WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 5, 2166-2172. Research Article ISSN 2277– 7105

SCREENING OF ANTIMICROBIAL POTENTIAL OF OLEANOLIC ACID ISOLATED FROM *VISCUM ARTICULATUM* BURM.

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Article Received on
07 March 2015,

Revised on 30 March 2015,
Accepted on 22 April 2015

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ABSTRACT

Pentacyclic triterpenoids show promising antibacterial activities. In its free acid form, oleanolic acid (OA) also constitutes an imperative part of cuticular barrier for insect and microbial herbivory in many plants. Its diverse pharmacological properties and low toxicity together with potential as a molecular scaffold for new drug discovery generate remarkable interest in pharmaceutical researchers. With regard to structure-activity relationship of triterpene acids and derivatives, it is suggested that both hydroxy and carboxy groups present in the triterpenes are important for their antibacterial activity against different pathogens. In present investigation isolated oleanolic acid from *Viscum articulatum* Burm. f. (Viscaceae) which is a parasitic plant and investigated for its antimicrobial activity against selected microbes.

KEYWORDS: Oleanolic acid, antimicrobial activity, MIC, MBC etc.

1. INTRODUCTION

Plant secondary metabolism covers all physiological and biochemical facets of ‘‘secondary products’’ including functional and evolutionary aspects. Oleanolic acid (3β-hydroxy-olea-12-en-28-oic acid) is pentacyclic triterpenoid that exists widely in plants in the form of free acid and/or aglycones of triterpenoid saponins.[1] In its free acid form, oleanolic acid (OA) also constitutes an imperative part of cuticular barrier for insect and microbial herbivory in many plants.[2] In present investigation, three mistletoe species were screened for their cuticular OA content. The term ‘mistletoe’ is used to refer to a large number of hemiparasite (partial parasitic) flowering plants from the families Viscaceae and Loranthaceae. These hemiparasitic plants are considered as invasive pests for forest trees since they take up water, nutrients and solutes from the host plants and thus drastically reduce their growth.[3] Most of the mistletoes, have wide distributions, are locally abundant and significantly alter the environment.[4] The members of family Viscaceae are reported to contain higher amount of OA in cuticular wax.[5] The cuticle layer of Viscaceae members is unique and comparatively thick (hence known as cuticular epithelium) than other mistletoes.[6] However, synthetic and semi-synthetic medicine available for use with reported adverse reaction as studies reviles a significant increase in the incidence of bacterial resistance to sam. Therefore, there is a need to isolate antimicrobial phytoconstituent from very own mother earth. Plant materials remain an important recourse to combat serious diseases in the world. According to WHO (1993), 80% of the world's population is dependent on the traditional medicine and a major part of the traditional therapies involves the use of plant extracts or their active constituents. These plant secondary metabolites are remarkable mile stone in search of potent natural moiety.

Oleanolic acid is a substance of current biochemical, physiological and therapeutic interest due to its diverse pharmacological properties, low toxicity and ability to interact with large number of biological targets.[7,8,9] OA and its semi-synthetic derivatives have been widely reported for several biological activities.[7,8] In present study commonly found Asian mistletoes have been explored for the content of OA and its antimicrobial activity.

2. MATERIALS AND METHODS

2.1. MATERIALS

The plant material of *Viscum articulatum* Burm. f. (Viscaceae) parasitic on *Cordia macleodii* (Grift) Hook and Thoms., Boraginaceae was collected from tropical dry deciduous forests of Satpuda hills, India (20'38' and 22'3' N Latitude and 72'11' E Longitude) in November, 2012. The plant specimens (Voucher no. LOT 5 and SJM 11231) were authenticated by Dr. S. C. Majumdar of Botanical Survey of India, Pune. The extraction and isolation of oleanolic acid has been done as per procedure

A simple and convenient approach for isolation of oleanolic acid from the squamate mistletoe species Patil S D1. *, Agrwal S A1, Biyani K. 2. *International journal of universal pharmacy and bio sciences 3(2): march-april 2014, 53-62.*

Bacterial strains and growth conditions

Bacillus subtilis (ATCC 6633), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8739), *Salmonella typhi* (ATCC 23654) and *Candida albicans* (ATCC 2091) were used for the investigation of antimicrobial activity. All the strains were procured from National Collection of Industrial Micro-organisms (NCIM), National Chemical Laboratory, Pune, India. Bacterial strains were maintained on Nutrient Agar slants and fungal strain was maintained on Sabouraud Dextrose Agar slants (Hi Media Laboratories Pvt. Ltd.) at 4º.

Controls and standards

Amoxicillin, tetracycline, ciprofloxacin and streptomycin were used as standards for antibacterial activity while tioconazole was used as standard for antifungal activity for positive control. Antimicrobial standards were procured from Abbot Healthcare Pvt. Ltd., Mumbai.

Antimicrobial activity test

Antimicrobial activity was done by agar well diffusion method. The stock solutions of each extracts were prepared in dimethylsulfoxide (DMSO). Concentrations of each extracts were taken as 100, 200, 300 and 400 mg/ml. The inoculums were prepared in 0.9% saline solution and were adjusted to yield approximately 10^8 CFU/ml using 0.5 McFarland's standard. 100 μl of inoculums was spread on respective agar plates. A sterile cork borer of diameter 6 mm was used to make wells on agar plates. 100 μl of each extract of different concentrations were poured into respective wells. Agar well diffusion assay was performed in triplicate and the antimicrobial activity was expressed as the mean of inhibition diameters (mm) ± standard deviation produced by the extracts. Standard antibiotics were taken as positive control. Negative control agar plates contain agar, inoculums and solvent used for dilutions.[10.11]

Determination of minimum inhibitory, minimum bactericidal concentrations and minimum fungicidal concentrations

The minimum inhibitory concentration (MIC) values for the micro-organisms were determined as sensitivity to the extracts in tube dilution assay. Two fold serial dilutions of each extracts having concentrations ranging from 100-0.78 mg/ml were prepared. The cultures were diluted in nutrient broth at a density adjusted using McFarland's standard and 0.5 ml of bacterial suspension was added to 4.5 ml of susceptibility test broth containing diluted extract solution. Positive controls were made of broth and suspension only. The negative control tubes were containing broth, inoculums and solvents used to dilute the extracts. Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) was determined by subculturing the test dilutions on to a fresh agar medium and incubating further for 24-48 h. The highest dilution that yielded no bacterial growth on agar medium was taken as MBC where as the highest dilution that yielded no fungal growth on agar medium was taken as MFC.[12]

STATISTICAL ANALYSIS

All values are expressed as mean ± standard deviation. Numerical data were analyzed using one way ANNOVA using GraphPad Prism® 6.04 for Windows (Graphpad Software Inc., San Diego, California, USA). $P< 0.05$ was considered to indicate statistically significant differences.

RESULTS AND DISCUSSION

The antimicrobial activity of isolated oleanolic acid against *B. subtilis, S. auerus, E. coli, S. typhi* and *C. albicans* was investigated by agar well diffusion method. The results showed that 10 µg/ml and 20 µg/ml of drug could inhibit and showed zone of inhibition against above all microorganisms. Antimicrobial activity Table 1, "Fig. 1". Antimicrobial activity was also assessed for the antimicrobial standards ("Fig. 2"and Table 3). Range of MIC and MBC of extracts recorded were 8.25-25 µg/ml and 11.5 - 39 mg/ml respectively. Both concentratiobs indicating significant antimicrobial potential of isolated oleanolic acid. Bactericidal and fungicidal effect of each dose was recorded against *B. subtilis, S. aureus, E. coli, S.typhi* and *C. albicans respectively*. The MIC and MBC value against each microorganism were summarized in Table 2. The antimicrobial activity of isolated oleanolic acid is not so strong as compared with antimicrobial drugs that are clinically used, although isolated oleanolic acid showed fairly high activity. Nonetheless, it seems that compound is not so toxic. In fact, oleanolic acid has been successfully isolation of pharmacologically versatile molecules which can be commercially and medicinally important, might be used for the treatment of infections.

Table 1. Antimicrobial activity oleanolic acid by agar well diffusion method

ISOLATED OLEANOLIC ACID	Concentration (µg/ml)	Zone of inhibition (mm)				
		a	b	c	d	e
	10	9.67±1.21	8.1±1.17	13.0±2.19	8.00±0.89	9.67±0.82
	20	10.0±0.89	10.3±1.21	10.2±0.75	9.33±1.21	9.0±0.89

a- *Bacillus subtilis* (ATCC 6633), **b-** *Staphylococcus aureus* (ATCC 6538), **c-** *Escherichia coli* (ATCC 8739), **d-** *Salmonella typhi* (ATCC 23654) and **e-** *Candida albicans* (ATCC 2091).

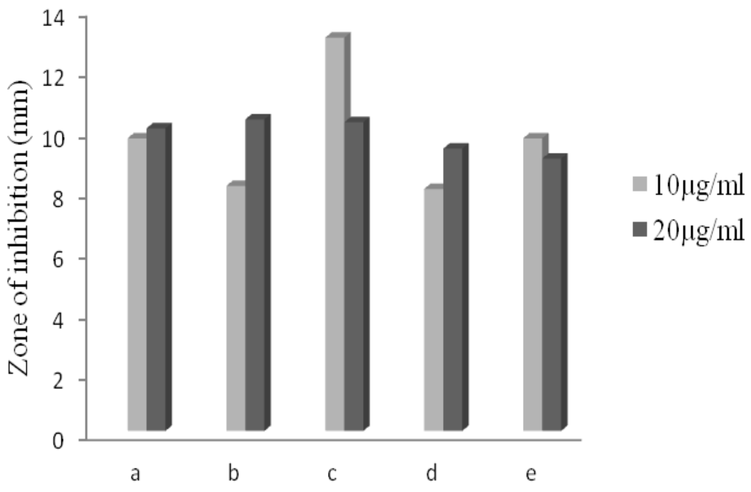

Fig. 1: Antimicrobial activity of isolated olenoilc acid

Fig. 1 antimicrobial activity of ethanol isolated oleanolic acid by agar well diffusion method Results were expressed in mean ± standard deviation of three replicates.

a is *B. subtilis*. b is *S. aureus*. c is *E. coli*. d is *S. typhi*. e is *C. albicans*.

Concentrations of each extract were taken as 10, 20 µg/ml

Table 2. Antimicrobial susceptibility of plants extracts

Organisms		Antimicrobial susceptibility (µg/ml)
B. subtilis	MIC	25.0
	MBC	39.0
S. aureus	MIC	25.0
	MBC	34.0
E. coli	MIC	12.5
	MBC	25.0
S. typhi	MIC	8.25
	MBC	11.5
C. albicans	MIC	12.5
	MFC	22.5

Table 3. Antimicrobial activity of antimicrobial standards.

Standards	Concentration µg/ml	Zone of inhibition (mm)				
		a	B	c	D	E
Amoxicillin	50	18.6±1.6	12.6±1.5	13±1.0	10.1±0.2	NA
Tetracycline	10	15.3±1.1	24.3±2.4	21.8±1.7	22.3±1.1	NA
Streptomycin	10	19.5±3.9	16.3±1.8	15.1±0.2	13.8±1.2	NA
Ciprofloxacin	10	28±4.5	29.6±2.4	29.5±3.4	30±4.5	NA
Tioconazole	10	NA	NA	NA	NA	28.1±3.5

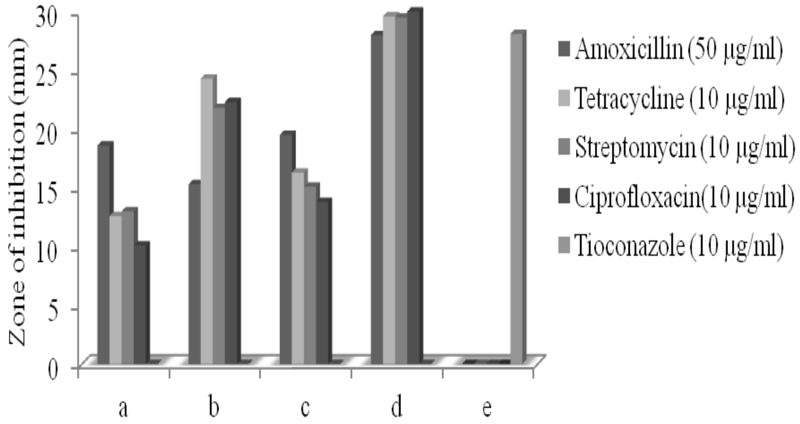

Fig. 2: Antimicrobial activity of standards

CONCLUSION: This study also showed isolated oleanolic acid could be potential sources of new antimicrobial agents. This study provides scientific insight to further determine the antimicrobial principles and investigate other different pharmacological activity of oleanolic acid. On the basis of the present finding, isolated oleanolic acids possess the capabilities of being a good candidate in the search for a natural antimicrobial agent for different bacterial and fungal infections.

ACKNOWLEDGEMENT: I would like to acknowledge Mr Dipesh Patil for constant support.

ACKNOWLEDGEMENT

The authors are thankful to Mrs. Dipesh K. Patil and Ms. Swati R. Dhande, Navi Mumbai, India for providing an encouragement throughout research work.

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