CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187
https://doi.org/10.5281/zenodo.6959244

Available online at: http://www.iajps.com *Research Article*

EXTRACTION AND ANTIMICROBIAL ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *TEPHROSIA PURPUREA*

Adil Parvez Mansoori*, Ruchi Acharya, Jaswinder Mehta, Bhawna Sharma, Peenu Mahendra Joshi

Article Received: July 2022 **Accepted:** July 2022 **Published: August** 2022

Abstract

In Present research work evaluate the antimicrobial activity of Hydroalcoholic extract of Tephrosia purpurea. *Tephrosia purpurea L. belongs to family Fabaceae, commonly known as KattuKolingi in Tamil and Sharapunka in Sanskrit. It is indigenous to India and isalso found in Ceylon, Mauritius, Tropical Africa and Subtropical regions. It is one of the most important plants used in the traditional system of medicine. Roots andseeds are insecticidal and pisicicidal. Dried extracts were subjected to the phytochemical test using standard methods to test for alkaloids, glycosides, phenol, saponins, flavonoids and teroids separately for extracts of all samples. The content of total flavonoid compounds (TFC) content was expressed as mg/100mg of quercetin equivalent of dry extract sample using the equation obtained from the calibration curve: $Y = 0.06X+0.019$, $R^2= 0.999$, where X is the quercetin equivalent (QE) and Y is the absorbance, The present investigation in this research work, the antimicrobial activity of extract obtained from Tephrosia purpurea was evaluated against bacterial pathogens used under present study.*

Key words: *Tephrosia purpurea, Antimicrobial activity, Phytochemical analysis*

Corresponding author:
Adil Parvez Mansoori,
aadilmansoori78692@gmail.com

QR code

Please cite this article in press Adil Parvez Mansooriet al, ***Extraction And Antimicrobial Activity Of Hydroalcoholic Extract Of Tephrosia Purpurea****., Indo Am. J. P. Sci, 2022; 09(8).*

INTRODUCTION:

Antimicrobial agents have become a significant global concern. Microorganisms resistant to more than two groups of antibiotics are regarded as multi-drug resistant (MDR). The global emergence of MDR bacteria is gradually elevated morbidity and mortality rates as well as in the increased treatment costs which limit the effectiveness of existing drugs and significantly cause treatment failure[1]. It often stops to respond to conventional antimicrobial agents and treatment, resulting in adverse effects on the patients, greater threat of death and higher costs. Hospital acquired infections caused by MDR bacteria is major challenge for clinicians and it creates problems in cancer and AIDS patients. Most widespread multidrug resistant bacteria include Gram-positive methicillin- resistant, *Staphylococcus aureus*, *enterococci* and Gram-negative bacteria i.e. members of *Enterobacteriaceae* and others like Pseudomonas aeruginosa, Mycobacterium tuberculosis[2].

The most wide spread multidrug resistant fungal pathogens which cause the nosocomial infections belonging to the genera *Candida, Aspergillus, Rhizopus, Penicillium, Fusarium, Cryptococcus* and Mucormycoses show high resistance to antifungal agents The indiscriminate exposure of antibiotics caused resistance and this imposed microorganisms to have a superior ability to stay alive even the strongest antibiotics. Therefore, it becomes necessary to enforce and implement some measures to reduce this problem of MDR; it can be achieved by some actions like to control the use of antibiotics, understand the genetic mechanisms of resistance, continue search for drugs with novel mechanisms of action, either synthetic or natural and take accuracy of diagnostic procedures or reduce the length of treatment[3].

In current scenario, number and types of infectious diseases are increasing at an alarming rate. This knowledge of risk associated with the use of antimicrobial agents or antibiotics has prompted research to explore medicinal properties of plants and their extracts which can serve as herbal sources of antimicrobial agents for protection against a wide range of bacteria (Gram-negative and Gram-positive) including antibiotic resistant species and fungal species. Plants contain active metabolites which may serve as alternative source of folk medicines and useful in treating various infectious diseases[4]. Plant secondary metabolites and essential oils can be used as an alternative remedy for the treatment of many infectious diseases.

The antimicrobial actions of "carqueja" (*Baccharis trimera* Less.) decoction on grampositive (*Staphylococcus aureus* and *Streptococcus uberis*) and gram-negative (*Salmonella gallinarum*and *Escherichia coli*) bacterial strains were evaluated and it was found that the former microorganisms are more sensitive to this herb than the latter, which corroborates previous studies[5]. Similarly, antimicrobial assays with plant extracts used in Asia (*Ruta graveolens* and *Zingiber officinale)* revealed an inhibitory capacity against Bacillus cereus strains[6]. In another study, the inhibitory activity of concentrates from 14 Brazilian plants against methicillin-resistant Staphylococcus aureus (MRSA) strains was analyzed[7]. The substances that demonstrated inhibitory activity were ethanol extract and its fractions (n-hexane, water, chloroform, dichloromethane, ethyl acetate and n-butanol) from *Punica granatum* fruit (pomegranate) and parts of T. avellanedae wood (purple trumpet tree). The greatest activities were found in the ethyl acetate fraction from *P. granatum* and hexane and chloroform fractions from T. avellanedae. millefolium), its essential oil (obtained from stem and leaves) presents higher antimicrobial activity than its respective extracts (methanol extract separated by chloroform into parts that were not all soluble). The oils prevented the growth of Streptococcus pneumoniae, Clostridium perfringes and Candida albicans and slightly inhibited Mycobacterium smegmatis, Acinetobacter lwoffii and Candida krussei[8]. In Present research work evaluate the antimicrobialactivity of Hydroalcoholic extract of *Tephrosia purpurea*. Tephrosia purpurea L. belongs to family Fabaceae, commonly known as KattuKolingi in Tamil and Sharapunka in Sanskrit. It is indigenous to India and is also found in Ceylon, Mauritius, Tropical Africa and Subtropical regions. It is one of the most important plants used in the traditional system of medicine.

MATERIAL AND METHODS:

Extraction by Soxhletion method

35.5 gm dried powdered stems of *Tephrosia purpurea*has been extracted with hydroalcoholic solvent (methanol: aqueous; 80:20) by soxhletion method usingsoxhlet's apparatus for 48 hrs, filtered and dried using vaccum evaporator at 40^0C.

Phytochemical Screening

Phytochemical examinations were carried out for all the extracts as per the standard methods.

1. Detection of alkaloids: Extract were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test: Filtrates was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a

yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrates was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test: Filtrates was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test: Filtrates was treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extract was dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's Test: Filtrates was treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

Benedict's Test: Filtrates was treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's Test: Filtrates was hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extract was hydrolysed with dil. HCl, and then subjected to test for glycosides.

Legal's Test: Extract was treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

4. Detection of saponins

Froth Test: Extract was diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. Detection of phenols

Ferric Chloride Test: Extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6.Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

7. Detection of flavonoids

Alkaline Reagent Test: Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test: Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

8. Detection of proteins and amino acids

Xanthoproteic Test: The extract was treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

Ninhydrin Test: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

9. Detection of diterpenes

Copper acetate Test: Extract was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes[9-10].

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride.10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol.10mg of dried extract of plant material was extracted with 10 ml methanol and filter. 3 ml (1mg/ml) of this extract was for the estimation of flavonoid.1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

Antimicrobial activity

Broth cultures of the pure culture isolates of those test microorganisms which are sensitive towards the phytoextracts used in present study were prepared by transferring a loop of culture into sterile nutrient broth and incubated at 37°C for 24hours. A loop full was taken from these broths and seeded onto sterile nutrient agar plates through sterile cotton swab to develop diffused heavy lawn culture.

The well diffusion method was used to determine the antibacterial activity of the extract prepared from *Tephrosia purpurea*using standard procedure[11]. There were 3 concentrations used which are 25, 50 and 100 mg/ml for each extracted phytochemical in antibiogram studies. It's essential feature is the placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted over night broth cultures should never be used as an inoculum. The plates were incubated at 37°C for 24 hr. and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug.

RESULTS AND DISCUSSION:

The crude extract so obtained after the maceration extraction process, extract was further concentrated on water bath evaporation the solvents completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from different extraction method using hydroalcoholic as solvents are depicted in the table 1.

A small portion of the dried extracts were subjected to the phytochemical test using standard methods to test for alkaloids, glycosides, phenol, saponins, flavonoids and steroids separately for extracts of all samples. Small amount of each extract is suitably resuspended into the sterile distilled water to make the concentration of 1 mg per ml. The outcomes of the results are discussed separately in the table 2.

The content of total flavonoid compounds (TFC) content was expressed as mg/100mg of quercetin equivalent of dry extract sample using the equation obtained from the calibration curve: $Y = 0.06X+0.019$, $R^2= 0.999$, where X is the quercetin equivalent (QE) and Y is the absorbance, Results of total flavamoid Content shown in table 3.

The present investigation in this research work, the antimicrobial activity of extract obtained from *Tephrosia purpurea* was evaluated against bacterial pathogens used under present study. The fresh pure 100% extracts obtained from plant used to suitably dilute upto the concentrations of 100, 50 and 25 mg per ml and applied on to the test organism using well diffusion method. Results of the experiment are being concluded in the Table 4-5, which clearly shows the antibacterial activity of extracts of *Tephrosia purpurea.*

Table 1: % Yield of Stems of *Tephrosia purpurea*

S. No.	Solvent	% Yield (W/W)
1	Hydroalcoholic	3.65

Table 2: Phytochemical Screening of *Tephrosia purpurea* extract

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids**	
	Dragendroff's test	-ve
	Wagner's test	-ve
	Mayer's test	-ve
	Hager's test	-ve
2.	**Glycosides**	
	Legal's test	-ve
3.	**Flavonoids**	
	Lead acetate	+ve
	Alkaline test	-ve
4.	**Phenol**	
	$fecl_3$ test	-ve
5.	**Amino acids**	
	Ninhydrin test	-ve
6.	**Carbohydrates**	
	Molisch's test	-ve
	Benedict's Test	-ve
	Fehling's Test	-ve
7.	**Tannins**	
	Gelatin Test	-ve
8.	**Proteins**	
	Xanthoproteic Test	-ve
9.	**Saponins**	
	Froth Test	+ve
10.	**Diterpenes**	
	Copper acetate Test	-ve

Table 3: Total flavonoid content of hydroalcoholic extract of *Tephrosia purpurea*

S. No.	Extract	Total flavonoid (QE)(mg/100mg)
1.	**Hydroalcoholic extract**	0.472

Table 4: Antimicrobial activity of *standard drug*againstselected microbes

S. No.	Name of drug	Microbes	Zone of inhibition		
			10 µg/ml	**20 µg/ml**	**30 µg/ml**
1	Ciprofloxacin	*Staphylococcus aureus*	17±1.69	18±2.62	22±2.16
		E. coli	16±0.86	21±0.57	28±0.5

Table 5: Antimicrobial activity of hydroalcoholic extract of *Tephrosia purpurea* againstselected microbes

S. No.	Name of microbes	Zone of inhibition		
		25mg/ml	50 mg/ml	100mg/ml
1.	*Staphylococcus aureus*	6±0	6±0	10±0.86
2.	*E. coli*	6±0	6±0	11±0.57

***(n=3, mean±SD)**

CONCLUSION:

From the above study, it can be concluded that the selected medicinal plants have great potential as antimicrobial agents against *Staphylococcus aureus* and *E. coli*. Hence, this study would lead to the development of some stable, biologically active compounds which can be employed in the formulation of antimicrobial agents.

REFERENCES:

1. Levy SB, Marshall B. Antibacterial resistance worldwide: causes, challenges and responses. Nature Medicine Supplement. 2007; 10(12):122-129.
2. Walsh FM, Amyes SGB. Microbiology and drug resistance mechanisms of fully resistant pathogens. Current Opinion in Microbiology. 2004; 7:439–444.
3. Paul R, Prasad M, Sah NK. Anticancer biology of Azadirachta indica L (neem): A mini review. Cancer Biology and Therapy. 2011; 12(6):467-476.
4. Cowan MM. Plant products as antimicrobial agents. Clinical Microbiology Reviews. 1999; 12:564–582.
5. Avancini CAM, Wiest JM, Mundstock EA. Bacteriostatic and bactericidal activity of the Baccharis trimera (Less.) D.C. Compositae decocto, as disinfectant or antisseptic. Arq Bras Med Vet Zootec. 2000; 52(3):230-4.
6. Alzoreky NS, Nakahara K. Antibacterial activity of extracts from some edible plants commonly consumed in Asia. Int J Food Microbiol. 2003; 80(3):223-30.
7. Machado TB, Pinto AV, Pinto MC, Leal ICR, Silva MG, Amaral ACF, et al. In vitro activity of Brazilian medicinal plants, naturally occurring naphthoquinones and their analogues, against methicillin-resistant Staphylococcus aureus. Int J Antimicrob Agents. 2003; 21(3):279-84.
8. Candan F, Unlu M, Tepe B, Daferera D, Polissiou M, Sokmen A, et al. Antioxidant and antimicrobial activity of the essential oil and methanol extracts of Achillea millefolium subsp. millefolium Afan. (Asteraceae). J Ethnopharmacol. 2003; 87, 2- 3.
9. Ahmad I, Beg AZ. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. J Ethnopharmacol 2001;74:113-23.
10. Reddy NS, Navanesan S, Sinniah SK, Wahab NA, Sim KS. Phenoliccontent, antioxidant effect and cytotoxic activity of Leea indica leaves, BMC Complement Altern Med 2012;12:128.
11. Mohamed, E.A.A., Muddathir, A.M. & Osman, M.A. Antimicrobial activity, phytochemical screening of crude extracts, and essential oils constituents of two *Pulicaria spp*. growing in Sudan. *Sci Rep*, 2020, 10, 17148.