ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 10, Issue, 12, pp.76887-76890, December, 2018

DOI: https://doi.org/10.24941/ijcr.40003.12.2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

IN VITRO EVALUATION OF ANTIMICROBIAL ACTIVITY OF TWO MEDICINAL PLANTS AGAINST HUMAN PATHOGENIC BACTERIA

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ARTICLE INFO

Article History:
Received 18th September, 2018
Received in revised form
15th October, 2018
Accepted 10th November, 2018
Published online 31st December, 2018

Key Words:
Antibacterial activity;
Trianthemapentandra and *Rubiacordifolia*

ABSTRACT

Effective treatment of a disease entails the development of new pharmaceuticals or some potential source of novel drugs. Commonly used medicinal plants of our community could be an excellent source of drugs to fight off this problem. This study is focused on exploring the antimicrobial properties of the plants that are commonly being used as traditional medicines. The antimicrobial potential of four different plant extracts was screened against twelve pathogenic microorganisms and two reference bacterial strains. Methanol extracts of *Trianthemapentandra* and *Rubiacordifolia* were subjected to a test of their antimicrobial properties by agarwell diffusion method. The result indicated that most of the extracts exhibited antimicrobial properties. The highest potential was observed in the methanol extract of *Rubiacordifolia* against *Escherichia coli, Enterococcus faecalis* with zone of inhibition (ZOI) of 15.4 ± 1.43, 15.3± 2.43 mm, respectively. *Rubiacordifolia* also showed the highest MIC against test organisms. The methanolic extract of of *Trianthemapentandra* showed efficacy against *Enterococcus faecalis*. The experiment confirmed the efficacy of two selected plant extracts as natural antimicrobials and suggested the possibility of employing them in drugs for the treatment of infectious diseases caused by the test organisms.

Citation: ***Manjula, M. and Prakash, K.*** **2018.** "In vitro evaluation of antimicrobial activity of two medicinal plants against human pathogenic bacteria", *International Journal of Current Research*, 10, (12), 76887-76890.

INTRODUCTION

Plants used in old-fashioned medicinal performs against infections have been found to prevent growth and virulence of numerous microbes (Cioch *et al*., 2017). Traditional medicine is one of the most simplyaccessibleconduct methods in emergingnations, with almost 80% of the population in about regions using outdated medicine to come across their primary healthcare needs (Maroyi, 2013). Plants createanassorted array of chemicals, known as secondary metabolites, as aversion for self-defense and statement with other organisms in their ecosystems. These secondary metabolites possess many advantages for anti-infective drug development, including being generally bioactive, being drug-like and metabolite-like, and harboring potential for synergy with other secondary metabolites as part of a plant's multicomponent defense system (Harvey *et al*., 2015). Furthermost studies on the against bacterial activity of plant extracts have focused on their growth inhibitory possible, and without great success; there are no classic (bacteriostatic or bactericidal) antibiotics derived from plant secondary metabolites on the market. Investigation into alternative anti-infective mechanisms of action can open new avenues in drug development to combat antibiotic resistance (Schroeder *et al*., 2017), and natural products may serve as a critical reservoir of antibiotic adjuvants to overcome resistance mechanisms (Wright, 2017). One target of great interest, for example, is biofilm production. Biofilms are surface-associated microbial communities that can survive high concentrations of treatment by physically occluding drug entry via the biofilm matrix and through reduction in the rate of cellular metabolism (Wu *et al*., 2015). Inhibiting the formation of biofilms could allow biofilm-associated infections to be additional efficiently determined with antibiotic treatment. For example, handling with an ellagic acid glycoside-rich blackberry extract enhanced the capability of several functionally distinct classes of antibiotics to pointedly reduce the number of biofilm-associated *S. aureus* cells on a medical device (Quave *et al*., 2012). The present study was aimed to determine the potential antibacterial activities of hydromethanolic extracts from nine selected medicinal plants organs belonging to different families on human pathogenic bacteria, in order to valorize them in the light of previous works for further application in food and pharmaceutical industries as natural valuable

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products. *Rubiacordifolia* is a perennial, climber with a stem quadrangular, growing up to 12 m long. Roots are perennial, long, cylindrical, and rusty brown in colour Leaves are highly variable, ovate lanceolate, 5-7 nerved, 2-10 cm long and 2-5 cm broad, occurring in whorls of 4-6. Flowers are fragrant, minute, whitish or greenish yellow. Fruit is minute, glabrous, 1-2 seeded, dark purplish or blackish when mature. The roots contain quinones derivative glycosides like rubiadin,1-hydroxy, 2-methoxy anthraquinone,3-dimethoxy 2 carboxyanthraquinone, rubiprasin A,B,C, and other phytochemical are mangistin, alizarin, garancin, mollugin, furomollugin specifically contained in root. Powdered dried roots decoction taken internally for the treatment of skin diseases and disorder of spleen, In Siddha and ayurvedic medicine recommended the roots of *Rubiacordifolia* used internally in the treatment of abnormal uterine bleeding, internal and external haemorrhage, bronchitis, rheumatism, stones in the kidney, gall bladder etc (Nadkarni, 2000). The plant is used in the treatment of blood disorders. *Trianthemadecandra* is a prostrate, glabrous, succulent and annual found almost throughout India. The plant belongs to the family Aizoaceae. The leaves are usual fleshy, opposite, unequal, smooth-margined leaves; prostrate growth form; the flowers with five perianth segments; flowers subtended by a pair of bracts; stamens 5 or 10; superior fruit a circumscissile capsule with a winged lid. The whole plant contained flavonoid, Cmethylflavone, tetraterpenoid 1 (Trianthenol), alkaloid trianthemine and sitosterol- (+)-glucoside and stigmasterol- (+)-glucoside. The whole plant popularly use for skin diseases, wound healing, fever and tooth aches also the bitter roots are used for curing bacterial infections and it's also given in combination with ginger as a cathartic (Jaswanth *et al.,* 2002).

MATERIALS AND METHODS

Plant material: The leaves of *Trianthemapentandra* and root of *Rubiacordifolia* were collected from Government siddha medical college, Arumbakkam, Chennai-600 106, Tamilnadu, India. The botanical identification of the plants was done by Dr. Sankaranarayanan, Head, Department of Medicinal Botany. A voucher specimen (GSMC-MB/235 and GSMC-MB/246) was deposited in the herbarium of Department of Medicinal Botany. These plant materials were air-dried at room temperature and powdered. Then 500 g of each powder were macerated in methanol (2.5 l) at room temperature for 48 h. The filtrate was then concentrated under vacuum to give crude extracts from leaves of *Trianthemapentandra* and root of *Rubiacordifolia*. These extracts were stored at room temperature till further use.

Phyto chemical Analysis: Phyto chemical analysis was carried out by using the standard procedures to identify the constituents qualitatively in plant extracts, fractions and quantitatively in dried whole plant as described by Edeoga *et al.*

Bacterial strains: Antibacterial activities were conducted by Disc Diffusion method and Minimal Inhibitory Concentration Test (MIC) for all the selected plants against pathogenic bacteria (Gram positive and Gram negative). Bacteria used for the determination of antibacterial activities were Gram positive viz; *Staphylococcus aureus* MTCC 29213, *Klebsiellapneumoniae* MTCC 1771 and *Enterococcus faecalis* MTCC 439 and gram negative viz; *Pseudomonas aeruginosa* MTCC 2488, and *Escherichia coli* MTCC 25922.

Antibacterial assay by Disc Diffusion method: The antibacterial activity of *Trianthemapentandra*, *Rubiacordifolia*, *Wedilia*was determined by the disc-diffusion method (Velickovic and Smelcerovic, 2003). *S. aureus* and *E.* faecalis (Gram-positive), *E. coli*, *P. aeruginosa*, and K. pneumoniae (Gram-negative) were grown overnight on Mueller Hinton Agar plates. Young colonies were selected from the plate and suspended with 5 mL of sterile saline (0.9%). The density of the suspension was adjusted to approximately 3×10^8 Colony Forming Units (CFU). A sterile cotton swab was dipped into the inoculum suspension and the swab rotated several times with firm pressure on the inside wall of the tube to remove the excess fluid. The swab was used to inoculate the dried surface of Muller Hinton Agar plate by swabbing over the surface of the agar, rotating the plate approximately by 90°C to ensure an even distribution of the inoculums. Different concentrations (5, 10, 15 and 20 µL/mL) of the methanol and ethyl acetate extracts of the above mentioned plants (1mg of crude extract in 1 mL of methanol) were added on to separate, sterile paper disc of 5 mm diameter. Thereafter, the discs were allowed to dry. Each of these disc were tapped gently down onto the agar to provide uniform contact. The plates were incubated at 37 °C for 24 hours. Clear zones of inhibition was measured and calculated.

Minimum Inhibitory Concentrations (MICS): The minimum inhibitory concentrations were estimated using the dilution method (Sathyabama *et al.,* 2011). The bacterial strains were grown in Mueller Hinton Broth until they reached their exponential phase with an *A*560 of 0.8, representing 3×10^8 CFU/mL. Different concentrations (5, 10, 15 and 20 µL/mL) of the methanol extracts from the leaves of *Trianthemapentandra* and root of *Rubiacordifolia*, (1mg of crude extract in 1 mL of methanol and ethyl acetate) were added on to separate test tubes. Each test tube contained 4 mL of Muller Hington Broth inoculated with 0.5 mL bacterial suspension at a final concentration of 10^8 CFU/mL. The tubes containing 4.5 mL of bacterial inoculates and 0.5 mL of 7% methanol and 7% ethyl acetate were used as bacterial control. The treated bacterial cultures were incubated for 18 hours at 37°C. The growth suppression of the bacterial cultures was determined by measuring the absorbance at *A*560 nm.

Thin Layer Chromatography: Thin layer chromatography of the aqueous methanol extract from the leaves *Trianthemapentandra* and root of *Rubiacordifolia* was executed using standard procedures (Markham, 1975). The aqueous methanol extract was placed carefully in precoated aluminum silica gel 60 F, Merck F_{254} using a microcapillary tube. The spots were allowed to dry for few minutes and TLC plate was placed in the solvent mixture (Toluene, Acetone and Formic acid in the ratio of 6:6:1). After running the experiment, the TLC plates were dried and observed under UV at 240 nm and 360 nm in a UV TLC viewer. The Rf value of the spots was calculated by using the standard formula, Distance travelled by solute Distance travelled by solvent

RESULT AND DISCUSSION

Phytochemical screening: The phytochemical screening of the aqueous methanol extract of *Trianthemapentandra* and *Rubiacordifolia* studied presently showed the presence of alkaloids, flavonoids, phenol, Terpenoids, glycosides and saponin, and absence of glycosides and Terpenoid (Table -1).

Table-1. Phytochemical screenings of aqueous methanol extract of *Trianthemapentandra and Rubiacordifolia*

Sl.No.	Phytochemical Constituents	Observation	Aqueous methanol extract of *Trianthemapentandra*	Aqueous methanol extract of *Rubiacordifolia*
1	**Alkaloids** • Dragendorff's test	Orange / red precipitate	+	+
	• Mayers test	Cream pie ppt	+	+
2.	**Flavonoids** • Alkalai Reagent	Intense yellow colour Precipitate formed	+	+
	• Lead aceate test		+	+
3.	**Glycosides** • Keller-Killiani test	Pink colour (Ammonia layers)	-	-
4.	**Tannin** • $FeCl_3$ test	Blue-black colour	+	+
5.	**Saponins** • Frothing test	Foam	+	+
6.	**Terpenoids** • Salkowski test	Reddish brown colour ring formed in interface	-	-
7.	**Polyphenols** • Ferrozine test	Raddish blue	+	+
8.	**Anthocyanin** • Ammonia test	Pink color in ammonia layer	+	+

Table 2.The antibacterial activity of methanol leaves extract of *Trianthemapentandra* disc diffusion method

Pathogenic bacteria	Methanol leaves extract of *T. pentandra* Zone of inhibition (mm)[a]				
	Positive control 10 µl Ampicillin	Different concentrations of methanol leaves extract of *W. trilobata* (µl/ml)			
		5 µl	10 µl	15 µl	20 µl
Staphylococcus aureus	12.4±1.8	7.6±1.46	10.3±2.3	12.4±1.46	13.4±1.32
Escherichia coli	11.3±1.6	7.3±1.28	8.9±1.78	10.8±1.78	12.4±1.78
Enterococcus faecalis	10.5±1.9	8.3±1.46	10.2±1.63	11.6±0.89	13.4±2.78
Klebsiellapneumoniae	11.9±2.1	8.6±0.89	10.5±1.47	11.7±1.47	12.7±1.89
Pseudomonas aeruginosa	10.8±2.3	7.5±1.36	9.8±2.46	10.9±2.12	12.1±1.63

[a]The inhibitory diameter was measured by means of calipers. All the assays were duplicated, and the mean values were recorded.

Table 3.The antibacterial activity of methanol leaves extract of *Rubiacordifolia* disc diffusion method

Pathogenic bacteria	Methanol leaves extract *R. cordifolia* Zone of inhibition (mm)[a]				
	Positive control 10 µl Ampicillin	Different concentrations of methanol leaves extract of *E. sonchifolia* (µl/ml)			
		5 µl	10 µl	15 µl	20 µl
Staphylococcus aureus	12.4±1.8	8.1±0.24	11.2±2.78	12.8±2.12	14.8±1.70
Escherichia coli	11.3±1.6	9.3±1.68	12.1±1.69	13.2±1.69	15.4±1.43
Enterococcus faecalis	10.5±1.9	9.2±1.47	11.7±1.89	13.0±0.98	15.3±2.46
Klebsiellapneumoniae	11.9±2.1	8.4±1.26	10.9±1.63	12.7±2.64	14.3±0.56
Pseudomonas aeruginosa	10.8±2.3	8.3±1.89	10.5±0.89	12.4±1.63	14.5±0.36

[a]The inhibitory diameter was measured by means of calipers. All the assays were duplicated, and the mean values were recorded.

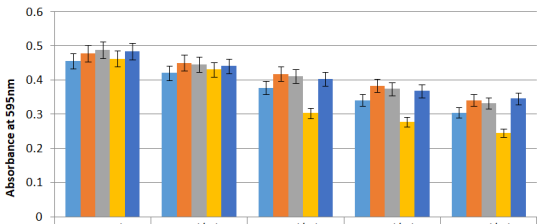
Accordingly Phytochemical analysis revealed the presence of secondary metabolites that include alkaloids, flavonoids, saponins, and anthraquinones in *Rubiacordifolia* root extract.

Tlc Finger Print: The methanol root extracts of *R. cordifolia* and leaves of *Trianthemapentandra* spotted on the TLC plates precoated with silica gel- G with best resolving solvent system showed four and five prominent spots respectively. Therefore the extracts subjected to column chromatography over silica gel, using different solvent systems fractions of mixtures were eluted.

Antimicrobial activity: The results of this study are an influence to the two medicinal plants parts that are used in Indian traditional medicine. Their antimicrobial properties to fight gainst various bacterial infections have been reported in several studies. The present outcomes of antimicrobial potential of methanol, extract of *Trianthemapentandra*, *Rubiacordifolia* against five pathogenic microorganisms K. pneumoniae**,** *E. coli,* E. faecalis, *S. aureus* and *P. aeruginosa* are shown in Table-2. The one investigated extracts exhibited considerable antimicrobial effects against all tested microorganisms. Maximum antibacterial activity was observed in *R. cordifolia* against *E. coli,* E. faecalis**,** while sensible activity was observed in *T.pentandra* against *E. coli* and *P. aeruginosa*. The highest activity of methanol extract of *R. cordifolia* was observed against *E. coli,* E. faecalis and *S. aureus* with inhibition zone of 15.4 ± 1.43, 15.3± 2.43, 14.8 ± 1.70mm respectively. This is in agreement with the earlier studies of antimicrobial activity of isolated compound Rc III- Hydroxy - 1- methyl -2 anthraquinone from the root of *R. Cordifolia* was active only against phytopathogen have been recorded (Naidu *et al*., 2009). In the present study, antibacterial potential may be attributed to bioactive components present in the plant. Previously Nawaz *et al*., 2001 experimentally proved Tetraterpenoidtrianthenol and chloroform extract of plant both showed antifungal activity. The use of this herb has been reported in Indian traditional system of medicine, and currently its application is receiving wide spread attention. In this statement, methanol extract of both plants were found excellent against both gram positive and negative associated bacteria.

Minimum Inhibitory Concentration (Mic): Results for the extracts and fractions are displayed in Graph-1 and 2. The methanol extract with best activity was chosen and fractionation was done.

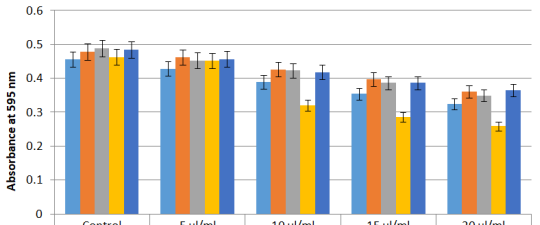
GRAPH-1. MINIMUM INHIBITORY CONCENTRATION OF METHANOL EXTRACT FROM THE ROOT OF RUBIA CORDIFOLIA



	Control	5 µl/ml	10 µl/ml	15 µl/ml	20 µl/ml
E. coli	0.456	0.421	0.378	0.341	0.305
E. faecalis	0.478	0.451	0.418	0.384	0.341
K. pneumoniae	0.489	0.446	0.412	0.374	0.332
P. aeruginosa	0.463	0.431	0.304	0.278	0.246
S. aereus	0.485	0.441	0.403	0.368	0.346

E. coli E. faecalis K. pneumoniae P. aeruginosa S. aereus

GRAPH-2. MINIMUM INHIBITORY CONCENTRATION OF METHANOL EXTRACT FROM THE ROOT OF *TRIANTHEMA PENTANDRA*



	Control	5 µl/ml	10 µl/ml	15 µl/ml	20 µl/ml
E. coli	0.456	0.428	0.389	0.354	0.324
E. faecalis	0.478	0.462	0.426	0.397	0.361
K. pneumoniae	0.489	0.453	0.423	0.387	0.349
P. aeruginosa	0.463	0.452	0.321	0.286	0.259
S. aereus	0.485	0.457	0.418	0.387	0.364

E. coli E. faecalis K. pneumoniae P. aeruginosa S. aereus

The leaves of *Trianthemapentandra*, root of *Rubiacordifolia* methanol extract could be identified as the most active extract with MICs ranging from 2–20 µg/ml on five microbial strains investigated.The root extract of *Rubiacordifolia* demonstrated highest antibacterial activity against *E. coli,* E. faecalis with MIC of 5 µg/ml. In generallyplant extracts demonstrated concentration-dependent antimicrobial activity. Aligiannis *et.al.*, (2001) projected a classification system based on MIC results gained for plant materials, which was consequently designated and executed by Duarte *et al*. (2005). All plant species with MIC values of up to 8 mg/ml are considered to possess at least some degree of inhibitory outcome, and any concentration beyond this should not be reflected effective, according to Fabry *et al.* (1998).

Conclusion

Thus, the evidence of in vitro antibacterial suggests the possibility of simultaneous use of cost effective conventional anti-bacterial antibiotics, in combination with the plant extracts, in treating human infection due to antimicrobial resistant drug bacteria, when the agents no longer effective during monotherapy. However, future research based on animal models, may resolve in vivo efficacy of methanol, extract from the leaves of *Trianthemapentandra* and root of *Rubiacordifolia*, either alone, or in combination with antibiotics.

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