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Evaluation of Anti microbial Activity of Extracts of *Pedalium murex*

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ABSTRACT

As part of search for new biological active compounds from higher plants the crude organic extracts of *Pedalium murex*as screened. The purpose of this study was to investigate Indian plants potential antibiotic activity by preliminary bio-screening. That is why to evaluate the anti-bacterial potential of different extracts of *Pedalium murex* was carried out. .In the present study, extracts of *Pedalium murex*was evaluated for preliminary phytochemical screening and antibacterial and antifungal activity. The various extracts of the powdered leaves of *Pedalium murex* (L.) were subjected to anti-bacterial studies one gram+ve and one gram-ve bacteria. The organisms used were *Staphylococcus epidermidis* (gm+ve) and *Escherischia coli* (gm-ve) and for evaluation of anti-fungal studies against the fungi like *Candida albicans* and *Aspergillus niger* are taken. The organisms were maintained on sabouraud's broth. Different extracts were tested at dose 40 mg/ml for both bacteria and Fungi where ciprofloxacin and ketoconazole were taken as standard respectively. The results showed among of all extracts ethanolic extract possess good antimicrobial activity against bacteria and fungi when the zone of inhibition is evaluated after 24 hrs of incubation at 37^0C.

1. Introduction

Worldwide interest in natural products as preventing and therapeutic agents has led to a greater appreciation of the rich heritage of traditional systems of medicine. Dietary and lifestyle modifications are the basis of Ayurvedic medicine, with herbalformulas rounding out therapeutic programs. Ayurvedic formulas contain many balancing herbs offering a high degree of safety and efficacy**[1]**. While these plant remedies are being used orally and by local application, the mechanism whereby such effects elicited has not been looked into. These effects have been brought about by their inherent antibacterial and anti-fungal activity by different plants.

As part of search for new biological active compounds from higher plants the crude organic extracts of *Pedalium murex* as screened. The purpose of this study was to investigate Indian plants potential antibiotic activity by preliminary bio-screening. That is why to evaluate the anti-bacterial potential of different extracts of *Pedalium murex* was carried out band reported here under.The main constituents of fruit are saponins/furastanol alkaloids 1%. Others are 2',4',5'-trihydroxy-5,7-dimethoxyflavone, triacontanyldotriacontanoate, rubusic acid, nonacosane, tritriacontane, triacontanoic acid, tritriacontanoic acid and sitosterol-β-D-glucoside. The fruit contains 3.5 to 5 % stable oil, aromatic oil, resins, tannins, glycosides,sterol and some nitrates.An infusion or extract of fresh leaves and stem in cold water, is demulcent and diuretic, useful in disorders of the urinary system such as the gonorrhoea, dysuria, spermatorrhoea etc. It dissolves calculi. In gonorrhoea half a pint of the above infusion taken every morning for 10 days successively relieves the scalding (burning sensation during micturition in gonorrhoea), and in many cases, nocturnal emissions and impotency, a cure is affected. Leaves are used very largely as a healing application to ulcers**[2]**.

2. Material and Methods

2.1 Plant Material

The fresh leaves of *Pedalium murex* belonging to the family Pedaliaceae were collected from the outskirts of Alwar in December 2013. The plant of *Pedalium murex has* been authenticated from Rajasthan University, Jaipur, India. (Ref. RU/2014/431).The leaves was dried initially under shade. It was preserved in a tightly closed container and powdered as per requirements.

2.2 Preparations of Extracts

The leaves of plant was subjected to size reduction to a coarse powder by using dry grinder and passed through sieve. About 150g of this powder was packed into Soxhlet apparatus and extracted successively with Ethyl acetate, chloroform, ethanol and aqueous (yield 1.65%, 1.56%, 1.82%, and 1.71 % respectively). The solvent was recovered by distillation in vacuum and extracts were stored in desiccators and used for subsequent experiments.[3]

2.3 Anti - Microbial Evaluation

2.3.1 Test Micro-organisms

The various extracts of the powdered leaves of *Pedalium murex* were subjected to anti-bacterial studies one gram+ve and one gram-ve bacteria. The organisms used were

1. *Staphylococcus epidermidis* (gm+ve)

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2. *Escherischia coli* (gm-ve)

The organisms were maintained on Nutrient Agar slants. These were tested using Nutrient broth. One loop full of the respective cultures (S. epidermis and E. coli) in slants which were maintained below 4^0 c for were taken and inoculated in the broth and incubated at 37^0c or 24 hours and were observed for the growth of the organism with naked eye for their turbid nature and compared with sterile broth. The presence of turbidity indicates growth and suitability of the culture for further work.

2.3.2 Preparation of stock culture

From the cultures which were maintained on Nutrient Agar slants, one loopful of the respective organisms were taken and aseptically transferred to 100ml of sterile nutrient broth in an Erlenmeyer flask which was shaken thoroughly and incubated at 37^0c for 24 hours. This is the stock-culture or sub-culture**[4-6]**.

2.3.3 Standardization of stock culture

1ml of this seeded broth was then diluted with 9ml of sterile water in a culture tube with0.05% tween 80(containing 8 drops of tween 80 in every 1000ml of water). This was shaken thoroughly and about 1ml of this suspension was transferred to a second culture tube which in addition contains 9ml of sterile water. This was shaken thoroughly and thus was further diluted 10 times with sterile water till 10^{10} dilution was obtained (up to 10 culture tubes).

Standardization of the seeded broth was done by inoculating 0.2ml of each dilution on to solidified nutrient agar medium by spread plate method. After incubation at 37^0c for 48 hours, the numbers of well formed colonies on the plates were counted. The seeded broth was then suitably diluted to contain between 10^7-10^8 micro-organism c.f.u./ml(colony forming until per ml). This was designated as the working stock that was used for anti-bacterial studies**[7]**.

2.3.4 Preparation of test solution

The test solution of each extract was prepared by dissolving the dry extracts of powdered leaves of *Pedalium murex*in respective solvent of each extract and used as control. Concentration of the test extracts was 3mg/ml and the standard anti-bacterial antibiotic ciprofloxacin 1mg/ml concentration.

2.3.5 Preparation of Culture Media

The media used for growth of bacteria was

1. Nutrient agar medium
2. Nutrient broth medium

Medium was sterilized by autoclaving at 15lb/sq.mm pressure at 121^0 C for 15 minutes.

1. Nutrient Agar Medium

The nutrient agar medium was prepared by dissolving 28 gm of nutrient agar in 1000 ml of distilled water.

Formula

Peptone - 1%
Sodium chloride - 0.5 %
Beef extract -1%
Agar- 2%
The pH was adjusted to 7.4±0.2

2. Nutrient Broth Medium

The nutrient broth medium was prepared by dissolving 13gms of nutrient agar in 1000ml of distilled water.

Formula

Peptone - 1%
Sodium chloride - 0.5 %
Beef extract -1%
Agar- 2%
The pH was adjusted to 7.4±0.2

2.3.6 Screening of anti-bacterial activity

Anti-bacterial activity of different test extracts was screened by Filter Paper Disc Method. Petri dishes were filled to a depth of 4-5 mm with a nutrient agar medium that had previously inoculated with suitable inoculum of a suitable organism of *Staphylococcus epidermidis* and *Echerischia coli*. The temperature of the medium does not exceed 48^0c to 50^0c when it was inoculated and the dishes maintained at temperature of 37^0c. The dishes were specially selected with bottom and were placed on a level surface so as to ensure that the layer of the medium was of a uniform thickness. Than the plate was divided by marking with marker for section according to test, control and standard**[8]**.

Filter paper (no. 2 whatman's) is cut into small disc (6 mm diameter) and sterilized in a plugged container in a hot-air oven. Sterile disc of filter paper are dipped into the test liquid (40 mg/ml) of all the extract and standard antibiotic ciprofloxacin (1mg/ml). The excess is allowed to drain and each disc is laid on the solid medium. The zone of inhibition is recorded after 24 hrs of incubation at 37^0c in table no. The data are plotted for graphical representation of every extract against zone of inhibition**[9,10]**.

2.4 Antifungal activity

2.4.1 Test Micro- organisms

The various extracts of the powdered leaves of *Pedalium murex* were subjected to anti-fungal studies against the fungi like

1. *Candida albicans*
2. *Aspergillus niger*

The organisms were maintained on sabouraud's broth. One loopful of respective culture (Candida albicans and *Aspergillus niger*) in slants which were maintained below 4^oc was taken and inoculated in broth and incubated at 37^o c for 24 hours and were observed for the growth of the organism with the naked eye for their turbid nature and compared with sterile broth. The presence of turbidity indicates growth and suitability for further work with the culture**[11].**

2.4.2 Preparation of Stock-Culture

From the cultures which were maintained on sabauraud's agar slants, one loopful of the respective organisms were taken and aseptically transferred to 100ml of sterile sabouraud's broth in an Erlenmeyer flask which was plugged with sterile cotton wool. This was shaken thoroughly and incubated at 37 ^{o}c for 24 hours. This is the stock culture or sub-culture.

2.4.3 Standardization of Stock-Culture

From this sub culture 1ml was transferred to 99ml of fresh sterile sabouraud's broth and shaken thoroughly. This is referred to as seeded broth. Seeded broth of both the organism s to be tested was prepared.

1 ml of this seeded broth was then diluted with 9 ml of sterile water in a culture tube with 0.05% of tween 80 (containing 8 drops of tween 80 in every 1000ml of water). This was shaken thoroughly and about 1ml of this suspension was transferred to a second culture tube which in addition contains 9ml of sterile water.[12] This was further diluted 10 times with sterile water till 10^{10} dilutions were obtained (up to 10 culture tubes). Then standardization of seeded broth was done by taking 0.2 ml of solution from the first culture tube and plating in triplicate on the SDA plate. Plating in triplicate on the SDA plates were carried out for the various dilutions (for all the culture tubes). These plates were incubated then at 37^{0}c for 48 hours and the numbers of well form colonies on the plate were counted. The final resulting suspension that contained 10^{5} to 10^{7}c.f.u /ml were taken for in- vitro anti-fungal activity**[13]**.

2.4.4 Preparation of Test Solution

The test solution of each extract were prepared by dissolving the crude extracts of powdered leaves of *Pedalium murex*in respective solvents of each extracts and that solvent were used as control. Concentration of the test extract and the solvent were 40 mg/ml and the standard anti-fungal used was ketoconazole(1mg/ml)**[14]**.

2.4.5 Culture Media

Media used for the growth of fungi was Sabouraud's Dextrose Agar (SDA) and Sabouraud's Dextrose Broth (SDB).

1. Sabouraud's Dextrose Agar (SDA) Medium

Glucose - 40gms
Peptones - 10gms
Agar - 25gms
Distilled water - 1000ml

The above constituents were put together and maintaining the PH 5.4, autoclaved at 121^{0}C at 15lb/sq. mm pressure for 15 minutes and used for sub-culturing of *C. albicans* and *A. niger*.

2. Sabouraud's Dextrose Broth (SDB) Medium

Glucose - 40gms
Peptone - 10gms
Distilled water - 1000ml

The above constituents were put together and maintaining the pH 5.4, autoclaved at 121^{0}C at15 lb/sq.mm pressure for 15 minutes and used for sub-culturing of *C. albicans* and *A. niger*.

2.4.6 Screening of anti-fungal activity

Anti-fungal activity of different test extracts was screened by filter paper disc method. Petri dishes were filled to a depth of 4-5mm with SDA medium that had previously inoculated with suitable inoculum of a suitable organism of *C. albicans* and *A.niger*. the temperature of the medium do not exceed 48-50^{0}c when it was inoculated and the dishes were maintained at temperature of 37^{0} c the dishes were specially selected with flat bottom and were placed on a leveled surface so as to ensure that the layer of the medium was of uniform thickness. Then the plates were divided by marker for sections according to test, control and standard**[15]**.

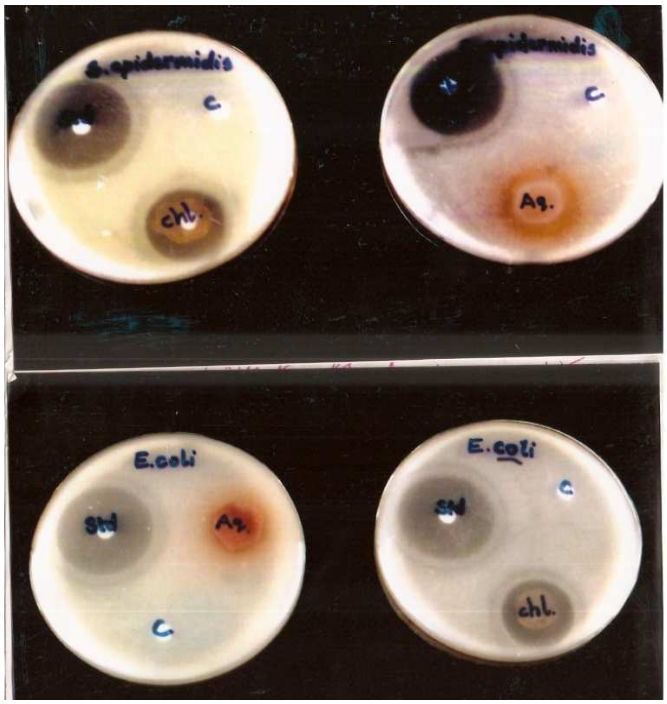
Whatman's filter paper disc were cut into small discs (6mm diameter) and sterilized in a plugged container in a hot air oven. Sterile discs of filter paper were dipped in to the test liquid (40 mg/ml) of all the test extracts and standard ketoconazole (1mg/ml). The excess is allowed to drain and each disc is laid on the solid medium. The zone of inhibition were recorded after 24 hours of incubation at 37^{0}C in table No.2 and the data are plotted in a graphical representation for every extract against zone of inhibition**[16]**.

3. Results and Discussion

Table No. 1: Anti-bacterial activity of various extract of powdered leaves of *Pedalium Murex*

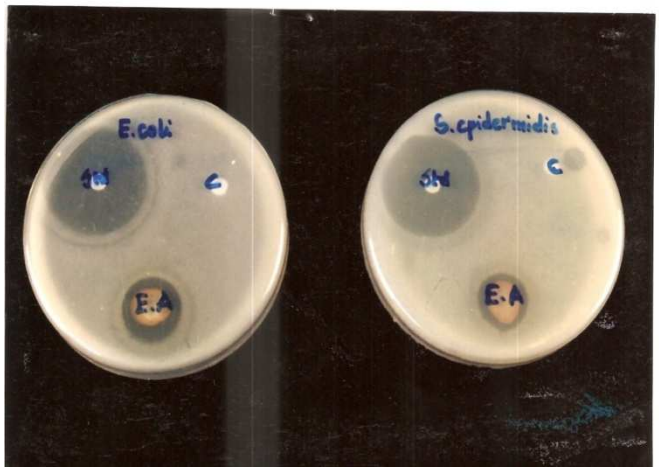
Test Microorganism	**Zone of Inhibition (mm)**				
	Standard(1mg/ml)	**Chloroform Extract(40mg/ml)**	**Ethyl acetate Extract (40mg/ml)**	**Ethanol Extract(40mg/ml)**	**Aqueous Extract(40mg/ml)**
Staphylococcus epidermidis	36	17	21	24	14
Escherischia coli	35	16	22	23	12

Figure No. 1: Antibacterial activity of various extracts of powdered leaves of *Pedalium murex*



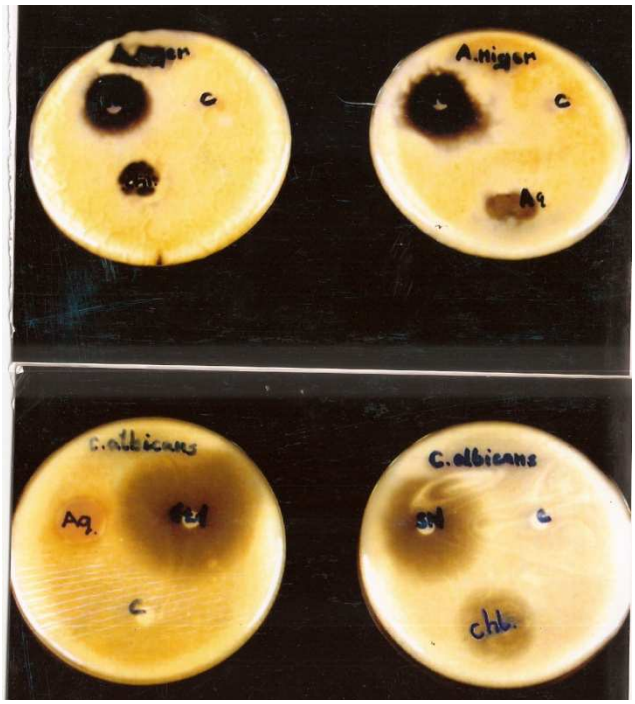
Std. : Standard (Ciprofloxacin), C : Control, Chl. : Chloroform extract, Aq. : Aqueous extract

Figure No. 2: Antibacterial activity of ethyl acetate extracts of powdered leaves of *Pedalium murex*



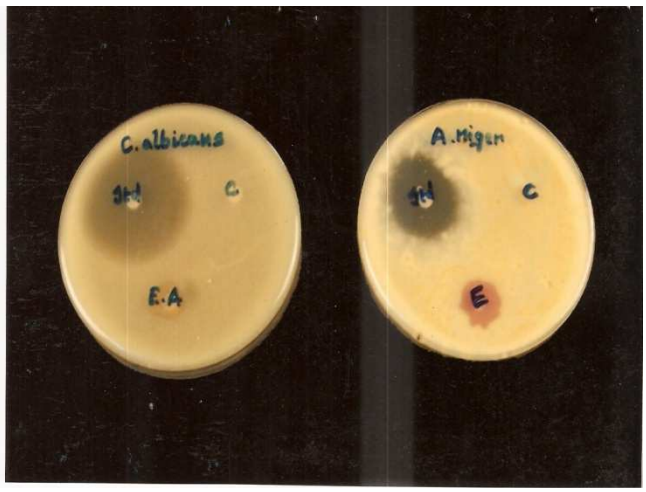
Std. : Standard (Ciprofloxacin), C : Control, E.A. : Ethyl Alcohol

Figure No. 3: Antifungal activity of various extracts of powdered leaves of *Pedalium murex*



Std. : Standard (Ketoconazole), C : Control, Chl. : Chloroform extract, Aq. : Aqueous extract

Figure No. 4: Antifungal activity of various extracts of powdered leaves of *Pedalium murex*

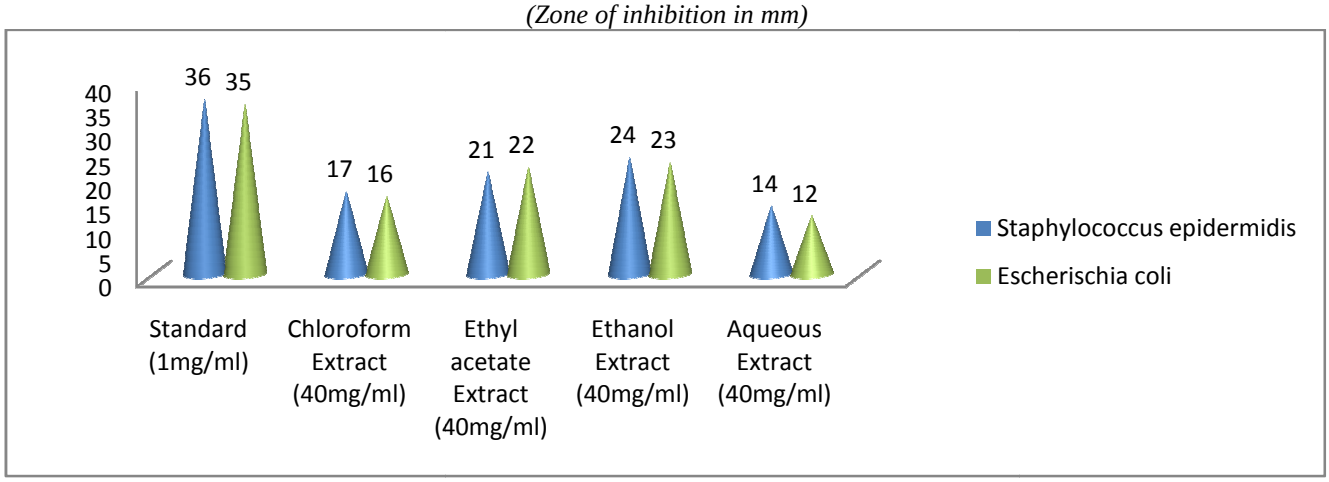


Std. : Standard (Ketoconazole), C : Control, E.A. : Ethyl Alcohol, E : Ethyl acetate

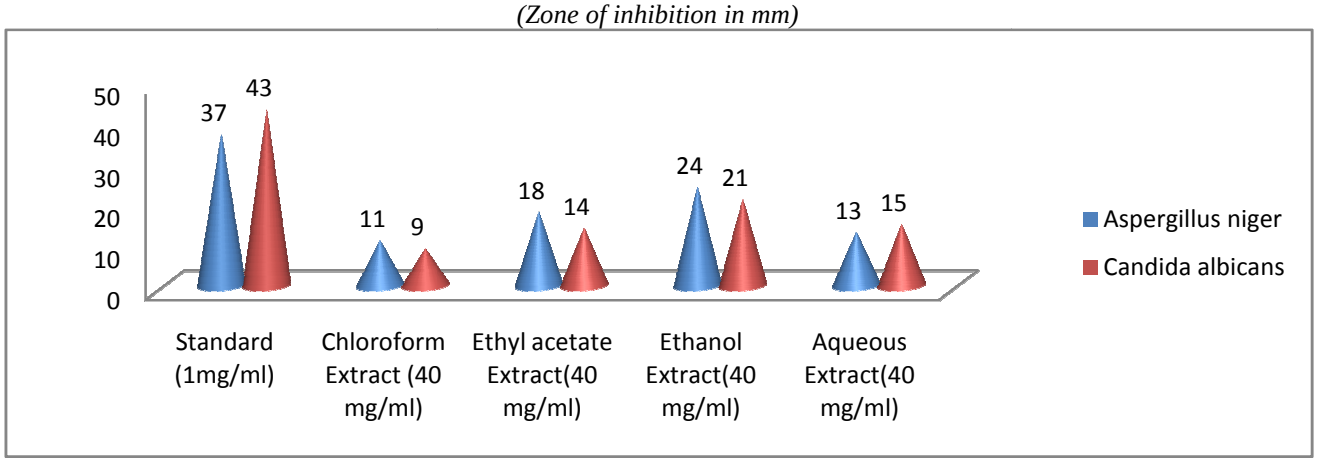
Table No.2: Anti-fungal activity of various extract of powdered leaves of *Pedalium murex*

Test microorganism	Zone of inhibition (mm)				
	Standard(1mg/ml)	Chloroform Extract(40 mg/ml)	Ethyl acetate Extract(40 mg/ml)	Ethanol Extract(40 mg/ml)	Aqueous Extract(40 mg/ml)
Aspergillus niger	37	11	18	24	13
Candida albicans	43	09	14	21	15

Graph No. 1: Graphical representation of antibacterial activity of various extract of powder leaves of *Pedalium murex (Zone of inhibition in mm)*



Graph No. 2: Graphical representation of antifungal activity of various extracts of powder of leaves of *Pedalium murex (Zone of inhibition in mm)*



4. Discussion

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The world health organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the words population. In the present work, various extracts obtained from *Pedalium murex*leaves shows strong activity against most of the tested bacterial and fungal strains. The results were compared with standard antibiotic drugs. In this screening work, no extracts of *Pedalium murex* were found to be inactive against any organism, such as Gram positive, Gram negative and fungal strains were resistant to all the extracts of *Pedalium murex*. From the above results the activities of ethanolic extract of *Pedalium murex* shows significant antibacterial and antifungal activity. The present study when we compare zone of inhibition of various extracts treated strain maximum zone of inhibition was observed for ethanolic extract treated strain for antibacterial and antifungal strains. The results claimed uses of leaves in the traditional system of medicine to treat various infectious disease caused by the microbes. Therefore, it may be concluded from the above results, that the crude extracts obtained from the leaves of *Pedalium murex* may be used enough as drug to treat disease caused by those bacteria, which are sensitive to the above mentioned samples. But before use in human being isolation of pure compound, toxicological study, and clinical trial in animal model should be carried out thereafter. However, further studies are needed to better evaluate the potential effectiveness of the crude extracts as the antimicrobial agents.

5. Conclusion

In conclusion, the results of present investigation suggest that, ethanolic extract of *Pedalium murex* have significant antibacterial and antifungal activity against various strain of bacteria and fungi. However, further studies are suggested to establish molecular mechanism and also to isolate and characterize the active principles responsible for the action.

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