

ANTIMICROBIAL EFFICACY OF *CASSIA TORA* AND *ACONITUM NAPELLUS*

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ABSTRACT

Pet ether, benzene, chloroform, ethyl acetate, methanol and distilled water extracts of two Indian Medicinal plants *Cassia tora* and *Aconitum napellus* were examined for their antimicrobial potential against the bacteria, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Trichophyton rubrum*, *Streptococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella sonnei* and fungi *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus*. All the plant extracts exhibited antibacterial and antifungal activities against almost all the microorganisms tested which explains that their use in daily life will generate a resistance or immunity to fight against microorganisms. The present results therefore offer a scientific basis for traditional use of the various extracts of *Cassia tora* and *Aconitum napellus*.

Keywords: *Cassia tora*, *Aconitum napellus*, Antimicrobial.

INTRODUCTION

From the beginning of human civilization people are indebted to nature in many ways¹. Medicinal plants and derived medicines are extensively used in traditional cultures all over the world and they are becoming progressively more popular in present society as natural alternatives to synthetic chemicals². Many of evidences are available to demonstrate the potential of medicinal plants used in various traditional, complementary and alternate system of medicines³. Medicinal values of many plants still remain uncharted for its enumerable activity of compounds responsible for later. Yet, plant materials remain a vital resource to combat severe diseases of the world. Pharmacognostic investigations of plants are carried out to find new drugs or templates for the development of new therapeutic agents⁴. Contrary to the synthetic drugs, antimicrobials of plant origin are not coupled with many side effects and have an huge therapeutic potential to cure many infectious diseases⁵. Plants are known to contain enumerable biological active compounds⁶ which possess antibacterial properties⁷. The WHO (World Health

Organization) considering phytotherapy in its health programs suggested basic measures for substantiation of drugs from plant origin in developing countries⁸.

The microorganisms have developed resistance to many antibiotics because of arbitrary use of antimicrobial drugs that create a big dilemma in the treatment of infectious diseases⁹. With the augment in the resistance of many microorganisms to the presently used antimicrobials and the high cost of production of synthetic compounds; in addition to many side effects; there is a need to look for the alternatives. Plants have provided a good source of anti-infective agents; emetine, quinine, berberine, tannins, terpenoids, alkaloids and flavonoids continue to be highly efficient instruments in the fight against microbial infections¹⁰.

Therefore, in present work attempts have been made to screen two medicinal plants *Cassia tora* and *Aconitum napellus* belonging to different families were evaluated for antimicrobial potentials to develop certain active fractions having therapeutic potentials to cure various diseases caused by MDR microorganisms.

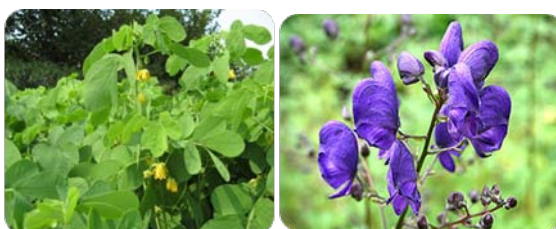


Fig. 1: (a) *C.tora*, (b) *A.napellus*

MATERIALS AND METHODS

Collection: Plant samples (*Cassia tora* and *Aconitum napellus*) were collected from various tribes living in tribal pockets of Mt. Abu, arid zone of Rajasthan. These plants were used by these tribes in their daily lives to cure various ailments and even from Chunnilal Attar Ayurvedic Store, Ghat Gate, Jaipur in the month of May, 2011.

Identification: Both the samples were authenticated and were given identification number *Cassia tora* (COE 213) and *Aconitum napellus* (COE 192). These samples were authenticated and submitted in Ethnomedicinal Herbarium, Centre of Excellence funded by DST, MGias, Jaipur (Rajasthan).

Sources of test organisms: Bacteria-Pure culture of all test organisms, namely *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*,

Shigella sonnei and *Trichophyton rubrum* and fungi *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus* were obtained through the courtesy of Mahatma Gandhi Institute of applied Sciences (MGias), Jaipur, which were maintained on Nutrient broth media.

Culture of test microbes: For the cultivation of bacteria, Nutrient Agar Medium (NAM) was prepared by using 20 g Agar, 5 g Peptone, 3 g beef extract and 3 g NaCl in 1 L distilled water and sterilized at 15 lbs pressure and 121°C temperature for 25-30 min. Agar test plates were prepared by pouring approximately 15 mL of NAM into the Petri dishes (10 mm) under aseptic conditions. A saline solution was prepared (by mixing 0.8% NaCl) in distilled water, followed by autoclaving and the bacterial cultures were maintained on this medium by regular sub-culturing and incubation at 37°C for 24-48 h. To prepare the test plates, in bacteria, 10-15 mL of the respective

medium was poured into the Petri plates and used for screening. For assessing the bactericidal efficacy, a fresh suspension of the test bacteria was prepared in saline solution from a freshly grown Agar slant.

Preparation of test extracts: Crushed powder (50 g) of all the species were successively soxhlet extracted with ethanol. Later, each of the homogenates was filtered and the residue was re-extracted twice for complete exhaustion, the extracts were pooled individually. Each filtrate was concentrated to dryness in vitro and redissolved in respective solvents, out of which 80 mg/10 disc i.e. 8 mg/disc concentration were stored at 4°C in a refrigerator, until screened for antibacterial activity.

Bactericidal assay: For both, bactericidal in vitro Disc diffusion method was adopted (Gould and Bowie, 1952), because of reproducibility and precision. The different test organisms were proceeded separately using a sterile swab over previously sterilized culture medium plates and the zone of inhibition were measured around sterilized dried discs of Whatman No. 1 paper (5 mm in diameter), which were containing 1mg, 5mg and 10mg of the text extracts and reference drugs (tetracycline and mycostatin for bacteria and fungi, respectively) separately. Such treated discs were

air-dried at room temperature to remove any residual solvent, which might interfere with the determination, sterilized and inoculated. These plates were initially placed at low temperature for 1 h so as to allow the maximum diffusion of the compounds from the test disc into the agar plate and later, incubated at 37°C for 24 h in case of bacteria and C in case of fungi, after which the zones of inhibition could be easily observed. Five replicates of each test extract were examined and the mean values were then referred. The Inhibition Zone (IZ) in each case were recorded and the Activity Index (AI) was calculated as compared with those of their respective standard reference drugs (AI = Inhibition Zone of test sample/Inhibition zone of standard).

RESULTS

The profile of the medicinal plants used in the present investigation. The results of antimicrobial activity of the crude extracts of selected Indian Medicinal Plants (*Cassia tora* and *Aconitum napellus*) showed good antimicrobial activity against selected test bacteria and fungi (Table 1 and 2). Overall, these extracts showed appreciable activity against selected test bacteria and fungi and hence, it justifies their use in our traditional system of medicine to cure various diseases (fig.1 and 2).

Table 1: Antibacterial Efficacy of *Cassia tora* in terms of inhibition zone against selected bacteria and fungi

<i>Cassia tora</i> Extracts	Measure	Bacteria						Fungi			
		<i>P. vulgaris</i>	<i>P. aeruginosa</i>	<i>T. rubrum</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>S. sonnei</i>	<i>C. albicans</i>	<i>A. niger</i>	<i>A. flavus</i>
	Standard I.Z.	9.1	9.9	10.9	17.9	9.8	12.3	11.9	11.4	9.7	11.8
Pet ether	I.Z. (mm)	6.3	7.5	4.3	8.3	16	16.6	11.6	11.3	11	6
	A.I.	0.692	0.757	0.394	0.463	1.632	1.349	0.974	0.991	1.134	0.508
Benzene	I.Z.*(mm)	8.6	9.6	12	13.6	8.3	11.3	11.6	9	14.6	13.3
	A.I.	0.945	0.969	1.100	0.759	0.846	0.918	0.974	0.789	1.505	1.127
Chloroform	I.Z. (mm)	12.3	8.5	6.6	9.6	9.3	7	8.6	20.3	11.3	6.6
	A.I.	1.351	0.858	0.65	0.536	0.948	0.569	0.722	1.780	1.164	0.559
Ethyl Acetate	I.Z. (mm)	12.6	10.6	10.6	13.6	10.6	9.3	9.6	6.6	7.5	7
	A.I.	1.384	1.707	0.972	0.759	1.081	0.756	0.806	0.578	0.773	0.593
Methanol	I.Z. (mm)	11	16	8.3	9.3	8	8	12.3	12.3	13.6	11.3
	A.I.	1.208	1.616	0.761	0.519	0.816	0.650	1.033	1.078	1.402	0.957
Distilled Water	I.Z. (mm)	7	8	7.5	12	7.5	10	10.6	14.3	8.3	10.3
	A.I.	0.769	0.808	0.688	0.670	0.765	0.813	0.890	1.254	0.855	0.872

I.Z. = Inhibition zone, A.I. = Activity index

*I.Z. in mm are the mean value of the triplicates *Aconitum napellus*

Table 2: Antibacterial Efficacy of *Aconitum napellus* in terms of inhibition zone against selected bacteria and fungi

<i>Aconitum napellus</i> Extracts	Measure	Bacteria						Fungi			
		<i>P. vulgaris</i>	<i>P. aeruginosa</i>	<i>T. rubrum</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>S. sonnei</i>	<i>C. albicans</i>	<i>A. niger</i>	<i>A. flavus</i>
	Standard I.Z.	9.1	9.9	10.9	17.9	9.8	12.3	11.9	11.4	9.7	11.8
Pet ether	I.Z.*(mm)	6.6	9.6	9.3	10	7.6	7.6	10	9.5	15.6	10
	A.I.	0.725	0.969	0.853	0.558	0.775	0.617	0.840	0.833	1.608	0.847
Benzene	I.Z. (mm)	7.3	10.5	7.6	8	7.6	8.3	10	14	17.3	10.5
	A.I.	0.802	1.606	0.697	0.446	0.775	0.674	0.840	1.228	1.783	0.889
Chloroform	I.Z. (mm)	6.6	8.3	11.6	8	11	6	9.3	10	7	8
	A.I.	0.725	0.838	1.064	0.446	1.122	0.487	0.781	0.877	0.721	0.677
Ethyl Acetate	I.Z. (mm)	7.6	9	5	9.6	9.6	9	15	9.3	6.5	6.3
	A.I.	0.835	0.909	0.458	0.536	0.979	0.731	1.260	0.815	0.670	0.533
Methanol	I.Z. (mm)	8	12	6.3	9.6	8.3	6	11.3	10	8	-
	A.I.	0.879	1.212	0.577	0.536	0.846	0.487	0.949	0.877	0.824	-
Distilled Water	I.Z. (mm)	11.3	7	5.3	10.3	8.3	8	12.6	11.3	13	12
	A.I.	1.241	0.707	0.486	0.575	0.846	0.650	1.058	0.991	1.340	1.016

I.Z. = Inhibition zone, A.I. = Activity index

*I.Z. in mm are the mean value of the triplicates

Cassia tora

While screening the extracts of *Cassia tora* the, good antimicrobial activity against the selected bacteria and fungi was observed. The various extracts were found active against all the bacteria and fungi tested. Results, comparable to the standards, were found against *P.aeruginosa* (7.5mm), *S.Sonnei* (11.6mm), *C.albicans* (11.3mm) (pet ether extract), *P.vulgaris* (8.6mm), *P.aeruginosa* (9.6mm), *S.aereus* (13.6mm), *E.coli* (8.3mm), *K.pneumoniae* (11.3mm), *S.sonnei* (11.6mm), *C.albicans* (9mm) (benzene extract), *P.aeruginosa* (8.5mm), *E.coli* (9.3mm), *S.sonnei* (8.6) (chloroform extract), *T.rubrum* (10.6mm), *S.aureus* (13.6mm), *K.pneumoniae* (9.3mm), *S.sonnei* (9.6mm), *A.niger* (7.5mm) (ethyl acetate extract), *T.rubrum* (8.3mm), *E.coli* (8mm) (methanol extract), *P.vulgaris* (7mm), *P.aeruginosa* (8mm), *E.coli* (7.5mm), *K.pneumoniae* (10mm), *S.sonnei* (10.6mm), *A.niger* (8.3mm) and *A.flavus* (10.3mm) (aqueous extract). Antimicrobial activity greater than the activity of the standard was observed against *E.coli* (16mm), *K.pneumoniae* (16.6mm), *A.niger* (11mm) (pet ether extract), *T.rubrum* (12mm), *A.niger* (14.6mm), *A.flavus* (13.3mm) (benzene extract), *P.vulgaris* (12.3mm), *C.albicans* (20.3mm), *A.niger* (11.3mm) (chloroform extract), *P.vulgaris* (12.6mm), *P.aeruginosa* (10.6mm), *E.coli* (10.6mm) (ethyl acetate), *P.vulgaris* (11mm), *P.aeruginosa* (16mm), *S.sonnei* (12.3mm), *C.albicans* (12.3mm) and *A.niger* (13.6mm) (methanol extract) and *C.albicans* (14.3mm) (aqueous extract).

While screening the extracts of *Aconitum napellus*, good antimicrobial activity against the selected bacteria and fungi was observed. The various extracts were found active against all the bacteria and fungi tested. Results, comparable to the standard, were obtained against *P.vulgaris* (6.6mm), *P.aeruginosa* (9.6mm), *T.rubrum* (9.3mm), *E.coli* (7.6mm), *S.sonnei* (10mm), *C.albicans* (9.5mm), *A.flavus* (10mm) (pet ether extract), *P.vulgaris* (7.3mm), *E.coli* (7.6mm), *S.sonnei* (10mm), *A.flavus* (10.5mm) (benzene extract), *P.vulgaris* (6.6mm), *P.aeruginosa* (8.3mm), *S.sonnei* (9.3mm), *C.albicans* (10mm), *A.niger* (7mm) (chloroform extract), *P.vulgaris* (7.6mm), *P.aeruginosa* (9mm), *E.coli* (9.6mm), *K.pneumoniae* (9mm), *C.albicans* (9.3mm) (ethyl acetate extract), *P.vulgaris* (8mm), *E.coli* (8.3mm), *S.sonnei* (11.3mm), *C.albicans* (10mm), *A.niger* (13mm) (methanol extract), *P.aeruginosa* (7mm), *E.coli* (8.3mm) and *C.albicans* (11.3mm) (aqueous extract). Antimicrobial activity, higher than the activity of the standard, was observed against *A.niger* (15.6mm) (pet ether), *P.aeruginosa* (10.5mm), *C.albicans* (14mm), *A.niger* (17.3mm) (benzene extract), *T.rubrum* (11.6mm), *E.coli* (11mm) (chloroform extract), *S.sonnei* (15mm) (ethyl acetate extract), *P.aeruginosa* (12mm) (methanol extract), *P.vulgaris* (11.3mm), *S.sonnei* (12.6mm), *A.niger* (13mm) and *A.flavus* (12mm) (aqueous extract). The methanol extract of the plant showed no activity against the fungus *A.flavus*.

DISCUSSION

The versatile medicinal plants are the unique source of various types of compounds having diverse chemical structures. Very little work has been done on the biological activity and plausible medicinal applications of these compounds and hence extensive investigation is needed to exploit their therapeutic utility to combat diseases.

The present results therefore offer a scientific basis for traditional use of the various extracts of *Cassia tora* and *Aconitum napellus*. These results explain that Indian Medicinal Plants have potentials as antimicrobials. Further, more or less both the selected Indian Medicinal Plants have also possessed antimicrobial potential against all test bacteria and fungi which explains that their use in daily life will generate a resistance or immunity to fight against microorganisms.

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