International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 7; Issue 6(F); June 2018; Page No. 13453-13456
DOI: http://dx.doi.org/10.24327/ijcar.2018.13456.2402

Research Article

AN EVALUATION OF PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITIES POSSESSED BY EXTRACTS OF INDIAN FLOWERS

Pinky Yadav[1]., Anita Khalkho[1]., Ankur Kashyap[1]., Nisha Mishra[1] and Madhulika Singh[2]

[1]Department of Biotechnology, Khandelwal College of Management Science and Technology, Bareilly, (U.P.)
[2]Division of Biotechnology, CytoGene Research & Development, Lucknow (U.P.)

A R T I C L E I N F O

Article History:

Received 9th March, 2018
Received in revised form 16th April, 2018 Accepted 26th May, 2018
Published online 28th June, 2018

Key words:

Vibrio sps., Flower extract, Phytochemical Activity, Antimicrobial Activity

A B S T R A C T

To evaluate Phytochemical and antimicrobial activity of different parts (Flower & Leaf) of *Tagetes erecta, Rosa, Nerium oleander*. Different Part of *Tagetes erecta, Rosa, Nerium oleander* were extracted with 20% , 30%, 50% (v/v) solvent (Acetone, Methanol, Ethanol)**.** The *Vibrio sps* (*V. parahaemolyticus & V. vulnificus*). used in this study were obtained on TCBS (Thiosulfate Citrate Bile sucrose) Media. The plant extracts were subjected to antimicrobial assay. In case of antimicrobial screening, crude extract of flower & leaves showed notable antibacterial activity against tested microorganisms. In Antibacterial Activity of the plant extract showed maximum zone of Inhibition was observed in 50 % Rose Leaves Acetone extract (28 mm) and minimum zone of inhibition was observed in 20 % Rose Flower Methanol extract (10 mm). From this study, it can be concluded that the species is effective in scavenging free radicals and has the potential to be a powerful antioxidant. These plant extracts which proved to be potentially effective can be used as natural alternative preventives to control cholera diseases and preserve food stuff avoiding healthy hazards of chemically antimicrobial agent applications.

INTRODUCTION

The prevalence of microbial infectious diseases and their complications are continuously increasing throughout the world mainly due to microbial drug resistance toward commonly used antimicrobials [1]. Plant derived antimicrobials are also considered to be safer compared with synthetic compounds because of their natural origin [2, 3]. It is well known that about quarter part of current medications is derived from compounds of plant origin [1, 4]. Plant-derived compounds could have other target sites than traditional antimicrobials and subsequently having different mechanisms of action against microbes [3, 5, 6].

Plant secondary metabolites are mostly responsible for their antimicrobial activity [7]. Major groups of phytochemicals which possess antimicrobial properties are phenolics and polyphenols (flavonoids, quinones, tannins, coumarins), terpenoids, alkaloids, lectins and polypeptides [8, 9, 3]. The preservative effect of many plant and herbs suggests the presence of antioxidative and antimicrobial constituents in their tissues. It has been reported that there is an inverse relationship between antioxidative status and incidence of human disease [9].

Corresponding author:* **Pinky Yadav
Department of Biotechnology, Khandelwal College of Management Science and Technology, Bareilly, (U.P.)

In recent years, multiple drug resustance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in treatment of infectious diseases. This situation forced scientists to search for few antimicrobial substances from various sources like medicinal plants which are good sources of noval antimicrobial agents [10].

More than 100 phytochemical compounds have been isolated from various parts of the plant, namely phenols, flavonoids, alkanoids, cardiac glycosides, saponines, terpenoids, steroids and tannins. These compounds are well known to possess biological and pharmacological activity against various chronic diseases such as cancer and cardiovascular and gastrointestinal disorder [11, 12, 13, 14].

Antioxidant, antiulcer, antidiabetic, anticancer, antihyperlipidaemic, anti-inflammatory, antimicrobial, antispermatogenic effects have also been reported on various animal models by the crude extracts of this plant [14, 15, 16, 14, 17, 18, 19, 20, 21, 22].

MATERIAL & METHOD

Sample Collection

Flower samples (Marigold, Rose, Oleander, Oleander leaves and Rose leaves) were collected from nearby area of B Block and Amrapali market, Indira Nagar (Lucknow). Flower samples were used for extraction of their active compounds using variable percentages of the solvents.

Extract Preperation

Flower sample were grinded into powdered form with a grinder. All the flower sample (100g) were then soaked in solvent such as acetone, methanol, ethanol in 20%, 30%, 50% separately in a beaker and let to soak for 4 days at room temperature (26-28ºC). Removal of dry plants parts was done by filtration through musclincloth and Whatman filter paper. The filtrate was then further concentrated using rotary evaporator. The extracts were all placed in glass petridishes. The dried plant extracts were then redissolved in solvent in order to obtain a solution containing 2.0 mg/ml of extract, respectively which are then used for assays.

Phytochemical Analysis

The tests were done to find the presence of the active chemical constituents such as alkaloids, glycosides, and steroids, flavonoids, reducing sugar and tannin.

Alkaloid test

1ml plant extract was taken and added ammonia solution (3ml). It was allowed to stand for few minutes to evaluated free alkaloids. Chloroform (10ml) was added to the test tube shaken by hand and then filtered.

The chloroform was evaporated from the crude extract by water bath. Mayer's reagent (3ml) was added. A cream color precipitation was obtained immediately that showed the presence of alkaloids.

Glycoside (Keller-Killiane) test

Test solution was treated with few drops of glacial acetic acid and Ferric chloride (.1M) solution and mixed. Concentrated sulphuric acid was added, and observed for the formation of 2 layers. Lower reddish brown layer and upper acetic acid layer which turns bluish green would indicate a positive test for glycosides.

Steroid test

1ml extracts were taken in a test tube and dissolved with Chloroform (10ml). Then added equal volume of concentrated sulphuric acid to the test tube by sides. The upper layer in the test tube was turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

Tannins (Gelatin) test

To the extracts 1% gelatin solution containing Sodium chloride was added. Formation of white precipitates the presence of tannins.

Phenols test (Ferric Chloride) test

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

Isolation and characterization of Bacterial sps.

Bacterial isolation was done by spreading the water sample on the TCBS media and colonies of bacterial growth were obtained after incubation at 37˚C for 24 hours. Water samples used for isolation were collected from Indira Nagar sewage water.

Antimicrobial activity of the flower extracts

Antimicrobial susceptibility test of the selected pathogens was done by well diffusion method using Kirby-Baeur technique [23]. All the tests were performed on Tryptone soya agar plates. Suspension of microbial cultures was inoculated on the entire surface of the Tryptone soya agar media in a Petri plate using sterile swab sticks. Inoculated plates were incubated at 37∘C for 24 hrs. On the second day, plates were read by taking measurement of zone of inhibition around each disc. The diameter of zone of inhibition of bacteria was recorded in millimeters. Pure acetone, methanol and ethanol were taken as negative control. The assay was done in triplicates and checked with the control plate. To determine the affectivity of extracts at different volumes, two different concentrations of extracts were taken on well diffusion method, on every Petri plate [24, 25].

RESULT & DISCUSSION

Isolation of Bacteria and Characterization

The *Vibrio sps.* used in this study were obtained on TCBS (Thiosulfate Citrate Bile sucrose) Media as shown in Figure 1.The morphological and biochemical characteristics of the isolates were examined according to the Bergey's manual of determinative Bacteriology (Kumar *et al.,* 2015) are given in Table 1.

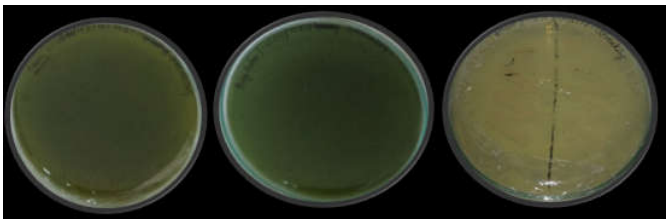


Figure 1 Isolation of vibrio species

Table 1 Identification of Vibrio species

S. No.	Test Name	B-A	B-B
1.	Indole test	Negative	Negative
2.	Sugar fermentation test		
	Glucose	Positive	Positive
	Sucrose	Negative	Positive
	Lactose	Negative	Negative
3.	MR test (Methyl-Red)	Negative	Positive
4.	VP test (Vogesproskauer)	Negative	Negative
5.	Urease test	Positive	Positive
6.	Citrate test	Positive	Positive
7.	Gram staining	Negative	Negative

Phytochemical Test

In the phytochemical screening, as shown in Table 2, we observed the presence of different phytochemicals like Phenol, Tannins, Glycosides, Steroids & Alkaloids.

Table 2 Phytochemical test of Plant extract (Rose flower, Rose leaves, Marigold Flower, Oleander flower, Oleander Leaves)

	Rose Flower								
Test name	Acetone			Methanol			Ethanol		
	20%	30%	50%	20%	30%	50%	20%	30%	50%
Phenols	A	P	A	P	P	P	P	P	P
Tannins	P	A	P	P	P	P	A	A	A
Glycosides	A	A	A	P	P	P	P	P	P
Steroids	A	P	P	P	A	A	P	A	P
Alkanoids	A	A	A	A	A	A	A	A	A
	Rose Leaves								
Test name	Acetone			Methanol			Ethanol		
	20%	30%	50%	20%	30%	50%	20%	30%	50%
Phenols	P	P	P	P	P	P	P	P	P
Tannins	P	P	P	A	A	A	P	P	A
Glycosides	A	A	A	P	P	A	A	A	P
Steroids	P	P	P	A	P	P	P	A	P
Alkanoids	A	P	P	P	A	A	P	P	P
	Marigold Flower								
Test name	Acetone			Methanol			Ethanol		
	20%	30%	50%	20%	30%	50%	20%	30%	50%
Phenols	P	P	P	P	P	P	P	P	P
Tannins	A	A	A	A	A	A	A	A	A
Glycosides	P	P	P	A	A	P	P	P	P
Steroids	A	A	A	A	P	A	A	A	P
Alkanoids	P	P	P	P	P	A	P	A	A
	Oleander Flower								
Test name	Acetone			Methanol			Ethanol		
	20%	30%	50%	20%	30%	50%	20%	30%	50%
Phenols	P	P	P	P	P	P	P	P	P
Tannins	A	A	A	A	A	A	A	A	A
Glycosides	A	A	A	A	A	A	P	A	A
Steroids	A	A	A	A	P	A	A	A	A
Alkanoids	P	P	P	P	P	P	P	P	P
	Oleander Leave								
Test name	Acetone			Methanol			Ethanol		
	20%	30%	50%	20%	30%	50%	20%	30%	50%
Phenols	P	P	P	P	P	P	P	P	P
Tannins	A	A	A	A	P	P	A	A	P
Glycosides	P	P	A	A	A	P	P	A	P
Steroids	P	P	A	P	A	A	P	P	P
Alkanoids	P	P	P	P	P	P	P	P	P

Antibacterial activity

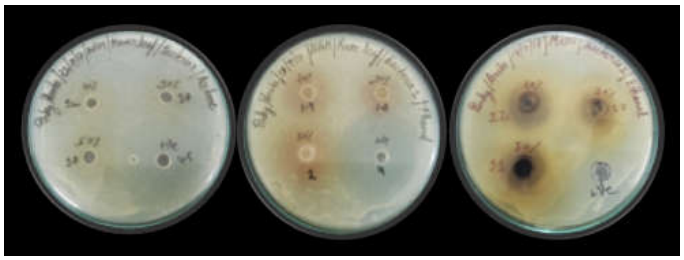
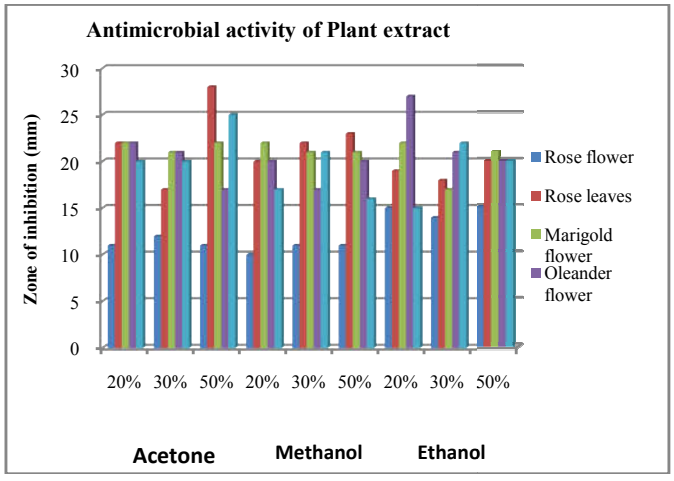


Figure 2 Antimicrobial Activity of Plant extract

Antibacterial activity results of solvent extracts of the different parts of flower such as Rose, Marigold & Oleander as shown in Figure 2. In general, the zone of inhibition produced by the Acetone, Methanol & Ethanol was between 28 to10 mm and was larger than those produced by acetone extracts which was 28 mm. Based on the results, the acetone extract of rose leaves showed the highest zone of inhibition compared with all the extracts against all the tested microorganisms.



Graph 1 Antimicrobial activity of Plant extract

In Antibacterial Activity of the plant extract showed maximum zone of Inhibition was observed in 50 % Rose Leaves Acetone extract (28 mm) and minimum zone of inhibition was observed in 20 % Rose Flower Methanol extract (10 mm) as shown in Graph 1.

CONCLUSION

In summary, plant extracts can be considered a good source of natural antioxidants and antimicrobials. Polyphenol extraction from plants using fast and appropriate techniques is a low-cost method due to the reduction in the amount of solvent used, in addition to avoiding the need for longer extraction times compared to the conventional extraction method. Moreover, natural bioactive compounds have been found to interfere with and prevent food borne disease. In fact, many studies have shown that flavonoids play significant multiple roles including Cholera, dysentery & Typhoid due to their acceleration of different aging factors. More comprehensive studies related to these compounds will enhance pharmaceutical exploration in the field of water borne disease prevention.

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How to cite this article:

Pinky Yadav *et al* (2018) 'An Evaluation of Phytochemical and Antimicrobial Activities Possessed by Extracts of Indian Flowers', *International Journal of Current Advanced Research,* 07(6), pp. 13453-13456.
DOI: http://dx.doi.org/10.24327/ijcar.2018.13456.2402

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