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Anti-bacterial activity of methanolic extracts of *Coldenia procumbens* from the Pranahita and Shivaram wild life sanctuaries, Mancherial, Telangana

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Abstract
Herbal medication is a part of Indian life. The present study was carried out to know the anti bacterial activity of *Coldenia procumbens* from Pranahita and Shivaram wild life sanctuaries of Mancherial, Telangana. All the bacterial cultures are human pathogenic and were procured from Primer Biotech Research Centre, Hyderabad. The micro organisms selected for the present study are; *E. coli, K. pneumonia, P. aeruginosa* (Gram -ve); *S. aureus, B. subtilis* and *E. faecalis* (Gram +ve). The present study indicated the anti-microbial activity of methanol leaf extracts. The present study validates use of the plant. Further it necessitates the pharmacological evaluation.

Keywords: Antimicrobial, Gram +ve, Gram -ve, ampicillin, *Coldenia procumbens*, *E. coli*, *K. pneumonia*, *P. aeruginosa*, *S. aureus*, *B. subtilis*, and *E. faecalis*

Introduction
Coldenia procumbens (Linn) is prostratic herb with small leaves in alternation, distributed through India ranging from barren lands to paddy fields and it is considered as a weed plant. Nature has been the source a large number curative compounds in addition to the resources.
India is a rich source of potential medicinal plants; especially the documented history demonstrates the rich legacy of indigenous herbs and their use in treating ailments in India. The Holy scripts like the Samaveda and the Atharvaveda (4500-1600 BC) have the mention of utilization of plants as source of drug.
The antibiotic treatment in some cases however resulted in some diseases. Moreover, drug induced mutations resulted in antibiotic resistant strains. In addition to this, the side effects may also afflict damage to many of human organs. Therefore, to overcome this limitation of synthetic drugs, researchers have focused on conventional medicinal plants that are part of the lifestyle of some countries. Plants are sources of numerous compounds with therapeutic value, like anti-diabetic, antioxidant, antibacterial, anti-inflammatory, antipyretic, gastro protective and hepato-protective nature etc. These plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, flavonoids, phenols and quinones. World Health Organization has regarded plants as potential sources of therapeutic compounds. In addition to this, bioactive compounds are also being used as antimicrobial and antioxidant additives in the food industry. Large numbers of pharmaceutical industries are investing a lot to devise cost effective natural drugs from plant extracts. Plant derived metabolites and pesticides are least harmful to the non targeted ecological parameters (Varma and Dubey, 1999; Gottlieb *et al*., 2002) [1-2].
Out of an estimated 17500 plant species in India, about 3000 species are being used and pat of ancient Indian traditional system of medicine. Anthropogenic activities and over exploitation forced the alteration in the very basic composition, vegetation, and some are at the verge of extinction or more vulnerable (medical plants). Plants being part of folk medicine can be screened for the phytochemical analysis and antimicrobial activity to ascertain the activity.
Plants have been explored for drugs for the therapeutic use, additives in food, agrochemicals and industrial chemicals and many others (Charu *et al.,* 2012, Habila *et al.,* 2011) [3, 4]. Ushimaru *et al*., (2007) [5] Chakraborthy, (2008) [6], Sathish, (1999) [7], Asha Devi *et al*., (2009) [8] and many workers studied the anti bacterial activity of plant derived bio active compounds. John De Britto *et al*., (2011) [9], Rajaiah *et al*., (2022) [10] assessed the methanol and fluid concentrates of leaves of many plants.

The present study was planned to know the antimicrobial activity and phytochemical analysis of *Coldenia procumbens a*gainst some bacterial pathogens.

Material and Methods

The selected plant *Coldenia procumbens*, Linn. (Boraginaceae) is popularly known as "Hamsapadu" and is used traditionally for the treatment of piles, fever, wounds, and other ailments by the Kolam and Mannevar tribes in and around the Pranahita and Shivaram wild life sanctuaries, Mancherial, Telangana. The area under study was located between longitudes 18° 52' 0.12" N & Longitude: 79° 45 "57.42"S.

The plant was authenticated by Dr. S. Nageswara Rao, Osmania University. The prepared herbarium specimens were kept in the herbarium of Botany Department, University college of Science, Saifabad, OU, Hyderabad, Telangana, India. All the reagents used were of analytical grade.

Bacterial cultures used

All the bacterial cultures are human pathogenic and were procured from Primer Biotech Research Centre, Hyderabad. The micro organisms selected for the present study are; *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* (Gram negative bacteria); *Proteus vulgaris*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Enterococcus faecalis* (Gram positive bacteria).

The organisms obtained from the laboratory stock were sub cultured into a sterile nutrient broth and incubated at 37 ºC for 18-24 h a day prior to the experiment. The culture growth obtained was used as inoculums for the antibacterial testing.

Phytochemical Analysis

Phytochemical screening was carried out by following standard procedure to assess the qualitative chemical composition of crude extracts and to ascertain major natural chemical groups such as alkaloids, Terpenoids, steroids, flavonoids, tannins, carbohydrates and amino acids. Qualitative phytochemical analyses of the extracts were performed by Adetuyi and Popoola (2001) [11].

Results and Discussion

The preliminary phytochemical analysis of leaf extracts of *Coldenia procumbens* revealed the presence of flavonoids, phenolic compounds, terpenoids, alkaloids, carbohydrates, steroids, glycosides and proteins. The results reveal the medicinal properties of the selected medicinal plants (Table no.1)

Table 1: Phytochemical Analysis of *Coldenia procumbens*

1	Flavonoids	+
2	Alkaloids	+
3	Terpenoids	+
4	Tannins	-
5	Carbohydrates	+
6	Glycosides	+
7	Amino acids and proteins	+
8	Phenols	+
9	Steroids	+

+ indicates Presence Negative – indicates absence

All the bacterial pathogens selected viz, *E. coli* (G ram -ve), *Klebsiella pneumonia* (Gram -ve), *Pseudomonas aeruginosa* (Gram -ve), *Staphylococcus aureus* (Gram +ve), *Bacillus subtilis* (Gram +ve), *Enterococcus faecalis* (Gram +ve) were tested against plant extracts of 25 µg/mL, 50 µg/ml, 75 µg/ml, 100 µg/mL concentrations impregnated on discs. The test compound *i,e* Ampicillin-antibacterial, were tested at 250 µg/mL concentration.

Table 2: Zone of inhibition of antibacterial activity of *Coldenia procumbens* against *selected microorganisms* (Values were with mean ± SE of three separate experiments).

Microorganism	25µg/ml/cm	50µg/ml/cm	75µg/ml/cm	100µg/ml/cm
Escherichia coli	6 ±0.1	7±0.2	9±0.2	12±0.1
Klebsiella pneumonia	2 ±0.3	3 ±0.4	5±0.3	7±0.5
Pseudomonas aerugenosa	1±0.2	2.5±0.5	4±0.5	5±0.2
Staphylococcus aureus	4±0.5	5±0.4	6±0.4	7±0.4
Bacillus subtilis	3±0.4	3.5±0.4	5±0.4	6±0.7
Enterococcus faecalis	2±0.4	4±0.6	5±0.8	7±0.5

The four different concentrations were used for the antimicrobial activity against the selected bacterial organisms. The methanolic *Coldenia procumbens* plant extract of 100µl concentration was more suitable for this investigation. The zone of inhibition in *E. coli* was 6, 7, 9 and 12 mm diameter in methanol (Table 2). The activity against *Klebsiella pneumonia has* given a higher 7 mm zone of inhibition at higher concentration and at a low concentration of 25 µg/ml/cm it was 2 mm. All the bacteria shown higher zone of inhibition at 100 µg/ml/cm concentration and it is 5, 7, 6, and 7 mm for *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *Enterococcus faecalis* respectively. Among all, it was more in case of *E. coli* at 100 µg/ml/cm the value were presented in table 1 and histogram 1. Increase in concentrations enhanced the zone of inhibition.

The present study findings are similar to that of Banso *et al.*, (1999) [12]. The present investigation revealed the anti-microbial nature of methanol leaf extracts of the *Coldenia procumbens*. The present study indicated the possible antimicrobial activity. Further it requires several detailed pharmacological assessment. Leaf extracts were tested against bacterial pathogens like *E. coli* (Gram-ve), *Klebsiella pneumonia* (Gram -ve), *Pseudomonas aeruginosa* (Gram -ve), *Proteus vulgaris* (Gram +ve), *Staphylococcus aureus* (Gram +ve), *Bacillus subtilis* (Gram +ve), *Enterococcus faecalis* (Gram +ve) (Table no2 and figure no1).

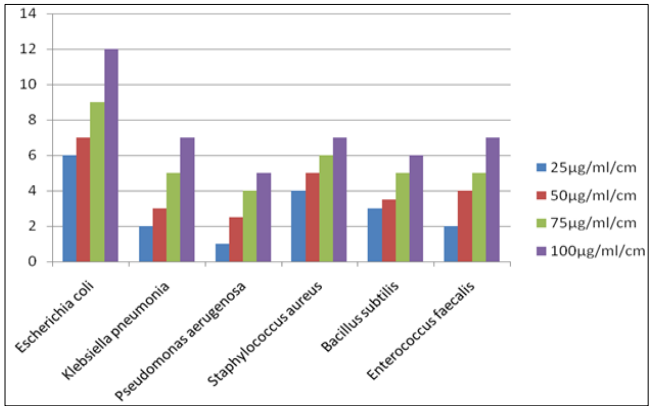

Fig 1: Anti-bacterial activity of Coldenia procumbens against selected bacteria.

The results indicated more inhibitory activity against gram-positive bacteria than gram-negative. The difference could be due to difference in chemical components, differential extraction and quantity of active components present in the extract. The findings are similar to previous studies (Nair *et al*., 2005 and Karou *et al.*, 2005, Rajaiah *et al*., 2022) [13, 14, 15]. This may be due to presence of inefficient peptidoglycan in outer layer of bacteria (Scherrer and Gerhardt, 1971; Kelmanson *et al.*, 2000; Masika *et al*., 2002; Kavitha *et al*., 2008) [16, 17, 18] where as in Gram-negative, the outer phospholipid is impermeable.

Tannins, saponins and alkaloids are antimicrobial in nature and the alcoholic extraction could have produced more number of components that are antibacterial (Akinyemi *et al*., 2005) [19]. The leaf extracts are antimicrobial and can be used in the treatment of infectious diseases, it may serve as a drug candidate for the development of new drugs.

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