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Invitro Antibacterial Activity of *Oldenlandia umbellata* an Indian medicinal Plant

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Abstract:

To find out the whether this medicinal plant has produce any antibacterial activity against any of common pathogenic organisms isolated from respiratory tract infections the whole plants (except leaves) of crude methanolic extract of *Oldenlandia umbellata* were tested against the pathogenic organisms isolated from the respiratory tract infections.*Oldenlandia umbellata* posses antibacterial activity against both gram positive and gram negative bacteria. It was found that the methanolic extracts of roots and aerial portion (except leaves) of *Oldenlandia umbellata* possessed high degree of antibacterial activity. However the leaves of this plant do not posses such activity.

It has been reported that the plant *Oldenlandia umbellata* contains about seven Anthraquinones, of which 1-2-dihydroxy anthraquinone known as Alizarin is the most predominant. Fractionation of the methanolic extract of *Oldenlandia umbellata* revealed five different fractions presence of of Alizarin was observed in all the five fractions by both qualitative and quantitative estimations. This compound was found to be the active principle and was separated from the plant *oldenlandia umbellata* by chromatographic procedures and identified as Alizarin. The plant derived Alizarin (OU-1) and the synthetic Alizarin (SA) exhibited similar antibacterial activity.

Keywords: Antibacterial activity, Alizarin, *Oldenlandia umbellata*, *Hedyotis umbellata* & chay root.

Introduction:

Plants are used as medicines since time immemorial. India has rich heritage of using medicinal plants in traditional medicines such as Ayurveda, Siddha, Unani besides folklore practices. The earliest mention of the medicinal uses of plants found in the Rigveda which is one of the oldest repositories of human knowledge[1]. A fairly comprehensive information of the curative properties of some of the herbs has been recorded in "CHARAK SAMHITA" and "SUSHRUTHA SAMHITA"[2]

Oldenlandia umbellata is known as *Hedyotis umbellata*. This genus comprises of herbs and shrubs distributed in the tropical and sub-tropical regions of the world. About seventy species occur in India, some of which are used in medicine. The plant *Hedyotis umbellata* belongs to the family Rubiaceae[3]. The leaves and roots are considered expectorant and used in asthma of bronchitis[4]. The root powder has been subjected to clinical trails and it has been proved to be an efficacious remedy for blood, particularly in the condition of Tuberculosis[5]. Methanol extracts of twenty one plant species belonging to different family have been screened for invitro antibacterial activity against multi-resistant bacterial isolates including gram positive and gram negative strains. *Oldenlandia umbellata* and *Oldenlandia corymbosa* are plants showing a maximum antibacterial activity[6]. Chay root dye was once used with a mordant to impart a red colour to fabrics such as wool & silk[8]. Regarding that the antibacterial activity of *Oldenlandia umbellata* has not been studied, the *invitro* antibacterial activity of *Oldenlandia umbellata* an Indian medicinal plant.

Materials & Methods:

The plant material were collected from chavadipalayam of Erode district (Tamilnadu), India,during the months of October and November. They were selected, identified and supplied by SKM Health and mind welfare charity trust siddha pharmaceuticals, Erode, Tamilnadu, India, one of the pioneers in the manufacture of siddha drugs in India. The voucher specimens are identified and deposited there.

Extract Preparation:

One kg of root material of *Oldenlandia umbellata* was shade dried coarsely powdered and soaked in methanol in aspirator bottles and exhaustively extracted at room temperature for 72 hrs. The solvent

was decanted, filtered and distilled off in Rotovoc apparatus. The methanol extract was completely dried from solvent under reduced pressure using high vaccum conditions. The collected extracts were then taken up for further investigations.

Media Preparation:

3.8 gms of Mueller-hinton agar powder was dissolved in 100 ml distilled water. Autoclaved at 121°c for 15 minutes. About 25 ml molten agar was poured into each petidish (9cm in diameter) and allowed to set.

Bacterial strains used:

Test strains - A freshly isolated strains of e.coli, klebsiella pneumoniae, pseudomonas aeruginosa, proteus sp and coagulase positive staphylococcus aureus from sputum sample. All the bacteria cultures were procured from ACS General hospital, Chennai, India.

Standard strains - *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirablis* ATCC 13315 , *Klebsiella pneumoniae* ATCC 12657.

Preparation of Dilutions of Plant Extracts:

50 mg of the plant extract was taken in a sterile test tube. 5ml of Dimethyl Formamide (DMF) was added to it. 1ml of this solution was added to 9ml of sterile distilled water. The concentration of this working solution was 1000 µgm.

Antibacterial activity:

Screening of the antibacterial activity of the plant extract-

Three Mueller Hinton agar plates were inoculated with fresh subcultures of staphylococcus aureus in Mueller-hinton broth and labeled as A, B and C. similarly, another set of three plates were inoculated with fresh subcultures of E.coli in MHA broth and labeled as D, E and F. To the first set three plates A, B and C. 0.2 ml of working solution of the plant extract was placed in the centre of the plate 'A'. Similarly, 0.1ml and 0.05ml of solution was of the plant extract solution. This gave a contration of 200 µgm, 100 µgm and 50 µgm. Then the plates were incubated at 37°c for 24-48 hrs.after the incubation period, a significant zone of inhibition was seen in plates A &D which received 200 µgm of methanol extract(Table-1). No zone of inhibition was seen in the plant extract showed activity only at a concentration of 200 µgm.

Table 1: Screening for antibacterial activity of *Oldenlandia umbellata*

Organisms	Concentration of Plant extract(OU)		
	200 µgm	100 µgm	50 µgm
Staph.aureus	A	NA	NA
E.coli	A	NA	NA

*Note- A - Activity, NA - No Activity

Invitro Antibacterial activity of Oldenlandia umbellate:

Antibacterial activity was done by cup-plate method[7]. *Invitro* antibacterial activity of clinical isoltes and standard ATCC strains of *E.coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirablis* were tested against the 200 µgm concentration of plant extract of *Oldenlandia umbellata*. 2-3 pure colonies of each of these organisms were inoculated into 1ml of Mueller-Hinton broth and incubated at 37°c for 2-4 hrs.

Table2: Zone of Inhibition

Organisms	Diameter of zone of inhibition (mm)		
	Plant extract of OU (200 µgm)	Standard Antibiotics	
		Ampicillin (A)	Chloramphenicol (C)
ATCC *Staph.aureus*	18	20	22
TEST *Staph.aureus*	20	22	18
ATCC *E.coli*	18	20	15
TEST *E.coli*	21	24	15
ATCC *Proteus sp*	18	26	18
TEST *Proteus sp*	18	26	22
ATCC *Klebsiella sp*	20	18	20
TEST *Klebsiella sp*	20	17	20
ATCC *Pseudomonas sp*	18	20	21
TEST *Pseudomonas sp*	18	0	0

*A-Ampicillin-(10 µgm) C- Chloramphenicol-(30 µgm); OU- Oldenlandia umbellata extract (200 µgm) O- No activity

Mueller-hinton agar plates were swabbed (sterile cotton swabs) with 4 hrs old broth culture of respective organisms. Using the sterile well-cutter, the well (3mm wide) was made into the each petriplate(8). 0.2 ml of the working plant extract solution were added into wells by using sterile micropipette, and simultaneously the standard antibiotics of Ampicillin(10µgm) and chloramphenicol(30 µgm) were placed in the each of the plates. Then the plates were incubated at 37°c for 24-48 hrs. After the incubation period, the diameter of the inhibition zones of each well was measured and the values were noted (Table-2)

Results and Discussion:

It was found that the *Oldenlandia umbellata* plant extract gave a zone of inhibition of around 18-21mm for all the strains. Its activity was almost similar against both the test strains as well as against the standard control strains.It showed activity against test strains of *pseudomonas* which failed to produce any zone of inhibition for Ampicillin(10 µgm) and Chloramphenicol (10 µgm). Crude methanolic extract of the leaves portion of the plant showed activity against *staphylococcus aureus*, *E.coli* at a concentration of 200 µg. when this extract was against standard ATCC strains of *Staphylococcus aureus* ATCC(29213),

E.coli ATCC(25922), *Klebsiella* ATCC(12657), *Proteus* ATCC(13315), and *Pseudomonas* ATCC(27853), and against the same group of organisms isolated from clinical specimens, plant extract almost similar activity against the test strains. So, that the leaves portion of *Oldenlandia umbellata* posses antibacterial activity against both gram positive and gram negative bacteria. The present study has revealed the importance of natural products to control antibiotic resistant bacteria which are being a threat to human health. This scientific study can serve as an important platform for the development of inexpensive, safe and effective medicines.

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