Int. J. Ayur. Pharma Research, 2014; 2(6): 24-27 **ISSN: 2322 - 0910**

International Journal of Ayurveda and Pharma Research

Research Article

EVALUATION OF ANTIMICROBIAL ACTIVITY OF SEED EXTRACT FROM PLANT *INGUDI* (*BALANITES AEGYPTIACA* LINN. DELILE)

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Received on: 09/09/2014 Revised on: 10/10/2014 Accepted on: 19/10/2014

ABSTRACT

Evaluation of antimicrobial activity of hydro-alcoholic extract of *Ingudi* (*Balanites Aegyptiaca* Linn. Delile.) was done against human pathogens. The antimicrobial activity of newly synthesized compound was first screened by disc diffusion method against Gram positive Enterococcus faecalis, Staphylococcus aureus ATCC 25323 and Gram negative Pseudomonas aeruginosa ATCC 27893, Escherichia coli ATCC 25922, human pathogenic bacteria and fungal strains of candida (C. albicans, C. tropicalis) according to the guidelines of National Committee for Clinical Laboratory Standards (NCCLS, 1997). Fresh grown bacteria were mixed in sterile saline (0.85%) and the turbidity was matched with McFarland No. 2 system to achieve concentration of 107 CFU/ml. Sterile petri plates containing 20 mL of Mueller Hinton agar (MHA, Hi-Media) were used for all bacterial culture. The bacterial innoculum suspension were spread on the surface of agar plates and allowed to solidify for 5 min. Sterile disc (5mm) of Whatman paper no. 1 was then placed on the surface of the media and the test compounds (25µl/ml) was put and allowed to diffuse and plates were incubated for 24 h at 37°C for bacterial cultures and fungal culture were incubated for 72 hr at 25°C. DMSO was used as negative control, Ciprofloxacin (5µg/disc, Hi-Media) was used as positive control for bacteria. The highest antibacterial potential was exhibited by Gram positive bacteria Staphylococcus aureus ATCC 25323 and Gram negative Pseudomonas aeruginosa ATCC 27893 among tested microorganisms.

KEYWORDS: Antimicrobial activity, Plant extract, *Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli, Ingudi*, *Balanites aegyptiaca* Linn. Delile.

INTRODUCTION

Infectious diseases are the world's leading cause of premature deaths, killing almost 50 thousand people every day. Moreover, the increasing emergence of resistant pathogenic strains to the existing drugs and new infectious diseases has necessitated the need for searching novel molecules with better antimicrobial properties than the existing ones[2] (Bhagat et al., 2012). Plant extracts and essential oils have been used as alternatives to antibiotic due to their antimicrobial activities and favorable effect on the animal intestinal system[3] (Al-Kassien, 2009). Spices and herbs and their constituents are generally recognized to be safe, either because of their traditional use without any documented detrimental impact or because of dedicated toxicological studies[4] (Frankic, 2009). Being a rich source of secondary biomolecules which exhibit significant pharmacological effects, spices and herbs appeal to many consumers who question the safety of synthetic food additives[5] (Craig, 1999). Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper cost than modern medicine that's why the fruit of *Ingudi* (*Balanites ageyptiaca* Linn. Delile) was collected from rural area Itawah district of Uttarpradesh state. The present study was

undertaken to evaluate antimicrobial effect of seed of *Ingudi* (*Balanites ageyptiaca* Linn. Delile).

MATERIAL AND METHOD

Source of the Drug

Drug was collected from rural area of district Itawah, U.P and identified by the teacher of Dravya Guna department in faculty of Ayurveda B.H.U. Ingudi bija oil (taila) was prepared in sesamum oil (*tila taila*) in Ayurvedic pharmacy of BHU according to authentic *taila paka vidhi* (Reference No DG/2013-14/351, date 18-11-13)(Form No 429).

Table 1: Properties of seed of *Ingudi* having the *Krimigna* or *Krumigna* (antimicrobial activity)

Name	Formulation Name	Karma	Reference
Ingudi	*Lauhadi rasayana*	*Krimihara*	C.Ci 1-3/15
Ingudi	*Kalayanaka lavana*	*Krimi*	S.Ci.4/32
Ingudi	*Ingudadya taila*	*Kriminasaka*	A.H.Ut 24/16
Ingudi	*Ingudi taila*	*krimi*	A.S.Su 6/105

Table 2: Properties of *Ingudi Taila*

Name	Formulation Name	Karma	Reference
Ingudi	*Ingudi Taila*	*Kustha,*	S.Su.45/118
Ingudi	*Ingudi Taila*	*Vrana ropana*	S.Ci.18/28
Ingudi	*Ingudi Taila*	*Dustavrana*	S.Ci 31/5
Ingudi	*Ingudi Taila*	*K. Karnashula*	S.Ut.21/31
Ingudi	*Ingudi Taila*	*Arsha, Kustha, krimi*	A.S.Su 6/105
Ingudi	*Ingudi Taila*	*Prameha*	A.S.Ci.14/3

Determination of Ethanol -soluble Extractive

5 gm of the air-dried seeds were coarsely powdered (figure1), macerated with 100 ml of ethanol of the specified strength in a closed flask for twenty-four hours shaking frequently during six hours, and allowed to stand for 18 hours. It was filtered rapidly taking precautions against loss of ethanol. 25 ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish (Temp at 105^0 C) and weighed. After that the percentage of ethanol-soluble extract was calculated with reference to the air dried drug. (Figure2)

Collection of test bacteria

Test bacteria were procured from Department of Micro-biology, Institute of Medical Sciences BHU Varanasi, India.

Maintenance and preservation of pure culture

Pure cultures of the bacterial species were maintained on nutrient agar media and were preserved in refrigerator. Sub culturing was done at regular intervals in order to maintain the cultures.

Methodology for antibacterial screening

Fresh grown bacteria were mixed in sterile saline (0.85%) and the turbidity was matched with McFarland No. 2 system to achive concentration of 10^7 CFU/ml. Sterile petri plates containing 20 mL of Mueller Hinton agar (MHA, Hi-Media) were used for all bacterial culture. The bacterial inoculum suspension were spread on the surface of agar plates and allowed to solidify for 5 min. Sterile disc (5mm) of Whatman paper no. 1 was then placed on the surface of the media and the test compounds (25µl/ml) was put and allowed to diffuse and plates were incubated for 24 h at 37°C for bacterial cultures and fungal culture were incubated for 72 hr at 25°C. DMSO was used as negative control, Ciprofloxacin (5µg/disc, Hi-Media) was used as positive control for bacteria. Zone of inhibition was measured in millimeters after 24 h. All test were performed in triplicate.

Table 1: Antimicrobial activity of plant extract

Microorganism	Zone of inhibition (in mm) Extract concentration (mg/ml)		Standard drugs
	100	200	
E. coli ATCC 25922	-	-	20 (Ciprofloxacin)
Enterococcus faecalis	-	-	22 (Ciprofloxacin)
P. aeruginosa ATCC 27893	9.60 ± 0.47	11.34 ± 0.47	20 (Ciprofloxacin)
S. aureus ATCC 25323	10.50 ± 1.63	12.43 ± 0.84	22 (Norfloxacin)
Candida albicans	-	-	18 (Amphotericin B)
Candida tropicalis	-	-	18 (Amphotericin B)

Determination of minimum inhibitory concentration (MIC)

According to National Committee for Clinical Laboratory Standards (NCCLS, 2000), MIC was defined as the minimum/lowest concentration that will inhibit the visible growth of microorganism (measured by turbidity on liquid medium) which was calculated by serial double dilution method on micro titer plate. Serial dilution (100, 50 mg/ml) were prepared on titer plate from stock solution with equal volume of nutrient broth. Specifically, standardized inoculums (1-2 x10^7CFU/ml) were added in each well. DMSO was used as negative control and all the plates were incubated and at the end of incubation period, MIC was determined. All observations were done in triplicate[6].

Table 2: Determination of MIC (mg/ml)

S.No	Bacterial Strains	MIC
1	*P. aeruginosa* ATCC 27893	25
2	*S. aureus* ATCC 25323	12.5

RESULTS AND DISCUSSION

Study on the growth inhibition of pathogenic bacteria is necessary to find out extract concentration to inhibit the bacterial growth. Antimicrobial activity finding revealed significant role of hydro alcoholic seed extract of *Ingudi* (*Balanites ageyptiaca* Linn. Delile) against human pathogens. In the present investigation antimicrobial result showed that extract significantly inhibits the growth of Gram positive bacteria *Staphylococcus aureus* ATCC 25323 and Gram negative *Pseudomonas aeruginosa* ATCC 27893. The results are presented in table 1 and 2. The results of this study also supported the point of view that different pathogenic bacterial species exhibited different sensitivities towards medicinal plants. Scientists from divergent fields are investigating plants a new with an eye to their antimicrobial usefulness. Laboratories of world have found literally thousands of phytochemical which have inhibitory effect on bacteria *in vitro*. It would be advantageous to standardize methods of extraction and *in-vitro* testing so that search could be more systematic and interpretation of results would be facilitated. This study confirms that seed extract have *in vitro* antibacterial activity. This obviously justifies the use of above seed extract of this plant in traditional medicine and further studies are needed to isolate and characterize antibacterial moieties in these for practical disease control *in vivo*.

CONCLUSION

Antimicrobial activity finding revealed significant role of hydroalcoholic seed extract of *Ingudi* against human pathogens. Antimicrobial result showed that extract significantly inhibits the growth of Gram-positive bacteria *Staphylococcus aureus* ATCC 25323 and Gram negative *Pseudomonas aeruginosa* ATCC 27893. This study supports that crude extracts of *Ingudi* oil may be used for treatment of infected wounds.

ACKNOWLEDGMENT

Authors are thankful to the Prof. G. Nath, Mr. Mayank Gangwar, Department of Micro-biology, Institute of Medical Sciences BHU Varanasi, India. providing laboratory facilities.

REFERENCES

1. Gangwar M, Kumar D, Tilak R, Singh TD, Singh SK, Goel RK, Nath G. Qualitative phytochemical characterization and antibacterial evaluation of glandular hairs of *Mallotus philippinensis* fruit extract. *Journal of Pharmacy Research* 2011; 4: 4214-4216.
2. Bhagat J, Kaur A, Sharma M, Saxena AK, Chadha BS. Molecular and functional characterization of endophytic fungi from traditional medicinal plants. World J Microbiol Biotechnol. 2012; 28: 963–71.
3. Al-Kassien, G.A.M. (2009). Influence of two plants extracts derived from thyme and cinnamon of broiler performance. Pakistan Vet. J., 29 (4), 169-173.
4. Frankič, T., Voljč, M., Salobir, J., Rezar, V. (2009). Use of herbs and spices and their extracts in animal nutrition. Acta agriculturae Slovenica, 94/2, 95-102.
5. Craig, J.W. (1999). Health promoting properties of common herbs. American Journal of Clinical Nutrition, 70 (3), 491S-499S.
6. Gautam MK, Gangwar M, Nath G, Rao CV, Goel RK (2012). In-vitro antibacterial activity on Human pathogens and total phenolic, flavonoid contents of Murraya paniculata (L.) leaves extract. Asian Pacific Journal of Tropical Biomedicine 2012; 2(3): S1660-S1663. http://dx.doi.org/10.1016/S2221-1691(12)60472-9.

Cite this article as:
Kumar Sanjeev, Singh Deepika, Abhishek Kumar, Dwivedi Kamal Nayan. Evaluation of Antimicrobial Activity of Seed Extract from Plant Ingudi. Int. J. Ayur. Pharma Research. 2014;2(6):1-5.
Source of support: Nil, Conflict of interest: None Declared

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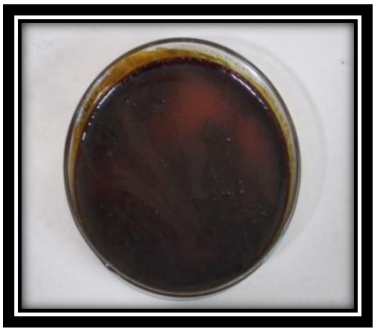
PHOTOGRAPHS

***Yavakuta*-Drug**

Ingudi Taila



Hydro-alcoholic Extract