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Antibactirial and Phytochemical Evaluation of *Oldenlandia Biflora* L. and *Pergularia Daemia*

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Abstract

The petroleum ether, ethyl acetate and aqueous extracts of *oldenlandia biflora* and *pergularia daemia* were examined for possible sources of antibacterial activities and phytochemical constituents. Antibacterial activity of the extracts was determined by cup plate method on nutrient agar medium. Two gram- positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*) and four gram – negative bacteria (*Pseudomonas epidermis, Escherichia coli*, *Shigella dysenteriae* and *Salmonella typhi*) are used as test organisms. *Oldenlandia biflora* extracts showed better activity than the *Pergularia daemia* extracts. Both the plant extracts exhibited more activity against *Bacillus subtilis* and *Staphylococcus aureus*. The ethyl acetate extracts of both the plants exhibited more antibacterial activity compared to petroleum ether and aqueous extracts. *Pergularia daemia* dose not exhibited antibacterial activity against the *Shigella dysenteriae*. Both the plants were found to contain alkaloids, tannins and flavonoids.

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Key words:
oldenlandia biflora, pergularia daemia, antibacterial activity, cup plate method.

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Introduction:

Plants are the source of most complex organic molecules. These molecules are showing wide structural diversities and are serving as templates for many semi synthetic and synthetic drug molecules. Plant derived drug molecules are frequently showing their role in treating the disease conditions with minimal side effects comparing to the synthetic molecules. Plants have a limitless ability to synthesize aromatic substances mainly secondary metabolites, of which at least 12,000 have been

isolated, a number estimated to be less than 10% of the total [1]. So, still more drug molecules that are hiding in the plants want to be investigated for isolating the new drugs having less toxic and more therapeutic effects their by decreasing the cost of the drugs and increasing the treatment efficiency.

Many antibacterial agents are available in the market, which are developed by the great scientists. But still the microbes are challenging the scientists by developing the resistance to the presently available drugs. Plants are known to produce a variety of compounds to protect themselves against a variety of their pathogens and therefore considered as potential source for different classes of antimicrobial substances [2]. So, our present study was aimed to identify new anti bacterial agent from two selected medicinal plants.

Oldenlandia biflora is a perennial herb belongs to the family rubiaceae. It mainly grows on seaside rocks up to 5-20cm height. The leaves are waxy, 1- 2.5cm long and 7- 12mm wide. It contains white colored flowers [3]. By our ethno- botanical survey we found that this plant was using as folk medicine in treating dysentery, fever and also gastric ulcers.

Pergularia daemia is a perennial twining herb belongs to the family asclepiadaceae. It was mainly found in tropical and sub- tropical areas secreting milky latex. Leaves are thin, broadly ovate and heart-shaped 2-12 cm long, covered with soft hairs. The literature review shows that it was reported to have anti-fertility [4], hepatoprotective [5], wound healing [6], anti diabetic [7] and anti-inflammatory activity [8].

Materials and methods:

Plant materials:

The leaves of the plants are collected from Kakinada, Andhra Pradesh and authenticated by department of pharmacognosy, Koringa College of pharmacy, korangi. The collected leaves are shade dried and powdered. Then the powder was passed through sieve no. 40 and then stored in an air tight container until the use.

Bacterial cultures:

Gram- positive bacterial cultures like *Bacillus subtilis*, *Staphylococcus aureus* and gram – negative organisms like *Pseudomonas epidermis, Escherichia coli*, *Shigella dysenteriae* and *Salmonella typhi* are used as test organisms. All the test strains were maintained on nutrient agar slopes and were sub cultured once in every two-week.

Extraction of plant materials:

The leaf powders of the two plants were extracted successively in soxhlet apparatus, using petroleum ether and ethyl acetate respectively. The aqueous extract was prepared by maceration method. The extracts were distilled, concentrated and dried. These extracts are stored in desecrator at room temperature until the use. At the time of testing, the extracts were reconstituted to a concentration of 100mg/ml in Dimethyl Sulphoxide (DMSO).

Preliminary phytochemical analysis:

Qualitative phytochemical analysis of the crude powder of the 12 plants collected was determined as follows: Tannins (200 mg plant material in 10 ml distilled water, filtered); a 2 ml filtrate + 2 ml $FeCl_3$, blue-black precipitate indicated the presence of Tannins. Alkaloids (200 mg plant material in 10 ml methanol, filtered); a 2 ml filtrate + 1% HCl + steam, 1 ml filtrate + 6 drops of Mayor's reagents /Wagner's reagent/Dragendroff reagent, creamish precipitate /brownish-red precipitate/orange precipitate indicated the presence of respective alkaloids. Saponins (frothing test: 0.5 ml filtrate + 5 ml distilled water); frothing persistence indicated presence of saponins. Cardiac glycosides (Keller-Kiliani test: 2 ml filtrate + 1 ml glacial acetic acid + $FeCl_3$ + conc. H_2SO_4); green-blue color indicated the presence of cardiac glycosides. Steroids (Liebermann-Burchard reaction: 200 mg

plant material in 10 ml chloroform, filtered); a 2 ml filtrate + 2 ml acetic anhydride + conc. H_2SO_4. blue-green ring indicated the presence of terpenoids. Flavonoids (200 mg plant material in 10 ml ethanol, filtered); a 2 ml filtrate + conc. HCl + magnesium ribbon pink-tomato red color indicated the presence of flavonoids [9].

Antibacterial assay:

Antibacterial activity of the extracts was determined by cup plate method on nutrient agar medium [10]. Cups are made in nutrient agar plate using cork borer (6 mm) and inoculums containing 10^8 CFU/ml of bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. Then 50 µl of each extract was placed in the cups made in inoculated plates. 50 µl of control (DMSO) was also maintained with extracts which served as control. After introducing the sample, standard and control in the cups the plates were kept in refrigerator at 4°C for 2 hrs, for diffusion and then the plates were incubated for 24 h at 37°C. After proper incubation, antibacterial activity was determined by measuring the diameter of the zone of the inhibition around the well and the activity was compared with Ciprofloxacin (10 µg/ml). Three replicates were carried out for each extract against each of the test organism.

Determination of Minimal Inhibitory Concentration (MIC):

MIC was determined by broth dilution method. In this method 0.1ml of standardized suspension of bacteria (10^8 CFU/ml) was added to each tube containing different concentrations of the extracts (prepared by two fold dilution method) and incubated for 24h at 37°C. The lowest concentration of the tube that did not show any visible growth by macroscopic evaluation was considered as the MIC.

Results and Discussion:

The petroleum ether, ethyl acetate and aqueous extracts of *oldenlandia biflora* and *pergularia daemia* were examined for possible sources of antibacterial activities and phytochemical constituents. Both the plants were found to contain alkaloids, tannins and flavonoids (Table: I). In addition to these phytochemicals *Pergularia daemia* also contains cardiac glycosides. Saponins and steroids are found to be absent in both the plant materials.

Both the plants exhibited significant antibacterial activity. All the extracts of *Oldenlandia biflora* show antibacterial activity but petroleum ether extracts of *Pergularia daemia* dose not exhibited any activity. Both the plant extracts exhibited more activity against *Bacillus subtilis* and *Staphylococcus aureus*. The ethyl acetate extracts of both the plants exhibited more antibacterial activity compared to petroleum ether and aqueous extracts. *Pergularia daemia* dose not exhibited antibacterial activity against the *Shigella dysenteriae*.

The MIC of each extract of both the plants was determined by broth dilution method. The ethyl acetate extract of *Oldenlandia biflora* inhibited the growth of the *Bacillus subtilis* at a concentration of 125µg/ml. Both the plant extracts exhibited MIC of 500µg/ml against *Pseudomonas epidermis, Shigella dysenteriae, Salmonella typhi* and *Escherichia coli* and MIC of 250µg/ml against *Bacillus subtilis* and *Staphylococcus aureus*. The petroleum ether extracts of *Pergularia daemia* dose not exhibited any activity.

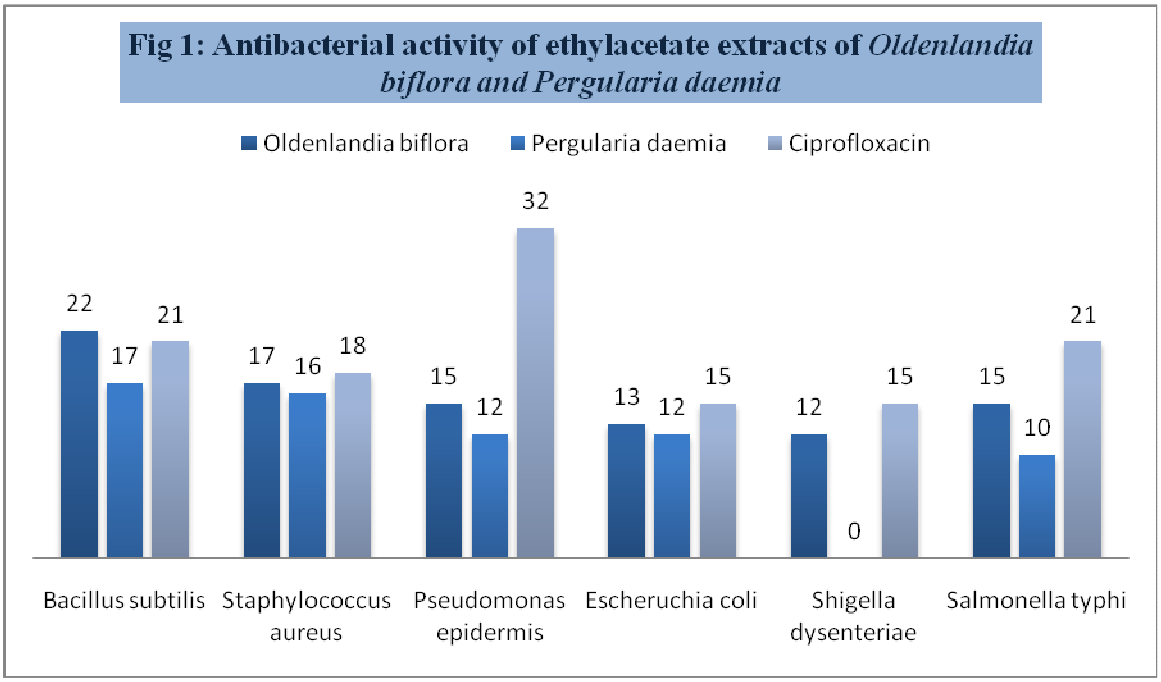
Phytoconstituents	*Oldenlandia biflora*	*Pergularia daemia*
Tannins	+	+
Alkaloids	+	+
Flavonoids	+	+
Saponins	-	-
Steroids	-	-
Cardiac Glycosides	-	+

Table 1: Phytochemical investigation of plant material

Test/Standard	Extract	Diameter of zone of inhibition (mm)					
		B.subtilis	*S. aureus*	*P.epidermis*	*E. coli*	*S.dysenteriae*	*S. typhi*
Oldenlandia biflora	Petroleum ether	18	16	13	9	10	11
	Ethyl acetate	22	17	15	13	12	15
	Aqueous	17	14	12	9	9	10
Pergularia daemia	Petroleum ether	NA	NA	NA	NA	NA	NA
	Ethyl acetate	17	16	12	12	NA	10
	Aqueous	14	11	11	11	NA	9
Standard drug	Ciprofloxacin (10 µg/ml).	21	18	32	15	15	21
Control	DMSO	NA	NA	NA	NA	NA	NA

Values are the mean of three replicates; NA: No activity.

Table 2: Antibacterial activity of *Oldenlandia biflora* and *Pergularia daemia*



Test organism	*Oldenlandia biflora* (µg/ml)			*Pergularia daemia* (µg/ml)		
	Petroleum ether	Ethyl acetate	Aqueous	Petroleum ether	Ethyl acetate	Aqueous
Bacillus subtilis	250	125	250	NA	250	250
Staphylococcus aureus	250	250	500	NA	250	500
Pseudomonas epidermis	500	250	500	NA	500	500
Escherichia coli	500	500	500	NA	500	500
Shigella dysenteriae	500	500	500	NA	500	500
Salmonella typhi	500	500	500	NA	500	500

Table 3: Minimum Inhibitory Concentrations of *Oldenlandia biflora* and *Pergularia daemia*

Conclusion:

From the present experiment it can be concluded that both the plant extracts are exhibiting anti bacterial activity. The plants may be further explored for its phytochemical profile to recognize the active constituent accountable for antibacterial activity.

References:

1) Mallikharjuna, P.B. et al., (2007): Phytochemical studies of *Strychnos potatorum* L.f. A medicinal plant. *E-jour. Chem*., 4(4): 510-518.
2) Micheal J Pelczer, chan ECS, Noel RK 1998, Microbilogy fourth edition Tata McGraw Hill Pg.no:535-537
3) http://flowers2.la.coocan.jp/Rubiaceae/Oldenlandia%20biflora.htm
4) Golam S, Gafur MA, Shah MAB, Khurshid AHM, Biswas MHU, Hassan P, Mannan A, Khan OF and Chowdhury AKA. Antifertility activity of *Pergularia daemia*. *J. Sci.* (2001) 1: 22-24.
5) Sureshkumar SV and Mishra SH. Hepatoprotective activity of extracts from *Pergularia daemia* (Forsk.) against carbon tetrachloride-induced toxicity in rats. *Pharmacog. Mag*. (2007) 3: 187-91.
6) Kumar B, Yadav DK, Govindarajan R and Pushpangadan P. Wound healing activity of *Pergularia daemia* (Forsk.) Chiov. *Pharmacog. Ethnopharmacol.* (2006) 1: 12-4.
7) Wahi AK, Ravi J, Hemalatha S and Singh PN. Antidiabetic activity of *Daemia extensa*. *J. Nat. Remedie* (2002) 2: 80-3.
8) Bhaskar VH and Balakrishnan N. Analgesic, anti-inflammatory and antipyretic activities of *Pergularia daemia* and Carissa carandas. *Daru* (2009) 17: 168-174.
9) Jigna PAREKH, Sumitra V. CHANDA, in vitro Antimicrobial Activity and Phytochemical Analysis of Some Indian Medicinal Plants; Turk J Biol 31 (2007) 53-58.
10) Anon. Pharmacopiea of India (The Indian Pharmacopiea), 3 edition, Govt. of India, New Delhi, Ministry of Health and Family Welfare. 1996.