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Evaluation of antimicrobial agent from leaves of *Synadenium grantii* Hk.f.Bot.Mag

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ABSTRACT

Higher plants have played a dominant role in the introduction of new therapeutic agents. In India, there are over 15000 kinds of naturally occuring higher plants[1]. About 2000 species of these are reported to posses medicinal values. Discovery and development of new therapeutic agents is a continuing process. In spite of the fact that at present we have our command a formidable array of modern drugs, the need to discover and invent new agents is geniune and urgent. Many of the members of family Euphorbiaceae are known because of their medicinal uses in tribals as well as in Ayurveda[2,3,4].

Present paper deals with one of such medicinally important plant *Synadenium grantii.* Antimicrobial studies of leaves extract of *S. grantii* shows strong antibacterial activity against gram positive bacteria viz. S. aureus and Streptococcus sp. and gram negative bacteria viz. *E. coli, S. dysentrae, S. typhi, P. aeruginosa* and *K. pneumoniae*. The IR of active fraction shows the presence of secondary amine, amide groups, Co-NH_2-C-N ring vibration. This proves its correlation with antimicrobial study.

Key words: *Synadenium grantii,* antimicrobial activity, therapeutic agents.

INTRODUCTION

Syndaneium grantii locally called as Badi Dudhi. This plant is recent introduction and found only on hedges Flrs. and Frts – Nov. – Feb. The plant is used as stimulant of central nervous system, and is used by tribals in many diseases viz. urinary tract infection, diarrhoea, dysentery, pains also used in bronchial affections.

The present work yielded useful information which can be exploited for the successful treatment of many diseases.

MATERIAL AND METHODS

The material for the present study was collected from Indore and its surrounding. 100 gm. of the shade dried plant material is extracted in "Soxhlet Extraction Appartus" successively with 500 ml. of each of the following solvents – P. ether, Benzene, Chloroform, Acetone, Ethanol (95%). Each time before extracting with next solvent the plant material is dried. Finally, the drug is macerated with water for 24 hrs. to obtain the aquous extract[5,6,7]. Each extract is concentrated by distilling off the solvent. The extract obtained with each solvent is weighted and its percentage is calculated in terms of the shade dried weight of the plant material. The colour and consistency of each extract is noted.

The extracts thus obtained are then subjected to qualitative test for identification of various plant constituents by the methods suggested by Finar (1962)[7], Farnsworth (1966)[8] and Harborne *et al.* (1979)[9]. Each extract sample was then tested for antimicrobial activity againste human pathogenic bacteria by "Cup Borrer Method".

The components of the ethanolic plant extract were seperated by column chromotography using silica gel column where Benzene and Hexane

are used as the solvent. The purity of each component was checked by Thin Layer Chromotography.

Each fraction are then again subjected for testing the antibacterial activity by "Disc Diffusion Method".[10,12]

RESULTS AND DISCUSSION

Antimicrobial studies of these extracts (P. ether, Benzene, Chloroform, Ethanol and water) is performed which includes various disease causing gram positive and gram negative organisms. Table 3(a) and 3(b) shows that ethanolic extract is

Table 1: Successive solvent extraction of *Synadenium grantii* (leaves) which show the colour and consistancy of each extract

S. No.	Solvent Used	Colour	Average Value of Extractive
1.	Petroleum ether (60-80)^{0}C	Dark Yellow	19.0%
2.	Benzene	Light Yellow	21.0%
3.	Chloroform	Light Green	23.5%
4.	Ethanol (95%)	Light Brown	17.5%
5.	Water	Light Green	24.0%

Table 2: Qualitative chemical examination of various extracts of *Synadenium grantii* (leaves)

S. No.	Plant Constituents Test/Reagents	P.ether extract	Benzene extract	Chloroform extract	Ethanol extract	Water extract
1.	Alkaloids					
	1. Mayer's Reagent	-	-	-	+	-
	2. Dragendraff Reagent	-	-	-	-	-
2.	Carbohydrates					
	1. Molish's test	-	-	-	+	+
	2. Fehling Soln. test	-	-	-	-	-
	Glucose	-	-	-	+	-
	Fructose	-	-	-	-	-
	Galactose	-	-	-	+	-
	Starch	-	-	-	-	-
3.	Glycosides					
	1. Borntrager's test	+	-	-	+	-
	2. Legal test	+	-	-	+	-
4.	Phytosterols					
	1. Liebermann's test	-	-	-	-	-
	2. Liebermann's Burchard's test	-	-	-	-	-
5.	Phenolic Compound					
	1. Ferric chloride test	-	-	-	+	-
	2. Lieberman test	-	-	-	+	-
6.	Proteins					
	1. Xanthoproteic test	-	-	-	+	-
	2. Biuret test	-	-	-	+	-
7.	Flavonoids	-	-	-	+	-

Note : Test for oil, saponin, aminoacids, gum and mucilage were also performed but found to be negative.

having strong antibacterial activity against gram positive and gram negative organisms. In case of gram positive bacteria extract shows antibacterial activity against *S. aureus* and *Streptococus* spp. but it does not show any response against *B. subtilis.* Extract shows strong antibacterial activity against gram negative organism viz *E.coli, S.dysenterae, S.typhi, P.aeruginosa* and *K. pneumoniae.*

Water extract also shows antibacterial activity against gram positive and gram negative bacteria. It shows strong antibacterial activity against *S. aureus* and *Streptococcus* spp. while extract is inactive against B. subtilis. In case of gram negative bacteria extract shows strong antibacterial activity against *S.dysenterae, E.coli* and *S.typhi* while extract shows positive response against *P. aerugionsa* and *K. pneumonia.*

Antimicrobial testing of P. ether, Benzene, Chlorform extract were also performed but there was no zone of inhibition found.

The ethanolic extract is the most effective than the extract of water. Fractionation of extract by column chromotography and antimicrobial susceptibility testing of individual fraction further suggest that not all the fractions are equally effective against micro ogranisms. Table No. 4(a) and 4(b) indicates that B_1, B_2, B_4, H_5, H_6, H_7, H_8, H_9, H_{10} does not show antibacterial activity against gram positive bacteria while B_3, H_1 shows strong antibacterial activity against *S. aureus* and *Streptococcus* spp., H_2 and H_3 fractions also shows antibacterial activity against S. aureus and Streptococcus spp. while all the fractions B_1 to B_5 and H_1 to H_{10} does not show any response against *B. subtilis.*

Table 4(b) shows that B_2, B_4, B_5, H_5, H_6, H_7, H_8, H_9, & H_{10} are inactive fractions against gram negative bacteria. Fraction No. B_1, B_3, H_1, H_2, H_3 & H_4 shows antibacterial activity against gram negative bacteria viz. *E. coli, S. dysenterae, S. typhi, P. aeruginosa* and *K. pneumoniae*. Out of all the studied fraction B_3 and H_3 shows strong antibacterial activity. In B_3 fraction shows strong antibacterial activity against *S. dysenterae, P. aeruginosa, S. typhi* while fraction shows positive response against *K. pneumoniae* and *E. coli*. H_3 fraction shows strong antibacterial activity in B_3 fraction shows strong

Table 3 (a): Antimicrobial testing of each extract of *Synadenium grantii* (leaves) against gram positive bacteria

S. No.	Extract Used	Quantity of extract used	*S. aureus*	*Streptococcus* Sp.	*Bacillus subtilis*
1	Ethanolic	0.05 ml	10 mm	12 mm	No Zone
		0.08 ml	13 mm	15 mm	No Zone
		0.11 ml	15 mm	17 mm	No Zone
		0.13 ml	18 mm	20 mm	No Zone
		0.16 ml	21 mm	23 mm	No Zone
	r		0.97	0.92	
		Zone Colour	- Light Brown		
2	Water	0.05 ml	09 mm	No Zone	No Zone
		0.08 ml	11 mm	10 mm	No Zone
		0.11 ml	14 mm	13 mm	No Zone
		0.15 ml	17 mm	15 mm	No Zone
		0.16 ml	19 mm	17 mm	No Zone
	r	-	0.92	0.93	
		Zone Colour	- Light Brown		

Note : * Antimicrobial testing of P. ether, Benzene and Chlorform extract were also performed but there was no zone of inhibition found.; * r = Correlation coefficient

antibacterial activity against *S. dysenterae, P. aeruginosa, S. typhi* while fraction shows positive response against *K. pneumoniae* and *E. coli*. H_3 fraction shows strong antibacterial activity against *E. coli, S. dysenterae, P. aeruginosa* while fraction H_3 shows positive response against *K. pneumoiae* and *S. typhi.*

Thus overall conclusion suggested that only B_3 and H_3 fractions are most effective among all the studied fractions. This fraction shows strong antibacterial activity against all the tested gram positive as well as gram negative organisms. However, the antimicrobial effect of a crude extract may be a combine action of all thee fractions.

The present study shows that there is a sharp and strong peak (broad) hump like at 3443 cm^{-1}. This peak is most probably due to secondary amine and nitrogen is in the ring. This is might be due to residual moiety of some alkaloid molecule as usual test also show the positive result (as seen in Table 1) about the presence of alkaloids. The peak is broaden which is might be due to some moisture in the compound.

Further strong peak at 2981 cm^{-1} is due to asymmetic stretching of C-H. Another strong peak at 1643 cm^{-1} is most probably due to –CONH– and also it is due to –CO stretching in –CONH– The presence of amide group is well reported in many of the alkaloids and the extract under study also shows the positive test for alkaloids.

The broader peak at 1393 cm^{-1} is most probably due –C-N- ring stretching vibration conclusively we can draw that extract under study have any of the compound which contain hetrocyclic ring with nitrogen as hetrocyclic atom.

The I.R. of the active fraction *Synadenium grantii* shows the presence of secondary amines and amide groups –CO-NH-C-N ring structural vibration. Amines and amides play important roles in our day-to-day lives. In many drugs viz. Amphetamines, Barbiturate, Analgesics, Anesthetics, Decongestants and antibiotics are such medicinal compounds which are amines or amine derivatives. Amphetamines stimulate the central nervous system and are also used to treat psychological disorders such as severe depression[12]. Many of the medicinal

Table 3 (b): Antimicrobial testing of each extract of *Synadenium grantii* (leaves) against gram negative bacteria

S. No.	Extract used	Quantity extract used	*E.coli*	Shigella dysenterae	*Salmonella typhi*	*Pseudo-monas aeruginosa*	*K. pneum-oniae*
1.	Ethanolic	0.05 ml	15 mm	17 mm	12 mm	10 mm	No Zone
		0.08 ml	17 mm	19 mm	15 mm	12 mm	8 mm
		0.11 ml	20 mm	22 mm	17 mm	14 mm	10 mm
		0.13 ml	23 mm	25 mm	20 mm	17 mm	13 mm
		0.16 ml	25 mm	28 mm	22 mm	20 mm	15 mm
	r	-	0.99	0.99	1.00	0.96	0.93
		Zone Colour - Light Brown					
2.	Water	0.05 mm	12 mm	14 mm	10 mm	No Zone	No Zone
		0.08 mm	14 mm	17 mm	12 mm	8 mm	No Zone
		0.11 mm	16 mm	19 mm	14 mm	10 mm	8 mm
		0.13 mm	18 mm	22 mm	17 mm	12 mm	11 mm
		0.16 mm	21 mm	25 mm	19 mm	15 mm	14 mm
	r	-	0.98	0.99	0.94	0.96	0.92
		Zone Colour - Light Brown					

Note : * Antimicrobial testing of P. ether, Benzene and Chlorform extract were also performed but there was no zone of inhibition found.; * r = Correlation coefficient

amines are also used as analygsis and anesthetics Novocaine and related compounds e.g. are used as local anesthetic while Demerol is a very strong pain reliever[11]. Phenacetin and accetaminophen which are common substitutes of aspirine and Dopa and Dopamine, the related compounds Epinephrine and Norenephrine are also amine and amide derivatives [5,12].

Sulfa drugs an important class of

Table 4(a): Antimicrobial testing of each fraction of benzene and hexane against gram positive bacteria

S. No.	Fractions	*S. aureus*	*Streptococcus* spp.	*Bacillus subtilis*
1.	B_1	No Zone	No Zone	No Zone
2.	B_2	No Zone	No Zone	No Zone
3.	B_3	20 mm	17 mm	No Zone
4.	B_4	No Zone	No Zone	No Zone
5.	B_5	No Zone	No Zone	No Zone
6.	H_1	15 mm	16 mm	No Zone
7.	H_2	12 mm	14 mm	No Zone
8.	H_3	10 mm	12 mm	No Zone
9.	H_4	No Zone	No Zone	No Zone
10.	H_5	No Zone	No Zone	No Zone
11.	H_6	No Zone	No Zone	No Zone
12.	H_7	No Zone	No Zone	No Zone
13.	H_8	No Zone	No Zone	No Zone
14.	H_9	No Zone	No Zone	No Zone
15.	H_{10}	No Zone	No Zone	No Zone
16.	St. Ben. & Hex	No Zone	No Zone	No Zone

Table 4(b): Antimicrobial testing of each fraction of benzene and hexane against gram negative bacteria

S. No.	Fractions	*E.coli*	*Shigella dysenterae*	*Salmonella typhi*	*Pseudomonas aeruginosa*	*K. pneum oniae*
1.	B_1	No Zone	22 mm	16 mm	18 mm	12 mm
2.	B_2	No Zone	No Zone	No Zone	No Zone	No Zone
3.	B_3	8 mm	25 mm	17 mm	21 mm	10 mm
4.	B_4	No Zone	No Zone	No Zone	No Zone	No Zone
5.	B_5	No Zone	No Zone	No Zone	No Zone	No Zone
6.	H_1	16 mm	20 mm	19 mm	13 mm	No Zone
7.	H_2	11 mm	12 mm	18 mm	No Zone	14 mm
8.	H_3	23 mm	20 mm	13 mm	16 mm	15 mm
9.	H_4	15 mm	14 mm	No Zone	No Zone	No Zone
10.	H_5	No Zone	No Zone	No Zone	No Zone	No Zone
11.	H_6	No Zone	No Zone	No Zone	No Zone	No Zone
12.	H_7	No Zone	No Zone	No Zone	No Zone	No Zone
13.	H_8	No Zone	No Zone	No Zone	No Zone	No Zone
14.	H_9	No Zone	No Zone	10 mm	No Zone	No Zone
15.	H_{10}	No Zone	No Zone	No Zone	No Zone	No Zone
16.	St. Ben. & Hex	No Zone	No Zone	No Zone	No Zone	No Zone

antibiotics are also systehsized from amine and amine related compounds[11]. This proves its correlation with antimicrobial activity. Thus, the results of present study not only confirm the correct usage of the plant by the tribals but also enhances the creditability of ethnobotanical explorations.

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