SINCE 2010

NAAS Rating
2012:1.3; 2013-16: 2.69
2017-2020: 3.98

IMPACT FACTOR
2019-20: 2.40; 2021:1.09

IPI Value 2.74
SJIF 6.783

Received on:
15th January 2023
Revised on:
25th January 2023
Accepted on:
26th January 2023
Published on:
1st February 2023

Volume No.
Online & Print
155 (2023)

Page No.
01 to 06

Life Sciences Leaflets is an international open access print & e journal, peer reviewed, worldwide abstract listed, published every month with ISSN, RNI Free- membership, downloads and access.

ANTIMICROBIAL ACTIVITY OF LEAF EXTRACT OF *RAUWOLFIA TETRAPHYLLA* L. (APOCYNACEAE)

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ABSTRACT:

Antimicrobial activity of leaf extract of *Rauwolfia tetraphylla* L., was studied using different solvent like chloroform, acetone, ethanol and water against bacterial strains like *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonus aeruginosa* and fungal strains *Candida albicans*, *Aspergillus niger* and *Penicillum chrysogenum*. The antimicrobial activity was determined by disc diffusion method. Out of the four-extract used, acetone and ethanol extracts were found to be highly active against *Staphylococcus aureus* and *Candida albicans*. The MIC values were obtained by serial dilution method.

KEYWORDS: *Antimicrobial activity, Leaf, Rauwolfia tetraphylla, Apocynaceae.*

INTRODUCTION:

Rauwolfia tetraphylla L., of the family Apocynaceae, a scandent herb, has its distribution mainly in the Indo Malyan region as a plant at edge of forest in wetter parts of most districts (Ramesh and Ajay Kumar 1984). The plant *Rauwolfia tetraphylla* is widely used in traditional medicine and often it is used as an adulterant or substitute of *Rauvolfia serpentina*. Traditional practitioners use remedy for snake and other poisonous bite, blood pressure, diabetes, piles, malaria, wound, helminthiasis, hypertension, vomiting, insomnia, skin diseases, mental disorders, cough, fever and as a herbal tonic (Yoganarasimhan,1996). The leaf, stem and root of *Rauwolfia tetraphylla* were pharmacognostically studied. Preliminary phytochemical study of different extracts revealed

the presence of various phytoconstituents. The Jaintis tribes in North Cachar hill districts of Assam use the plant extract mainly to counteract dyspepsia (Sajem and Gosai, 2006). As medicinal plants are gaining more importance in pharmaceutical industries for the preparation of new phytomedicines, this study was undertaken to check its properties as a drug.

MATERIAL AND METHOD:

Plant Material

Rauwolfia tetraphylla L., Leaves are whorled, medium to dark green in color, and occur in groups of 4 unequally-sized leaves at each node. The roots yield the drug deserpidine, which is an antihypertensive and tranquilizer. *Rauvolfia tetraphylla* need full sun to partial shade with a rich well-drained soil mix.

Extraction Procedure

The leaves of *Rauwolfia tetraphylla* L., were collected from Dandeli of Uttara Kannada district, Karnataka. The leaves were dried under shade and made in to coarse powder using an electrical grinder. The powder was subjected for successive extraction with chloroform, acetone, ethanol and water using Soxhlet apparatus separately. The extracts were dried and dissolved in DMF (Dimethyl formamide) solution and screened for antimicrobial activity.

Preliminary Phytochemical Screening

The compounds that are responsible for therapeutic effect are usually the secondary metabolites. The preliminary phytochemical analysis (Kokate 1993) was carried out by following procedures:

Test for Alkaloids

A small potion of the extract is stirred with few drops of 1% Hydrochloric acid and filtered. The filtrate is treated with Wagner's reagent. Reddish brown precipitate indicates the presence of alkaloids.

Test for Sapohins

One ml of extract is diluted with 20ml of distilled water and shaken vigorously for 15 min formation of stable foam indicates the presence of saponin

Test for Tannins

Development of blue green color in the extrac6t when treated with ferric chloride indicates the presence of tannins.

Test for Phenols

Phenol test small quantity of extract is diluted with 5% ferric chloride solution. Development of intense color indicates the presence of phenols.

Test for Steroids and Triterpenes

Leibermann- Burchards test- The extract is treated with 50% sulphuric acid and a few drops of acetic anhydride are added. The development of reddish-brown ring indicates the presence of steroids.

Salkowskis test- A few drops of chloroform and few drops of concentrated sulphuric acid was added to the extract. Appearance of yellow colour in the lower portion indicates the presence of triterpenes

Test for Flavonoids

Ferric chloride test- The extract is treated with few drops of 5% ferric chloride. The appearance of blackish green color indicates the presence of flavonoids.

Antimicrobial assay:

The antimicrobial screening was done by using three bacterial strains like *Bacillus subtilis, Staphylococcus aureus, Pseudomonus aeruginosa* and fungal strains *Candida albicans, Aspergillus niger* and *Penicillum chrysogenum*. All the bacterial strains and fungal strains were obtained from the stock culture Department of Botany Bangurnagar Degree College Dandeli. The antimicrobial activity was determined by disc diffusion method (Bauer et al 1966). Three different concentrations of 25mg/ml, 50mg/ml and 100mg/ml respectively were prepared. Each sterile disc was loaded with 10µl of test extract and placed on the agar plates inoculated with respective micro-organisms. The plates were kept for half an hour for pre incubation diffusion. Then the plates were kept for incubation at 37°C for 24 hrs for bacteria and 48 hrs for fungi. At the end of incubation zones around the discs were measured in mm using Hi Antibiotic Zone scale. The study was performed in triplicate. Streptomycin disc was used as standard for bacteria and Nystain disc for fungi.

Determination of Minimum concentration:

The minimum inhibitory concentration was determined by serial dilution method (Rollins and Joseph 2000). Serial dilution of the extract was prepared in the test tubes containing peptone water as diluent. Fifty mg of the extract was dissolved in one ml of DMF which is further subjected for two-fold dilution. Totally 10 test tubes were maintained. The final concentration of the extract was now one half of the original concentration in each test tube. Each bacterial isolate was inoculated at 37°C for 24hrs. The tubes were then examined for the presence of growth considering turbidity as criterion. The highest dilution in each series that did not show turbidity and thus no growth was considered to be the MIC of the organism.

RESULTS AND DISCUSSION:

Table 1 contains the phytochemical analysis of the leaf extract of *Rauwolfia tetraphylla* which shows the presence of alkaloids, saponins, tannins, flavonoids and phenolic compounds. Table 2 gives the antimicrobial activity of *Rauwolfia tetraphylla* leaf extract and the zone of inhibition in comparison

with the standard used. Acetone and alcohol extracts showed high activity against *Staphylococcus aureus* and *Candida albicans*. The heighest zone of inhibition in case of ethanol extract against *Staphylococcus aureus* is of 16mm which is very much nearer to the standard zone of inhibition (18mm) and against *Candida albicans* the zone of inhibition was 15mm. Acetone extract also showed good inhibitory activity against these strains and the zone of inhibition obtained were 14mm and 15mm respectively. Both of the extracts were inactive against rest of the strains used. The antimicrobial activity may be due to the presence of alkaloids, saponins, tannins, flavonoids and phenolic compounds. Present in the plant as secondary metabolites.

Table 3 shows the MIC values obtained against *Staphylococcus aureus* and *Candida albicans* which is same for both the strains (12.5mg/ml). The ethanolic, methanolic and chloroformic extracts of *Nerium oleander* (Apocynaceae) leaf and root showed considerable antimicrobial activity against Bacillus pumillus, Bacillus subtilis, *Staphylococcus* aureus and Escherichia coli (Hussain and Gorsi, 2004). A similar type of study made with root extract of *Pseudarthria viscida* (Sahare *et al*; 2008) showed that ethanol extract of was highly effective against *Staphylococcus aureus and Candida albicans* apart from other strains. Similarly the alcohol extract of *Woodfordia fruticosa* flower also inhibited the growth of these two strains (Khushalani *et al;* 2008). However, the extract here was found to be a broad-spectrum microbial inhibitor. The present study indicates that although the phytochemicals of *Rauwolfia tetraphylla* is not having the broad spectrum inhibition for microbes has significant inhibition for a gram positive microbe, *Staphylococcus aureus* and a fungus Candida *albicans*.

CONCLUSION:

The extracts of higher plants can be very good source of antibiotics (Fridous *et al*., 1990) against various fungal and bacterial pathogens. Plant based antimicrobial compounds have enormous therapeutical potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. The antimicrobial activity of the *Rauwolfia tetraphylla* leaf extract against Staphylococcus *aureus* and Candida *albicans* is an indication that the leaf extract is beneficial as a cure for skin diseases. The inhibiting nature of *Pseudarthria viscida* and *Woodfordia fruticosa* on the growth of *Staphylococcus aureus* and *Candida albicans* suggests that instead of a single drug treatment multiple drug formulation would be more effective.

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Table 1: Phytoconstituents of *Rauwolfia tetraphylla* L.leaf extract

Successive extracts				
Phytoconstituents	**Chloroform**	**Acetone**	**Ethanol**	Water
Alkaloids	-	+	+	-
Saponins	-	+	+	+
Tannins	-	+	+	+
Phenolic compounds	-	+	+	+
Steroids/Triterpenes	-	-	-	-
Flavonoids	-	+	+	-

Table 2: Zone of inhibition of different extracts of *Rauwolfia tetraphylla* L. against different pathogens

Test Organisms	Standard Zone	Zone of Inhibition in mm											
		Chloroform			**Acetone**			**Ethanol**			Water		
		25	50	100	25	50	100	25	50	100	25	50	**100**
Staphylococcus aureus	18	-	-	-	11	12	14	10	14	16	-	-	-
Pseudomonus aeruginosa	22	-	-	-	-	-	-	-	-	-	-	-	-
Bacillus subtilis	15	-	-	-	-	-	-	-	-	-	-	-	-
Candida albicans	20	-	-	-	11	14	15	10	13	15	-	-	-
Aspergillus niger	19	-	-	-	-	-	-	-	-	-	-	-	-
Penicillum chrysogenum	16	-	-	-	-	-	-	-	-	-	-	-	-

Table 3. Minimum Inhibitory Concentration (mg/ml) of Acetone and Ethanol of leaves of *Rauwolfia tetraphylla* L.

Extract	Bacteria *Staphylococcus aureus*	Fungus *Candida albicans*
Acetone	**12.5**	**12.5**
Ethanol	**12.5**	**12.5**