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### PRELIMINARY PHYTOCHEMICAL ANALYSIS AND SCREENING OF POTENTIAL ANTIBACTERIAL ACTIVITY OF PLANT EXTRACTS AGAINST PATHOGENIC STRAINS OF *NOCARDIA ASTEROIDS* AND *STREPTOCOCCUS PYOGENES*

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**Abstract:** The study was conducted to determine the phytoconstituents and to determine antibacterial potential including the minimum inhibitory concentration (MIC) of the leaf extracts of *Withania somnifera* and *Eucalyptus melliodora* against pathogenic strains of bacteria viz. *Nocardia asteroides* and *Streptococcus pyogenes*. Aqueous and methanolic plant extracts were screened for their antibacterial activity by *in vitro* agar well diffusion and disc diffusion methods against the microbial strains collected from MTCC, Chandigarh. MIC was determined by dilution series test. The zones of inhibition against *N. asteroides* by methanolic extracts of *E. melliodora* and *W. somnifera* by agar well diffusion were 16 and 20 mm whereas in case of disc diffusion method they were 10 and 15 mm respectively. Zones of inhibition noted against *S. pyogenes* were 22 mm and 23 mm via agar well diffusion method and 11 mm and 13 mm by disc diffusion method with extracts of *E. melliodora* and *W. somnifera* respectively. The aqueous extracts were not showed any reliable results against both the pathogens using disc diffusion method. Minimum Inhibitory Concentration (MIC) obtained were 31 µg/mL and 62 µg/mL against both the pathogens viz. *N. asteroides* and *S. pyogenes* with *E. melliodora* and *W. somnifera* respectively. The phytochemicals viz. alkaloids, flavanoids, saponins, tannins, carbohydrates and proteins were found to be present in extracts of both the plants.

**Keywords:** Antimicrobial activity, Methanolic extracts, *Nocardia asteroides*, *Withania somnifera*, Minimum Inhibitory Concentration (MIC).



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## INTRODUCTION

People in any region of this world suffer from variety of skin diseases among which some are superficial infections whereas some of them proved to be life threatening. India is a developing nation and presents demographic features quite similar to other developing countries. Emerging occupational and epidemiological health problems are major priorities that need to be tackled along with existing traditional public health problems like communicable diseases, malnutrition, poor environmental sanitation, and inadequate medical care. (Swaran J.S. Flora, 2008).

Plants have been used for the treatment of diseases all over the world before the advent of modern clinical drugs and are known to contain substances that can be used for therapeutic purposes or as precursors for the synthesis of useful drugs. (Sofowora, A.1982). Phytomedicines derived from plants have shown great promise in the treatment of infectious diseases including viral infections (Cowan, 1999). Considering plants as a source of potential antimicrobial drugs, a systematic investigation was carried out to screen antibacterial activity of *Eucalyptus melliodora* and *Withania somnifera* leaf extracts against *Nocardia asteroides* and *Streptococcus pyogenes*.

*Eucalyptus melliodora*: It has been used traditionally to treat diabetes (Abdullah D *et al*, 1996), in remedies to treat coughs and the common cold. It can be found in many

lozenges, cough syrups, rubs, and vapor baths. Herbalists recommend the use of fresh leaves in teas and gargles to soothe sore throats and treat bronchitis and sinusitis. On the skin, eucalyptus oil has been used to treat arthritis, boils, sores and wounds. The oil can also be rubbed on the skin as an insect repellent. Eucalyptus oil is also rich in cineole (a potent antiseptic that kills bacteria responsible for bad breath), so some professional herbalists may also recommend diluted eucalyptus tinctures to treat bad breath (Blumenthal M *et al*, 2000, Chao S C *et al*, 1998, Cermelli C *et al*, 2008).

*Withania somnifera*: It is used for tumors, inflammation (including arthritis), and a wide range of infectious diseases. Generally, ashwagandha stimulates the immune system. It has also been shown to inhibit inflammation and improve memory. Taken together, these actions support the traditional reputation of ashwagandha as a tonic or adaptogen. It counteracts the effects of stress and generally promotes wellness. The fruit is rich in saponins and can be used as a soap substitute (Emboden W, 1979). The leaves are an insect repellent (Buchanan R, 1987).

## Materials and Methods

### Plant extract preparation

The plant materials were collected from the local region of Ghaziabad, Uttar Pradesh. Fresh leaves were shade dried for 10 days.

The dried leaves were ground to coarse powder and sieved. 10 gm of each powder were suspended in 100 ml of methanol and water separately and kept overnight on a rotary shaker at 180 rpm. The extracts were filtered through four layered muslin cloth and then centrifuged at 10,000 rpm for 10 minutes. The supernatants obtained were filtered through Whatman's no. 1 filter paper. The filtrates were concentrated to one-fifth of the original volume by keeping them in water bath at the boiling point of solvent and stored in sterile glass bottles at 4°C for further use (P. Goyal *et al*, 2008, Ogunyemi A O *et al*, 1979).

#### Inoculum preparation

Loops full from the lyophilized cultures obtained from MTCC, Chandigarh were suspended in 50 ml of Nutrient Broth. *N. asteroides* (MTCC 927) culture was incubated at 25°C for 78 hrs and *S. pyogenes* (MTCC 1927) at 37°C for 48 hrs. The inoculums prior to use were maintained according to the McFarland standard (Dilnawaz Shaikh *et al*, 2005).

#### Antibacterial Assay

Agar Well Diffusion Method: Nutrient agar plates were inoculated with 100 µL of inoculum. Wells were created using cork borer with 6 mm diameter and filled with 100 µL of plant extracts. Plates seeded with *N. asteroides* allowed to incubate at 25°C and those with *S. pyogenes* at 48°C. Zones of inhibition were measured after 24 hrs of

incubation (J. Parekh *et al*, 2006, Dhia Hassawi *et al*, 2006).

Disc Diffusion Method: Discs were prepared by soaking them with 100 µL of plant extracts. Nutrient agar plates were inoculated with 100 µL of inoculum and discs placed over the surface and incubated at optimum temperatures. Zones of inhibition were measured after 24 hrs of incubation (R. Nair *et al*, 2004).

#### Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was determined using the tube dilution techniques. Varying amounts of the extract in the following concentration 5% to 100% were prepared using single dilution method. An 8 ml of the nutrient broth was pipetted into the various test tubes and sterilized at 121°C for 15 minutes, they were allowed to set. Later each tube was inoculated with an overnight standard inoculum size is of  $1-2 \times 10^8$  CFU/ml of *Nocardia asteroides* and *Streptococcus pyogenes*. This is equal to McFarland 0.5 turbidity standard and then transferred into the test tube containing the extract. The test tubes were incubated at 37°C for 24 hours. The least concentration of the plant extract that does not permit any visible growth or turbidity of the inoculated test organisms in broth culture were taken as the minimum inhibitory concentration in each case. Control experiment with plant extract and another tube with no plant extract were also performed (Olaleye, *et al*, 2007).

### Phytochemical Analysis

Phytochemical analysis to screen the plants for the presence of alkaloids, saponins, flavonoids and carbohydrates was performed according to the method described by Sofowora (1993) and Evans (1998).

#### Test for Alkanoids:

Few drops of the manager's reagent, Drangendorff's reagent, Wanger's reagent or Hanger's reagent and 10% tannic acid solution were added. The presence of precipitate in at least 3 or all of the above reagents was indicated the presence of alkaloids Sofowora (1993).

#### Test for Carbohydrates:

A few drops of Molisch's reagent were added to 2ml of each of the water extract in two tubes. A small quantity of concentrated sulphuric acid was then added and allowed to form a lower layer. A purple ring at the interface of the liquids indicates the presence of carbohydrates. Each mixture was then shaken and allowed to stand for 2 minutes and diluted with 5ml of water. A purple precipitate also showed the presence of carbohydrates Sofowora (1993).

#### Test for Tannins (Ferric chloride test):

A portion of the water extract was diluted with distilled water in a ratio of 1:4 and few drops of 10% ferric chloride solution was added. A blue or green colour was indicated the presence of tannins Sofowora (1993).

#### Test for Saponins:

A small quantity of the ethanolic extract was boiled. The mixture was filtered and 2.5ml of the filtrate was added to 10ml of the distilled water in a test tube. The test tube was corked and shaken vigorously for about 30 seconds then it was allowed to stand for half an hour. A honeycomb forth was an indicator of the presence of saponins Sofowora (1993).

#### Test for Flavonoids (Shinoda test):

Four pieces of magnesium fillings was added in ethanolic extract followed by few drops of concentrated hydrochloric acid. A pink or red colour was indicated the presence of flavonoid Evans (1998).

#### Test for Proteins

One mL conc.  $\text{HNO}_3$  was added in 2 mL aqueous extract and then heated. Yellow precipitate wad determined the presence of proteins.

### Result and Discussion

The zones of inhibition with the methanolic extract of *E. melliodora* were 16 mm and 22 mm using agar well diffusion method, 10 mm and 11 mm using disc diffusion method against *N. asteroides* and *S. pyogenes* respectively. The zones of inhibition with methanolic extracts of *W. somnifera* were 20 mm and 23 mm using agar well diffusion method, 15 mm and 13 mm using disc diffusion method against *N. asteroides* and *S. pyogenes* respectively (Table 1). MIC obtained were 31 $\mu\text{g}/\text{mL}$  and 62 $\mu\text{g}/\text{mL}$

against both the pathogens viz. *N. asteroides* and *S. pyogenes* with *E. melliodora* and *W. somnifera* respectively (Table 2). The commercially important phytochemicals viz. alkaloids, flavanoids, saponins, tannins, carbohydrates and proteins were found to be present in the extracts of both the plants

as positive results for the tests conducted were obtained (Table 3). Non reliable results were produced with the test conducted with the aqueous extracts of both the plants using both the diffusion methods.

**Table 1: Zone of Inhibition (in mm) with organic extracts**

Microorganism	<i>E. melliodora</i>		<i>W. somnifera</i>	
	Agar Well Diffusion	Disc Diffusion	Agar Well Diffusion	Disc Diffusion
<i>N. asteroides</i>	16	10	20	15
<i>S. pyogenes</i>	22	11	23	13

**Table 2: MIC of Plant Extracts (µg/mL)**

Microorganisms	<i>E. melliodora</i>	<i>W. somnifera</i>
<i>N. asteroides</i>	31	31
<i>S. pyogenes</i>	62	62



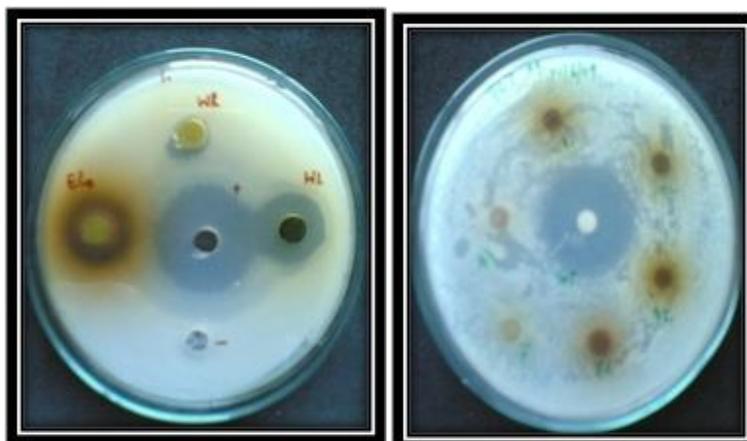


Figure1: Antibacterial activity of plant extracts of leaf and root extracts of *W. somnifera*, *E. melliodora* and positive control against *N. asteroides*

Table 3: Phytochemical Analysis of Plant extracts.

Test ↓	<i>E. melliodora</i>	<i>W. somnifera</i>
<b>Alkaloids</b>		
Mayer's test	+	-
Hager,s test	+	+
Wagner,s test	+	+
<b>Carbohydrates</b>		
Molisch's test	+	-
Barfoed's test	+	+
Biuret's test	+	+
<b>Proteins</b>		
Millon's test	-	+
Xanthoprotic	-	-
Flavanoids	-	+
Saponins	+	+
Tannins	+	+

## CONCLUSIONS

It can be concluded that the methanolic leaf extracts of *W. somnifera* and *E. melliodora* showed an excellent antimicrobial activity against *N. asteroides* and *S. pyogenes*. These can be explored further for their useful metabolites and can be utilized as a constituent of commercial chemotherapeutic preparations of natural origin with no adverse side effects and

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