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ANTIMICROBIAL ACTIVITY *OF WITHANIA SOMNIFERA, GYMNEMA SYLVESTRE* AND *CANNABIS SATIVA* AGAINST PATHOGENIC BACTERIA

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ABSTRACT

The organic extracts of *Withania somnifera*, *Gymnema sylvestre* and *Cannabis sativa* have been evaluated of antimicrobial activity against *P. aeruginosa*, *E. coli* and *C. albicans*. The antimicrobial activity of ethanolic extracts (20 mg/mL) of *W. Somnifera* was (44, 45, 42 mm) against *P. aerouginos, E. coli* and *C. albicans*, respectively. The antimicrobial activity of *G. sylvestre* petroleum ether extract was 20 mm against *P. aeruginosa.* The antimicrobial activity of *Gymnema sylvestre* water: ethanol extract was 18 mm against *E. Coli.* The antimicrobial activity of *G. sylvestre* chloroform extract was 20 mm against *C. albicans.* The antimicrobial activity of *G. sylvestre* chlorofomic extract (10 mg/mL) was 21, 27 and 19 mm against *P. aerouginosa, E. coli* and *C. albicans* respectively. The *Cannabis sativa* antimicrobial activity of extracts (hexane and propanol) was 20 and 23 mm against *P. aerugenosa* and *C. albicans*, respectively. The antimicrobial activity of propanolic extracts (10 mg/mL) of *C. sativa* was 19, 17 and 21 mm against *P. aerugenosa, E. coli* and *C. albicans*, respectively.

Key words: Antimicrobial activity, *W. somnifera, G. sylvestre*, *C. sativa*.

INTRODUCTION

Among the various medicinal plants *Withania somnifera* Dunal (Wintercherry, Ashwagandha or Asgandh) is an important medicinal plant and its use in ayurvedic and unani extends back over 3000 to 4000 years. *Withania somnifera* Dunal belongs to the family *solanaceae.* It is a xerophytic plant, found in the drier parts of India, Sri Lanka, Afghanistan, Baluchistan and Sind and is distributed in the Mediterranean regions, the Canaries and Cape of Good Hope. The Gymnema sylvestre (Gurmar) is an herb native to the tropical forests of southern and central India. Chewing the leaves suppresses the sensation of sweet. This effect is attributed to the presence of the eponymously named gymnemic acids. *G. sylvestre* has been used in traditional medicine as a treatment for diabetes for

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nearly two millennia, but there is insufficient scientific evidence to draw definitive conclusions about its efficacy. The *Cannabis* (bhang) is a genus of flowering plants that includes three putative varieties, *Cannabis sativa*, *Cannabis indica*, and *Cannabis ruderalis*. These three taxa are indigenous to Central Asia, and South Asia. The *Cannabis* has long been used for fibre (hemp), for seed and seed oils, for medicinal purposes, and as a recreational drug. Industrial hemp products are made from *Cannabis* plants selected to produce an abundance of fiber. To satisfy the UN Narcotics Convention, some hemp varieties have been developed, which contain minimal levels of THC (Δ^9 tetrahydrocannabinol), one of the psychoactive molecules that produces the "high" associated with marijuana. The psychoactive product consists of dried flowers of plants selectively bred to produce high levels of THC and other psychoactive chemicals. Our aim was to evaluate the antimicrobial activity of *somnifera, Cannabis sativa and Gymnema sylvestre* against the pathogenic bactreia.

EXPERIMENTAL

Material and method

Plant and culture collection

The plants were collected (*Withania somnifera* from Madhya Pradesh, *Gymnema sylvestre* from Herbal Park, Yamuna Nagar, Haryana and *Cannabis sativa* from the roadside of NH1 Highway near Kundli, New Delhi). The various human pathogenic microorganisms were procured from Microbial Type Culture Collection (MTCC): Institute of Microbial Technology (IMTECH), Chandigarh; which included Gram-negative bacteria: *Escherichia coli* (MTCC 5704) *Pseudomonas aeruginosa* (MTCC 2295*);* and Yeast: *Candida albicans* (MTCC 3017.

Preparation of *Withania somnifera* root extract

The roots of *Withania somnifera* were thoroughly washed with clean water and allowed for sun drying for seven days and grounded into fine powder. The 5 g of *Withania somnifera* powder was soaked in 50 mL of ethanol (96%) and incubated for 72 hr at room temperature. The extract was filtered with whatman filter paper. The extra solvent from the filtrate was evaporated using water bath at 58^{o}C. The extracts were stored at 4^{o}C for further use.

Preparation of *Gymnema sylvestre* leaves extracts

The leaves of *Gymnema sylvestre* were thoroughly washed with water then allowed for shadow drying for four days at room temperature and grounded into fine powder. The 5 g

of *Gymnema sylvestre* powder was soaked in 50 mL of petroleum ether, chloroform, water: ethanol (1 : 1) and incubated for 48 hr at room temperature. The extracts were filtered with Whatman filter paper. The extra solvent from the filtrate were evaporated by using water bath at different temperature (petroleum ether at 55^{o}C, chloroform at 77^{o}C and water: ethanol at 80^{o}C). The extracts were stored at 4^{o}C further use.

Preparation of *Cannabis sativa leaves* extracts

The leaves of *Cannabis sativa* were thoroughly washed with water then dried for three days at room temperature and grounded into fine powder. The 5 g of *Cannabis sativa* powder was soaked in 50 mL of each solvent (propanol, hexane) and incubated for 72 hrs at room temperature. The extract was filtered with watman. The extra solvent from the filtrate were evaporated by using water bath at different temperature (propanol at 65^{o}C, hexane at 70^{o}C). The extracts were stored at 4^{o}C further use.

Phytochemical screening of *Withania somnifera Gymnema sylvestre* and *Cannabis sativa*

The crude extract of *Withania somnifera Gymnema sylvestre* and *Cannabis sativa* were subjected to qualitative phytochemical screening for identification of various classes of active chemical constituents by using the following methods.

Test for saponin: The 5 mL of extract was shaken vigorously with 10 mL of distilled water for 2 min the appearance of foam that persisted for at least 15 min, or the foaming emulsion when olive oil was added, confirmed the presence of saponins.

Test for Tannins: A few drops of 5% $FeCl_3$ solution were added to 2 to 3 mL of ethanolic extract. Appearance of deep blue black colour, indicated the presence of tannins.

Test for Steroids: The 2 mL of chloroform was added to 2 mL of extract followed by addition of 2 mL of conc. H_2SO_4 and shaken well. Chloroform layers appearing red and acid layer showing greenish yellow flourescence, indicated the presence of steroid in the test extract.

Test for Flavonoids: A few drops of lead acetate were added to 2 to 3 mL of extract. Formation of yellow colour indicated the presence of flavonoid.

Antimicrobial activity of *Withania somnifera, Gymnema sylvestre* and *Cannabis sativa*

The antimicrobial activity of various extracts was determined by the Agar well diffusion method. Microbial inoculum was aseptically spread on the surface of pre solidified

Mueller Hinton agar plates using spreader. A well of about 6.0 mm was aseptically punctured using sterile cock borer. 100 µL of each extract of different dilutions was poured into wells. DMSO was used as negative control whereas ciprofloxacin and ketokanozole were used as positive controls. The plates were kept in laminar flow for 30 minutes for pre-diffusion to occur and then incubated at 37^{o}C for 24 hours. The zone of inhibition was measured using Hi-media zone scale for analysis of antimicrobial activity.

RESULTS AND DISCUSSION

The extracts of *W. somnifera, G. sylvestre and C. sativa* have been assessed for antimicrobial activity against *P. aeruginosa, E. coli* and *C. albicans*. The antimicrobial activity of ethanolic extracts of *W. Somnifera* at varying concentration is shown in Table 1. The ethanolic extracts (20 mg/mL) of *W. Somnifera* was showing zone formation (44, 45, 42 mm) against *P. aerouginosa*, *E. coli* and *C. albicans*, respectively. The antimicrobial activity of *W. Somnifera* was reported earlier[1,2]. The antimicrobial activity of *G. sylvestre* extract (petroleum ether, chloroform and water: ethanolic) is shown in Table 2. The zone formation of *G. sylvestre* petroleum ether extract was 20 mm against *P. aeruginosa.* The zone formation of *G. sylvestre* water: ethanol extract was 18 mm against *E. coli.* The zone formation of *G. sylvestre* chloroform extract was 20 mm against *C. albicans.* The antimicrobial activity of *G. sylvestre* was reported by Natrajan and Ramachandran[3]. The zone formation of *G. sylvestre* chloroform extract is shown in Table 3. The zone formation of *G. sylvestre* chlorofomic extract (10 mg/mL) was (21, 27, 19 mm) against *P. aerouginosa, E. coli and C. albicans*, respectively. The zone formation of hexane and propanolic extract of *C. sativa* is shown in Table 4. The antimicrobial activity of propanolic extract of *C. sativa* is shown in Table 5.

Table 1: Antimicrobial activity of ethanolic extracts of *Withania Somnifera*

	Diameter of zone of inhibition (mm)						
***Withania Somnifera* extracts**	**Ethanol 20 mg/mL**	**Ethanol 10 mg/mL**	**Ethanol 5 mg/mL**	**Ethanol 2.5 mg/mL**	**Cipro-floxacin (+) control**	**Flucanazole Positive control**	**DMSO (-) control**
P. aeruginosa	44 ± 1.0	31 ± 0.5	26	19	29	-	-
E. coli	45 ± 0.5	32 ± 0.3	24 ± 1.0	18 ± 0.6	27	-	-
C. albicans	42 ± 0.5	30	26	18 ± 1.0	-	24	-

(-) = no activity, ± mean deviation, Experiments were performed in triplicates

Table 2: Antimicrobial activity of various extracts of *Gymnema Sylvestre*

	Diameter of zone of inhibition (mm)					
***G. Sylvestre* extracts**	**Petroleum ether**	**Chloro-form**	**Water : Ehanol**	**Ciprofloxacin (+) control**	**Flucanazole positive control**	**DMSO Negative control**
P. aeruginosa	20	17	16	29	-	-
E. coli	14	17	18	27	-	-
C. albicans	16	20	11	-	24	-

(-) = no activity, ± mean deviation, Experiment was performed in triplicates

Table 3: Antimicrobial activity of chlorofomic extract of *Gymnema Sylvestre*

	Diameter of zone of inhibition (mm)					
***G. Sylvestre* extracts**	**Chloro-form 10 mg/mL**	**Chloro-form 5 mg/mL**	**Chloro-form 1 mg/mL**	**Cipro-floxacin (+) control**	**Flucanazole Positive control**	**DMSO Negative control**
P. aeruginosa	21	19	-	29	-	-
E. coli	27	23	-	27	-	-
C. albicans	19	16	-	-	24	-

(-) = no activity, ± mean deviation, Experiment was performed in triplicates.

Table 4: Antimicrobial activity of various extracts of *Cannabis sativa*

Cannabis sativa	**Hexane**	**Propanol**	**Ciprofloxacin (+) control**	**Flucanazole (+) control**	**DMSO (-) control**
P. aeruginosa	20	15	29	-	-
E. coli	18	17	27	-	-
C. albicans	20	23	-	24	-

(-) = no activity, ± mean deviation, Experiment was performed in triplicates

Table 5: Antimicrobial activity of propanolic extracts of *Cannabis sativa*

	Diameter of zone of inhibition (mm)					
***Cannabis sativa* extracts**	**Propanol 10 mg/mL**	**Propanol 5 mg/mL**	**Propanol 2.5 mg/mL**	**Ciprofloxacin (+) control**	**Flucanazole Positive control**	**DMSO Negative control**
P. aeruginosa	19	17	15	29	-	-
E. coli	17	13	11	27	-	-
C. albicans	21	19	12	-	24	-

(-)= no activity, ± mean deviation, Experiments was performed in triplicates

The zone formation of *C. sativa* extracts (hexane and propanol) was 20 and 23 mm against *P. aerugenosa* and *C. albicans*, respectively. The zone formation of *C. sativa* propanolic extracts (10 mg/mL) was 19, 17 and 21 mm against *P. aerugenosa, E. coli* and *C. albicans*, respectively. The antimicrobial activity of *C. Sativa* was reported by Esra *et al.*[4] The positive control for bacterial culture was ciprofoxacin and for fungal it was flucanazole, both were taken at 5 mg/mL concentration. The DMSO was taken as negative control. The phytochemical analysis of *W. somnifera, G. sylvestre* and *C. sativa* is shown in Table 6.

Table 6: Phytochemical analysis of *Withania somnifera, Gymnema sylvestre* and *Cannabis sativa*

Extract/ solvent	**Phytoconstituent**				
	Saponin	**Flavonoid**	**Cardiac Glycosides**	**Steroid**	**Tannin**
Withania somnifera (ethanol)	+	-	N.D	-	-
Gymnema sylvestre (chloroform)	+	+	+	+	+
Gymnema sylvestre (petrolium ether)	+	-	+	+	-
Gymnema sylvestre (water : ethanol)	+	+	+	+	-
Cannabis sativa (propanol)	+	+	N.D	-	N.D
Cannabis sativa (hexane)	+	-	N.D	-	N.D

N.D. Indicates not determined of bioactive compound

The bioactive substances from these plants can be employed in the formulation of antimicrobial agents for the treatment of various bacterial infections. The results of present investigation indicate that antibacterial activity varies with plant extract concentration. The purification of these phytoconstituents and determination of their respective antimicrobial potencies should be the prospect route for examination.

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