Phytochemical Screening and Antimicrobial activity of *Tephrosia villosa*

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Abstract
Phytochemical screening, antibacterial activity and anti-inflammatory properties of Tephrosiavillosa root, stem and leaves were assessed in this study. Phytochemical screening of the plant extracts (root, stem and leaf) of Tephrosia villosa was made by running the root, stem and leaf through solvent such as ethanol. The ethanolic extract of root indicated positive tests for steroids, alkaloids, amino acids and proteins, saponins, flavones, anthocyanines and phenolic compounds. Ethanolic extract of stem and leaf indicated positive tests for steroids, alkaloids, carbohydrates, glycosides, amino acids and proteins, saponins, flavones, anthocyanines and phenolic compounds. Ethanolic extracts of root, stem and leaves tested for their antibacterial activity, the extracts in each series indicate modest to higher activity against both Gram negative and Gram positive pathogenic microorganisms'.

The leaves extract of Tephrosia villosa indicated promising activity against the dangerous pathogens such as K. pneumonia, Salmonella typhi and Salmonilla paratyphi, by producing 13mm, 13mm and 12mm zone of inhibition around the inoculation. Plant root extract was effective against the Staphylococcus aureus (12mm), Salmonella typhi (12mm) and Salmonilla paratyphi (11mm).

Keywords: Phytochemical screening, antimicrobial activity, zone of inhibition, alkaloids, steroids.

Introduction

With over 400 species, the genus *Tephrosia* belongs to the family leguminosae and is extensively dispersed throughout the world's tropical, subtropical and desert regions[4,12,15]. According to reports, thirty of the fifty species that are native to equatorial, are found in Kenya, seventy-five are located in South Africa, thirty are native to South America and thirty appear in India[2,14]. Morphologically these are tall, between 0.5 and 4 m, upright herbs or soft, woody shrubs with lush foliage. Because they can fix nitrogen, they may be able to improve soil fertility[12]. Compound leaves with inverted lance- or obovate-shaped leaflets are 7–15 cm in length and 0.3–1 cm in width. Phenotypically they are pea-shaped, few-flowered, leaf-opposed, raceme-like clusters of flowers with a 7 mm length are white, purple, or pinkish in colour. Self-pollinating plants yield linear, long pods that measure 2.5–4.0 cm in length and 3–4 mm in width. Dark brown, ellipsoid seeds have an average of 11 2n chromosomes. Most organisms are diploid. *Tephrosia candida* (Roxb.) DC., *Tephrosia elata*, Deflers, *Tephrosia purpurea* (L.) *Pers.*, *Tephrosia villosa* (L.) Pers., *Tephrosia virginiana* (L.) Pers. and *T. vogelii* are among the species that are frequently linked to insecticidal activity. Of the 400 species of *Tephrosia vogelii* that have been identified, *T. vogelii* has been examined the most. Native to tropical Africa, it is a shrub or small tree that grows to a height of 2 to 3 metres in 5 to 7 months.

A major global cause of death is antimicrobial resistance (AMR). An estimated 1.27 million deaths in 2019 were directly linked to AMR infections caused by bacteria[10]. African and South Asian regions had the greatest death rates per 100,000 people, with children under the age of five accounting for one in five deaths caused by antimicrobial resistance (AMR) illnesses. Like many other low- and middle-income nations, Nepal has a high burden of AMR infections[19]. A 23-year retrospective study from a Kathmandu tertiary care facility revealed progressively rising MDR infection rates[23].

In an additional investigation conducted by the same organization, MDR was found in 488/532 (91.7%) and 643/719 (89.4%). Tertiary care facilities frequently have high rates of multidrug resistance in *Acinetobacter* spp. and *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus* (MRSA) and are non-susceptible to third-generation cephalosporins, fluoroquinolones and aminoglycosides among *Klebsiella* spp. and *E. coli*[1,6].

Plants have been the basis of traditional medicines throughout the world for thousands of years and continue to provide new remedies to mankind[7]. Aromatic and medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment inhibiting bacterial or fungal growth[3]. For thousands of years, plants have served as the foundation for traditional medicines throughout the world and are now being used to develop innovative treatments for humankind[7]. Certain bioactive compounds produced by aromatic and medicinal plants are known to interact with other organisms in the environment to limit the growth of bacteria or fungi[3]. Worldwide, natural ingredients provide the basis for about 30% of pharmaceuticals[13]. The spectrum of incurable bacterial infections is expanded by the growing incidence of drug-resistant strains of bacteria and the recent emergence of

strains with decreased antibiotic susceptibility which increases the urgency of finding novel approaches to combat infections and potent therapeutic agents[18]. Plants contain medicinally significant secondary metabolites that present a new avenue for the development of innovative medicines with antimicrobial and anti-disease properties.

The general consensus is that the utilization of ethno-pharmacological data in phytochemical research is a successful method for identifying novel anti-infective drugs derived from higher plants[5]. Some medicinal plants may indeed be a source of novel antibacterial agents, even against strains of bacteria resistant to some antibiotics.

Native to India, the perennial herb of *Tephrosia villosa* has a multibranch, that grows up to 90 cm tall and is heavily covered with silky, white hair. Flavonoids such as villosin, tephrinone and tephcalostaan are produced by the roots and seedpods. Rotenoids, dehydrorotenone and prenylated flavonone are found in whole plants. Significant action was demonstrated by the ethanol extract of T. villosa's fruit, twigs, roots and leaves against the larvae of the southern house mosquito (*Culex quinquefasciatus*)[11]. Mealworm (*Tenebrio molitor*) was inhibited by TvD1 (a defensin) that was recovered from *T. villosa* [16]. It has been demonstrated that plant defensin, a tiny, cationic, cysteine-rich, broad-spectrum antimicrobial peptide with four or five disulfide bridges, is a part of the innate immune system.

Strong activity was shown by tobacco overexpressing the defensin gene (TvD1) against the first and second instar larvae of the taro caterpillar (*Spodoptera litura*), a significant polyphagous insect that attaches itself to 44 families of economically significant plants[17]. The current study looks at *Tephrosia villosa* antibacterial qualities and phytochemical content. Plant extracts' antimicrobial properties can be ascertained using a variety of approaches including twofold serial dilution, agar well diffusion and disc diffusion. Normally, the agar well diffusion technique is taken into consideration while evaluating medicinal plants for antibacterial activity. *Tephrosia villosa* collection, identification, extraction and antimicrobial assessment are the goals of our current study.

Material and Methods

Plant collection and identification: The whole plants of *Tephrosia villosa* (Root, Stem and Leaf) were collected from Gulbarga University Campus, Gulbarga. A voucher specimen (Voucher No. HGUG-8020) was kept at the Department of Botany, Gulbarga University, Gulbarga after identification of the plant.

Phytochemical studies of *Tephrosia villosa*

Alkaloids: Alkaloids such as atropine, quinine, morphine and others, are basic nitrogenous plant compounds that are primarily optically active and include nitrogen heterocycles as their structural units. They also have notable physiological effects. The presence of alkaloids in the plant extracts was examined by performing the below tests.

Mayer's Test: A few drops of Mayer's reagent were added to the plant extract. Alkaloids are present when a white or pale yellow precipitate is formed.

Dragendorff's Test: A few drops of Dragendorff's reagent were added to the plant extract. An orange-brown precipitate's formation suggested the presence of alkaloids.

Wagner's Test: To the plant extract, few drops of the Wagner's reagent were added. Development of brown precipitate indicated the presence of alkaloids.

Steroids: These belong to a broad class of organic compounds that are found in many different kinds of plants and animals. They are distinguished by the presence of 1, 2-cyclopentenophenanthrene ring structure, which can be partially reduced or altered in other ways, e.g. adrenocorticoids, bile acids, steroids, sex hormones etc.

Salkowski Test: Two millilitres of chloroform and two millilitres of strong sulfuric acid were added to the plant extract and after shaking, the red colour at the bottom layer showed the presence of steroids.

Liberman-Burchard's Test: After adding 2 millilitres of chloroform and 2 millilitres of strong sulfuric acid to the plant extract and shaking it, the presence of steroids was shown by the red colour in the lower layer.

Carbohydrates: The term "hydrates of carbon" refers to substances with the general formula $Cx(H_2O)y$ because they have the same proportion of hydrogen and oxygen as water such as sucrose, glucose etc.

Molisch's Test: Two drops of a recently made 10% alcoholic solution of α-naphthol were put to a test tube containing plant extract. After shaking it, 2 millilitres of concentrated sulfuric acid were added from the test tube's side. As a result, the violet ring that formed where two liquids met, suggested that carbohydrates were present.

Anthrone Test: Anthrone reagent (two millilitres) was added to two millilitres of water in a test tube containing plant extract. After that, it was shaken softly. The presence of carbohydrates was indicated by the formation of green or blue colour.

Glycosides: The acetyl derivatives, also referred to as glycosides, are created when an alcohol molecule combines with the hemiacetyl form of sugar. Glycosides are derived from sugars whereas fructosides are derived from fructose.

H_2SO_4 Test: Two millilitres of concentrated H_2SO_4 were added to a test tube containing plant extract, shaken and

allowed to stand for a few minutes. The development of a reddish colour signified the existence of glycosides.

Proteins and Amino Acids: Proteins are intricate nitrogenous substances found in both plant and animal cells. When proteins are hydrolyzed by enzymes or powerful inorganic acids, a mixture of amino acids is produced.

Ninhydrin Test: Two millilitres of ninhydrin's reagent were added to a test tube containing plant extract and the mixture was heated for two to three minutes. Amino acid content was determined by the formation of blue hue.

Millon Test: A test tube containing plant extract was filled with 2 millilitres of Millon's reagent. The presence of proteins was confirmed by the formation of a white precipitate that turns red when heated.

Saponins: These are steroidal glycosides derived from plants that have the ability to froth in water, much like soap solution. They work by reducing the water's surface tension.

Aqueous Test: In a test tube containing plant extract, 2 ml of water was added. Formation of foam indicated the presence of saponins.

Flavones: Known by another name, anthoxanthins, flavones are yellow pigments found in the kingdom of plants.

Aqueous Test: Two millilitres of aqueous NaOH were put to a test tube that contained plant extract. The appearance of a yellow tint suggested the existence of flavones.

H_2SO_4 Test: When 2 millilitres of concentrated H_2SO_4 were added to a test tube containing plant extract, the colour changed from yellow to orange, signifying the presence of flavones.

Anthocyanins Test: Anthocyanins are water-soluble, naturally occurring plant pigments that are made up of glucosides and their aglyones (i.e. sugar-free pigments). They are typically found in the aqueous cell sap of flowers and are the cause of their wide range of hues including red, violet, blue and others.

Aqueous Test: In a test tube containing plant extract, 2 ml of aqueous NaOH was added; change in colour from blue to violet colour orange indicated the presence of anthocyanins.

H_2SO_4 Test: In a test tube containing plant extract, 2 ml of conc. H_2SO_4 was added. Formation of yellowish orange colour indicated the presence of anthocyanins.

Oils and Fats: Press a tiny amount of plant extract between sheets of filter paper. The presence of oils was revealed by the formation of oil stains on the paper.

Saponification Test: One millilitre of 0.5 N alcoholic potassium hydroxide and one millilitre of phenopthalein were put to a test tube containing plant extract. The test tube was then heated on a water bath for one to two hours. The production of soap (froth) signified the existence of fats and oils.

Tannic acids and phenolic compounds $FeCl_3$ Test: Two millilitres of $FeCl_3$ solution were introduced to a test tube that contained plant extract. The presence of tannins and phenolic compounds was indicated by the formation of a precipitate or a violet colour.

Lead acetate Test: Two millilitres of lead acetate solution were introduced to a test tube that contained plant extract. Precipitate formation suggested the existence of tannins and phenolic chemicals.

Aqueous bromine test: Two millilitres of aqueous bromine solution were introduced to a test tube that contained plant extract. Precipitate formation suggested the existence of tannins and phenolic chemicals.

Preparation of micro-organism: The organisms used in this study were *Staphylococcus aureus* (ATCC 25923), *Klebsiella pneumoniae* (ATCC BAA-1706), *Klebsiella pneumonia* (ATCC 13883), *Pseudomonas aureginosa* (ATCC 10145), *Salmonella typhi*, (ATCC 700931), *Salmonella partatyphi* (ATCC 9150), *Vibrio cholera* (ATCC 39050), the strains were maintained on nutrient agar slants at 4ºC.

Antimicrobial Activity of *Tephrosia villosa*: The antimicrobial activity of the alcoholic extracts was carried out against the pathogenic bacteria's namely *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aureginosa*, *Salmonella typhi*, *Salmonella partatyphi* and *Vibrio cholera*.

Sub culturing of bacteria: 24 hours prior to the test, the stock culture of strains of microorganisms were aseptically inoculated in 5 ml of the nutrient broth and inoculated for 24 hours at 37˚C in an incubator. To detect the antibacterial activity of extract, the universally accepted agar medium was used for the cultivation and maintenance of the standard strains.

Nutrient agar: All the strains were inoculated on the agar medium (gm/litre) as peptone -5gm, beef extract- 3gm, NaCl-5gm, agar 20gm**,** dissolving all these ingredients in distilled water (1000ml) and the reaction mixture was adjusted to pH 7 and autoclaved at 15 lbs pressure (121˚C) for 20 minutes.

Preparation of standard solution: Standard drug used in this is ampicillin. Ampicillin is soluble in water. The concentration of this drug was made to 100µg/ml

Preparation of solution of extracts under test: Concentration of test extract is made to 200 µg/ml by

dissolving 20mg of extract in 20ml dimetyl formamide. Now the concentration of test extract is 200 µg/ml. From this stock solution, 2ml is pipetted out and diluted to 20ml with dimetylformamide (DMF) to get final concentration of 200 µg/ml.

Method employed: Method employed was dig-well method. In this method, array of antibiotic potency is based on measurement of diameter of upto 3cm of microbial growth inhibition surrounding cylinder containing solid nutrient medium, previously inoculated with culture of suitable microorganisms. Inhibition produced by test compound is compared by known concentration of standard.

A culture of test organisms is spread over the surface of an agar medium. Holes are then punched out of agar of 6mm diameter, some of these are fitted with fluid containing known concentration of ampicillin to be assayed while others are filled with standard fluid. Then plates are incubated at 37ºC in an incubator for 24 hours. Dimetylformamide (DMF) is filled in one hole to act as solvent control. The extent of diameter of inhibition after 24hours was measured as zone of inhibition in millimeters.

Results and Discussion

Phytochemical Activities: Phytochemical activities of the plant extracts (root, stem and leaf) of *Tephrosia villosa* were made by running the root, stem and leaf through solvent such as ethanol. The ethanolic extract of root indicated the positive tests for steroids, alkaloids, amino acids and proteins, saponins, flavones, anthocyanines and phenolic compounds. Ethanolic extract of stem and leaf indicated the positive tests for steroids, alkaloids, carbohydrates, glycosides, amino acids and proteins, saponins, flavones, anthocyanines and phenolic compounds.

Antimicrobial activity of various extracts of *Tephrosia villosa*: Antimicrobial activity was carried out by dig-well method. The standard solution was prepared to a concentration of 100µg/ml and the test solution was prepared to 200µg/ml. Amongst the extracts tested for their antibacterial activity, the extracts in each series showed moderate to high activity against both Gram positive and Gram negative organisms.

The series of ethanolic extracts of root, stem and leaf of *Tephrosia villosa* was active against *Klebsiella pneumonia*, *Salmonella typhi*, *Salmonella paratyphi* and *Staphylococcus aureus*. *Pseudomonas aerugenosa* and *Vibrio cholera* did not show any inhibition zone. The ethanolic extracts of root, stem and leaf of *Tephrosia villosa* did not show any inhibition zone on *Vibrio cholera* and *Pseudomonas aeruginosa*.

Phytochemical Screening: Phytochemical screening of ethanolic extracts of root, stem and leaf of *Tephrosia villosa* was used in this study. The ethanolic extract of root indicated the positive tests for steroids, alkaloids, amino acids and proteins, saponins, flavones, anthocyanines and phenolic compounds. Ethanolic extract of stem and leaf showed positive tests for alkaloids, steroids, carbohydrates, glycosides, amino acids and proteins, saponins, flavones, anthocyanines and phenolic compounds.

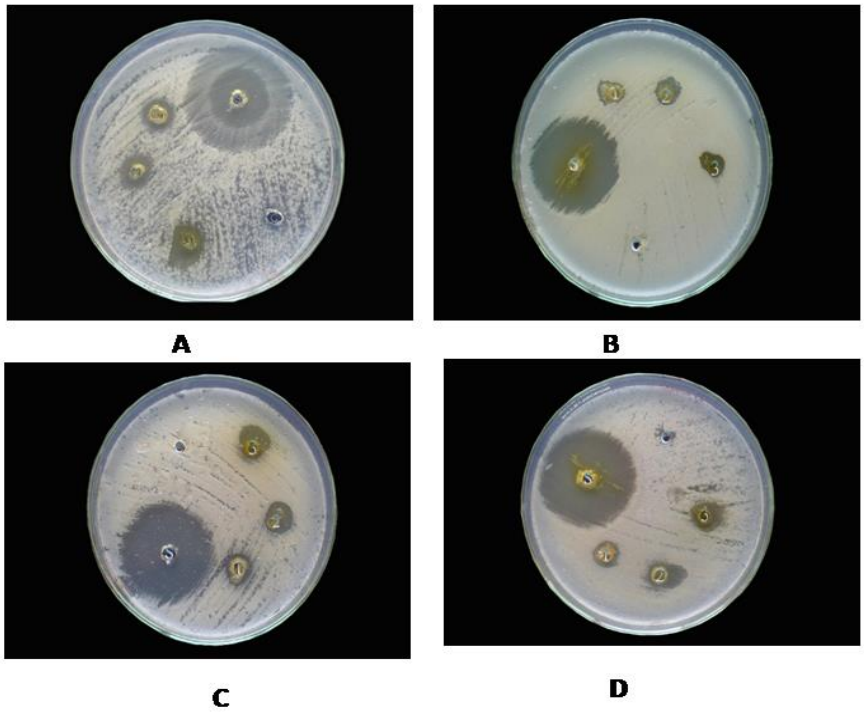

Figure 1: (A) Inhibition Zone against innoculated *Klebsiella pneumonia, Staphylococcus aureus* (Figure 1B), *Salmonella typhi* (Figure 1C), *Salmonella paratyphi* (Figure 1D), compared with standard Ampicillin antibiotic, Dimethyl Formamide (DMF) and followed with the ethanol extract of root, stem and leaf extracts

Table 1
Phytochemical studies of Tephrosia villosa Extracts (Leaf, Stem and Root)

Test	Ethanolic Extract or Root	Ethanolic Extract of Stem	Ethanolic Extract of Leaf
Alkaloids			
a) Mayer's Test:	+	+	+
b) Dragendroff's Test:	+	+	+
c) Wagner's Test:	+	+	+
Steroids			
a) Solkowski's Reaction	+	+	+
b) Libermann-Burchard's Test:	+	+	+
Carbohydrates			
a) Molisch's Test	-	+	+
b) Anthrone Test	-	+	+
Glycosides			
a) H_2SO_4 Test	-	+	+
b) Molisch's Test	-	+	+
Amino acids and Proteins			
a) Ninhydrin Test	+	+	+
b) Millon's test	+	+	+
Saponins			
a) Aqueous Test	+	+	+
Flavones			
a) Aqueous NaOH Test	+	+	+
b) Conc. H_2SO_4 Test	+	+	+
Anthocyanines			
a) Aqueous NaOH Test	+	+	+
b) Conc. H_2SO_4 Test	+	+	+
Oils and Fats			
a) Spot Test	-	-	-
b) Saponification Test	-	-	-
Phenolic Compounds			
a) $FeCl_3$ Test	+	+	+
b) Lead Acetate Test	+	+	+

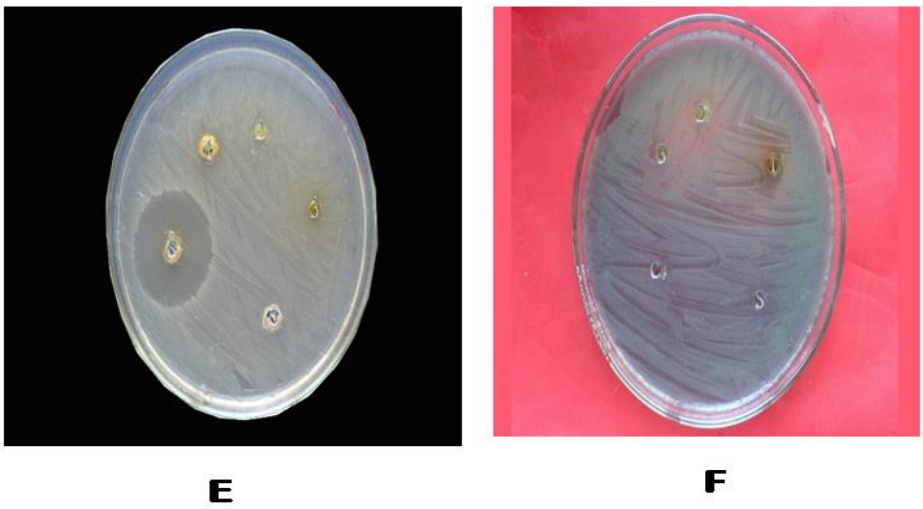

Figure 2: Showing the lower or reduced activity against innoculated *Vibrio cholera* (Figure 2E), *Pseudomonas areuginosa* (Figure 2F) compared with standard Ampicillin antibiotic, Dimethyl Formamide (DMF) and followed with the ethanol extract of root, stem and leaf extracts

Table 2
Antimicrobial activity of various extracts of *Tephrosia villosa* plant

Zone of inhibition in mm						
Ethanolic Extract	***K. pneumonia***	***S. aureus***	***S. typhi***	***S. paratyphi***	***V.cholera***	***P. aureginosa***
Root	9mm	12mm	12mm	11mm	-----	------
Stem	10mm	10mm	11mm	12mm	-----	------
Leaf	13mm	0.8mm	14mm	13mm	-----	-------
Antibiotic	20mm	28mm	30mm	35mm	28mm	-------

Antimicrobial activity: More number of compounds have been shown to exhibit antimicrobial properties by various methods, but the problem of bacterial resistance to nearly all antibiotics necessitates the discovery of novel medications. Despite ongoing reports on novel antimicrobial agents, the search is not exhaustive. In the present investigation, various extracts of *Tephrosia villosa* were tested for antibacterial activity by dig well method. The test solution was prepared to a concentration of 200µg/ml. Ethanolic extracts of root, stem and leaf were effective in causing inhibition in growth of *Klebsiella pneumonia*, *Salmonella typhii*, *Salmonella partatyphii* and *Staphylococcus aureus* and did not cause inhibition in the growth of *Vibrio cholera* and *Pseudomonas aerugenosa*.

Although only a small portion of medicinal phytochemicals have been researched, they undoubtedly play a significant role in the development of novel medications as widely distributed natural resources. For the purpose of drug discovery, additional small molecules with strong bioactivity need to be screened and identified. Finding more suitable and efficient drug delivery methods that allow the release of active compounds at the target region in infected human bodies is another challenge for future research on various phytochemicals. Developing more accurate techniques to identify the chemicals with antibacterial action in complicated plant extracts or herbal medicine formulations presents another challenge [9].

The mechanisms of action, pharmacodynamic material basis (i.e. medicinal composition), pharmacokinetics and synergistic action (e.g. interaction between a herb and a chemical antibacterial agent) as well as the development of novel products (e.g. novel drug delivery systems) should be the main areas of future research on the antibacterial properties of herbs. For accurate pharmacodynamic evaluation and quality assurance, antimicrobial components made from herbs must be screened and purified. Herbal nano-antimicrobial compounds can be made using the supramolecular self-assembly[20-22] and self-delivery approach (nanoparticles and nanofibers)[8]. These agents can be utilized to treat bacterial infections.

Conclusion

Many of the medicinal herbs that have long been employed by traditional Indian healers have recently been shown to have antibacterial and anti-inflammatory properties. The main goal of the present study was to investigate the phytochemical screening and antimicrobial effect of *Tephrosia villosa* plant extracts (leaf, stem and root). These are attributed to extracts of leaves, stems, root fractions and active compounds either the whole or part of plants. This investigation has been undertaken to study the phytochemical and antibacterial properties of ethanolic extracts of leaf, stem and root of *Tephrosia villosa* which is a traditional medicinal plant.

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