) or water (hydrophilic). Lipid-soluble antioxidants shield cell membranes from lipid peroxidation, whereas water-soluble antioxidants typically interact with oxidants in the blood plasma and the cytoplasm of cells.

Three distinct methods of antioxidant activity for small molecules are presented below for convenience: (I) the radical character is transferred with the creation of a reactive antioxidant-derived radical; (II) free radicals are trapped by the creation of a stable or inert free radical trap; and (III) compounds that replicate the actions of antioxidant enzymes.[3]

A substance that either destroys bacteria (microbicide) or inhibits their growth (bacteriostatic agent) is called an antibiotic. Antimicrobial drugs can be categorized based on the bacteria they mostly target. Antimicrobials are medical compounds that are used to treat or prevent infections. Antibiotics, antiviral, antifungal, antiseptics, and antiparasitics are among them. Antimicrobial agents are used as disinfectants on non-living surfaces. The three main classes of antimicrobial agents are antibiotics (which kill microorganisms inside the body), antiseptics (which are applied to living tissue and help reduce infection during surgery), and disinfectants (non-selective agents, such as bleach), which kill a wide range of microbes on non-living surfaces to prevent the spread of illness. Originally limited to formulations generated from living bacteria, the term "antibiotic" is increasingly used to refer to synthetic drugs such fluoroquinolones and sulfonamides. The in vitro antibacterial activity was assessed using cultures of Escherichia coli and Staphylococcus aureus by disc diffusion method, while the in vitro anti-inflammatory activity was assessed using human red blood cell (HRBC) membrane stabilization method. The crude extract and fractions' total phenolic (TPC) and total flavonoid contents (TFC) were also measured using the aluminum chloride method and Folin-Ciocalteu's phenol reagent, respectively.[4]

MATERIALS AND METHODS

Collection and Authentication

The fresh aerial part of *Tephrosia purpurea* aerial part was collected from adjoining area of Barpali (Dist-Bargarh, Odisha) in the month of July. The plant was authenticated by Mr.S. N. Badpanda, Botanist Sriram College, Rampur.The aerial part was dried under shade and powdered by the help of mechanical process. The coarse powder has stored in air tight container for further studies.

Fig. 01: *Tephrosia purpurea*

Extraction

Hot successive extraction (Soxhlet)

The shade dried coarse powder of aerial part (100 g) was subjected to continuous hot extraction with solvents i.e. - Pet. Ether (60–80o C), and Alcohol as per their polarity successively. The extracts were filtered and dried by Rotary evaporator, weighed and percentage of yield was calculated in terms of air-dried crude powdered materials.

Phytochemical Screening[7,8]

The different qualitative chemical tests can be performed for establishing profile of given extract for its chemical composition. The following tests may be performed on extracts to detect various phytoconstituents present in them.

Methods

Alkaloids

Two hundred milligram of solvent free extract was stirred with 50 ml of dilute hydrochloric acid and filtered. Then the filtrate was tested carefully with various alkaloidal reagents as follows:

Mayer's test: Two drops of Mayer's regent was added in a 2 ml of filtrate by the side of the test tube. A white precipitate was obtained which indicate the presence of alkaloids.

Wagner's test: Two drops of Wagner's regent was added in a 2 ml of filtrate by the side of the test tube. A reddish-brown precipitate was obtained which indicate the presence of alkaloids.

Hager's test: Two drops of Hager's reagent (saturated aqueous solution of picric acid) was added in a 2ml of filtrate by the side of the test tube. A prominent yellow precipitate was obtained which indicate the presence of alkaloids.

Dragendorff's test: Two drops of Dragendorff's regent were added in a 2 ml of filtrate by the side of the test tube. A prominent yellow precipitate was obtained which indicates the presence of alkaloids .

Carbohydrates and Glycosides

Two hundred milligram of extract was dissolved in 50 ml of water and filtered. The filtrate was subjected to the following tests.

Molish's test: Two drops of alcoholic solution of -naphthol were added in 2 ml of filtrate and the mixture was shaken well. Then one ml of concentrated sulphuric acid was added slowly along the sides of the test tube and allowed to stand. A violet ring was obtained which indicate the presence of carbohydrates.

Fehling's test: One ml of filtrate was boiled on water bath with one ml each of Fehling solutions A and B. A red precipitate was obtained which indicate the presence of sugar.

Barfoed's test: One milliliter of Barfoed's reagent was added in1 ml of filtrate and heated on a boiling water bath for 2 min. A red precipitate was obtained which indicates the presence of sugar.

Benedict's test: About 0.5 ml of Benedict's reagent was added in point 5 ml of filtrate. The mixture was heated on a boiling water bath for two min. A characteristic colour precipitate was obtained which indicates the presence of sugar (46).

Glycosides

Fifty milligram of extract was hydrolyzed with concentrated hydrochloric acid for 2 hrs. on a water bath and filtered. Then the hydrolysate was subjected to the following tests.

Borntrager's test: Ten milliliter of chloroform was added in 2 ml of filtered hydrolysate and shaken properly. Then chloroform layer was separated and 10% ammonia solution was added to it. Pink colour was obtained which indicates the presence of glycosides.

Saponin

Fifty milligram of extract was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for fifteen min. A layer of foam indicates the presence of saponin.

Proteins and Amino Acids

Two milligram of extract was dissolved in 10 ml of distilled water and filtered through Whatman no.1 filter paper and the filtrate were subjected to tests for proteins and amino acids.

Millon's test: Few drops of Millon's reagent were added in two ml of filtrate. A white precipitate was obtained which indicate the presence of proteins.

Biuret test: One drop of 2% copper sulphate solution was treated with 2 ml of filtrate. To this 1 ml of ethanol (95%) was added, followed by excess of potassium hydroxide pellets. Pink colour in the ethanolic layer was not obtained which indicates the absence of proteins.

Phenolic Compounds and Tannins

Ferric chloride test: Fifty milligram of extract was dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution was added. A dark green colour was obtained which indicates the presence of phenolic compound.

Lead acetate test: Fifty milligram of extract was dissolved in distilled water and to this, three ml of 10% lead acetate solution was added. A bulky white precipitate was obtained which indicates the presence of phenolic compound.

Alkaline reagent test: An aqueous solution of the extract was treated with 10% ammonium hydroxide solution. Yellow fluorescence was obtained, which showed the presence of flavonoids.

Pharmacological screening[12,13]

Antioxidant activity

Total phenolic content: The Gallic acid equivalence method (GAE), uses a mixture of phosphomolybdate and phosphotungstate for the colorimetric assay of phenolic and polyphenolic antioxidant.

Reagents used: Dilute Folin ciocalteu reagent with equal volume of distilled water, 20 % of sodium carbonate in water, gallic acid.

Procedure: The preparation was made the calibration curve of standard gallic acid 10-100 µg/ml in water. The test solution was made 1 mg/ml. The sample was mixed with 0.25 ml of folin ciocalteu reagent and 1.25 ml of 20% sodium carbonate solution. The sample was incubated for 40 minutes at room temperature, after that the samples were compared with standard at 725 nm.

Calculation: The number of total phenols from calibration curve as a Gallic acid equivalent by the formula; T = C. V. / M

Antimicrobial Screening[9,10,11]

Screening for antibacterial activity of the extracts was done by agar disk diffusion method followed by determination of minimum inhibitory concentration (MIC) of the shortlisted active plant extracts against the bacterial strains. Antimicrobial activity was determined against standard bacterial strains.The procedure of antimicrobial disk diffusion method was; Mueller Hinton agar (MHA) and Sabouraud's dextrose agar (SDA) were used for preparation of the assay plates for the bacteria and yeasts respectively. Overnight grown microbial cultures from MHA and SDA plates were used to prepare the inocula of the bacteria and yeasts respectively. Several similar looking colonies were picked from the culture plates and suspended in cation adjusted Mueller Hinton broth or Sabouraud's dextrose broth (SDB) accordingly for bacterial strains. Turbidity of the suspensions were adjusted to 0.5 McFarland using Densi- La-Meter or Densimat. Prepared microbial inocula were seeded in or swabbed on the surface of the media agar plates. 50, 100, 150, and 200 mg/ml of plant extracts (in all their extraction solvents) were dispensed.Plates were incubated at 35°C-37°C in the incubator for 18-24 hrs. for bacteria and 24-48 hrs. for yeasts. Antimicrobial activity was observed as the zone of inhibition of microbial growth around the wells (diameter in mm) which was measured using the vernier calipers. All the plant extracts were tested against standard Gram positive & Gram-negative bacteria.

Antibacterial drugs ciprofloxacin (25 µL, 50 µL, 75 µL, 100 µL) was included as the standard drug.The screening assays were done in triplicates and the results (diameters of zones of inhibition in mm) were statistically expressed as mean ± standard deviation (SD) using the statistical tool.

RESULT AND DISCUSSION

Extractive values

The dried aerial part of the plant material was extracted with Pet. Benzene and ethanol by hot continues percolation (Soxhlet extraction) method with increasing order of their polarity. The extracts were evaporated in distillation unit. The results of extractive values showed, the alcohol extract have higher quantity of extract (6.3%) in comparison to other solvent extracts. The extractive values were recorded in the table 01.

Table 01: Extractive values of *Tephrosia purpurea*

Sl. No.	Solvent used	Aerial part (%)
1	Pet. Ether	0.9

2	**Alcohol**	**6.3**

Qualitative phytochemical studies

Qualitative phytochemical studies of *Tephrosia purpurea* aerial part were performed on its pet, benzene and ethanol extracts to identify its Carbohydrate and Glycoside, Protein & Amino acid, Phenolic compounds & Flavonoids and Phytosterols by using suitable chemicals and reagents. Qualitative phytochemical studies of Carbohydrate & Glycoside showed a good characteristic colour and precipitate in all five tested reagents. Protein and amino acid were found slightly. Phenolic compounds and Flavonoids were abundantly present in all the extracts. The above qualitative phytochemical screening showed that the aerial part is a rich source of Phenols & Flavonoids (table 02).

Table 02: Qualitative Phytochemical Screening

Phytochemical test	**Pet. Ether**	**Alcohol**
1. Alkaloids		
Mayer's test	-	-
Wagner's test	-	-
Hager's test	-	-
Dragendorff's test	-	-
2. Carbohydrates & Glycosides		
Molish's test	-	+
Fehling's test	-	-
Barfoed's test	+	+
Benedict's test	-	+
Borntrager's test	-	+
3.Saponins		
Foam test	-	-
4. Proteins & amino acid		
Millon's test	-	-
Biuret's test	-	+
Ninhydrin test	-	-
5. Phenolic compounds & flavonoids		
Ferric chloride test	+	+
Lead acetate test	-	+
Alkaline test	-	+
6. Phytosterol		
Solkowski test	-	+
-, Absent; +, Present		

Pharmacological screening

Antioxidant activity

Determination of Total Phenolic Contents

The total phenolic content in the methanol extract of *Tephrosia purpurea* was calculated using standard Folin-Ciocalteu method. Phenols are very important plant constituent. There is highly positive relationship between total phenols and antioxidant activity of many plant species, because of the scavenging ability of their hydroxyl groups. It was reported that phenolic compounds have good antioxidant properties.

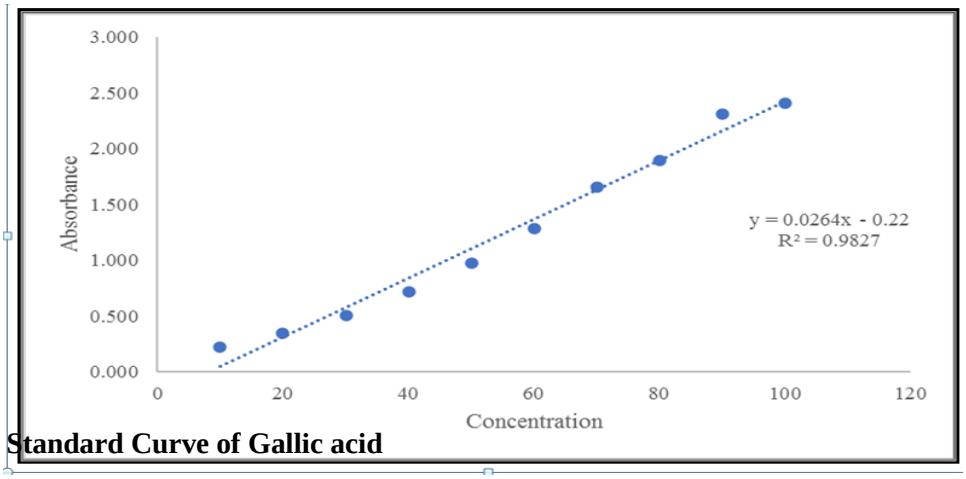

Fig 02: Standard Curve of Gallic acid

Table 03: Total phenolic contents of Ethanol extracts of *Tephrosia purpurea*

Sample extract	Sample solution µg/ml	Wt. of dry extract per ml, m(gm)	Absorbance	GAE conc. C (µg/ml)	GAE conc. C (mg/ml)	TPC as GAE $C=\frac{x \times V}{m}$
Ethanol	1000	0.001	0.512	18.4	0.0184	24.4

Antimicrobial activity

The chloroform extract of *Tephrosia purpurea* showed the satisfactory anti-microbial activity against E. coli, and S. aureus. The zone of inhibition of ethanolic extract of *Tephrosia purpurea* was effective at 200 mg/ml (**Table 10).**

Table 04: Antibacterial activity of ethanolic extract of *Tephrosia purpurea*

Treatment	Dose	Zone of inhibition (mm)			
		E. coli (Gram - negative)	**S. typhi (Gram negative)**	**P.aeruginos a (Gram-negative)**	**S.aureus (Gram - positive)**
Negative Control (DMSO +microorganism)	200 µl	0	0	0	0
	400 µl	0	0	0	0
	600 µl	0	0	0	0
	800 µl	0	0	0	0
Positive Standard (Ciprofloxacin+ microorganism)	25 µg/ml	5 ± 0.25	20 ± 0.13	4 ± 0.29	08 ± 0.19
	50 µg/ml	08 ± 0.14	20 ± 0.36	07 ± 0.29	09 ± 0.14
	75 µg/ml	11± 0.29	21 ± 0.32	07 ± 0.51	13 ± 0.22
	100 µg/ml	16 ± 0.11	22 ± 0.18	09 ± 0.23	15 ± 0.16
Petroleum ether extract	50 mg/ml	0	0	0	0
	100 mg/ml	0	0	0	0
	150 mg/ml	0	0	0	0
	200 mg/ml	2 ± 0.23	0	0	0
Ethanolic Extract	50 mg/ml	0	0	0	0
	100 mg/ml	0	0	0	0
	150 mg/ml	02 ± 0.29	0	0	0
	200 mg/ml	07 ± 0.33	5 ± 0.39	6 ± 0.24	03 ± 0.26

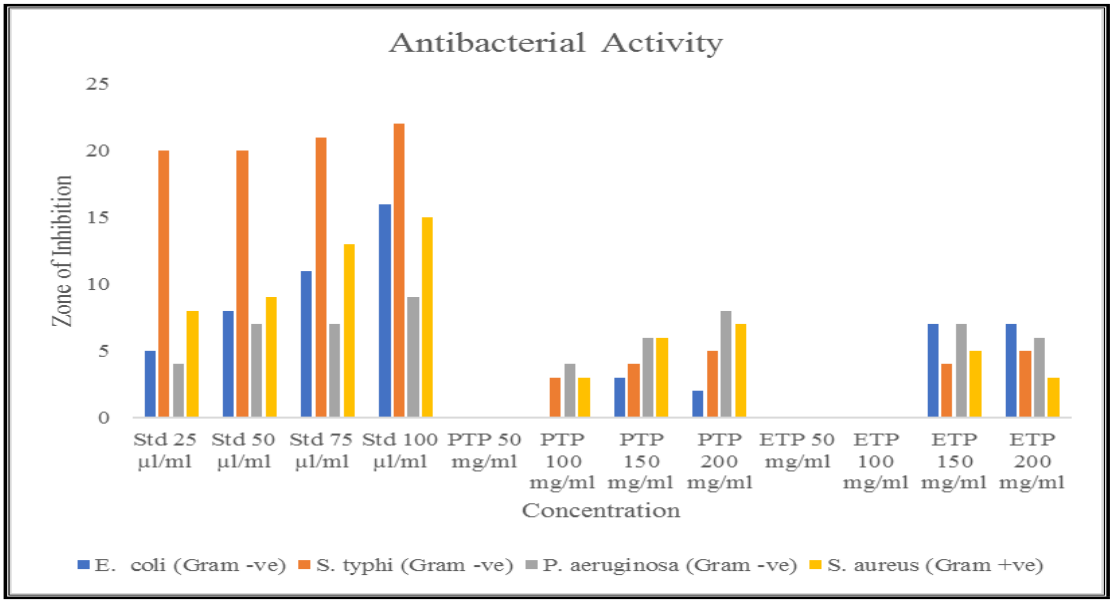

Fig.03: antibacterial activity plot of PTP and ETP

CONCLUSION

Based on the study, it can be concluded that the plant having different phytochemicals. From pharmacological result it is concluded that petroleum ether and ethanolic extracts of *Tephrosia purpurea* have good antimicrobial and antioxidant activity when compared with conventional and standard drug. affinity. It is comparable with standard drug Further studies using in vivo models are required to find out and established effectiveness and pharmacological rationale for use of leaves and whole plant as anthelmintic and antioxidant drug.

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