



COMBINATIONAL EFFECT OF *ASPARAGUS RACEMOSUS*, *ANDROGRAPHIS PANICULATA* AND *DISCOREA VILLOSA* FOR ANTHELMINTIC AND ANTIMICROBIAL POTENTIAL

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ABSTRACT

Asparagus racemosus, *Andrographis paniculata* and *Discorea villosa* are traditionally used as herbal medicine. In the present study, the aerial plant parts were subjected to hydroalcoholic extraction and the extracts were used to evaluate their antimicrobial and anthelmintic potential. The extracts of all the three plants at concentrations of 4, 8 and 16 mg/ml and their combination (concentration ratio 1:1:1) were used against six different bacterial strain and three fungal strain for assessment of antimicrobial activity. For anthelmintic study, the concentrations of 20 and 40 mg/ml were used against *Pheretima posthuma* (Earthworms). All the plant extracts as well as their combination showed significant anthelmintic and antimicrobial potential. The combinational study showed synergistic effect.

Keywords: *Asparagus racemosus*, *Andrographis paniculata*, *Discorea villosa*, Hydroalcoholic, Antimicrobial, Anthelmintic.

INTRODUCTION

Helmenthiasis is a highly prevalent disease mainly in the third world countries.¹ Inadequate sanitation and poor hygiene may be the main reason behind the helmenthic infection.² It is among the most important animal diseases inflicting heavy production losses. The gastro intestinal helmenthis has become resistant to the commonly used helmenthic drugs³ and hence, there is a need to find the anthelmintic property from natural resources like medicinal plants, which may be effective against helmenthiasis and may have less or no side effects. Also, the increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microorganism has been a major problem to mankind and this leads to the screening of several medicinal plants for their potential for antimicrobial activity.⁴⁻⁶ Beside this, plant originated antimicrobial drugs do not have much side effect and have an enormous therapeutic potential to heal many infectious diseases.⁷ Medicinal plants represent a rich source of antimicrobial agents. In recent years, screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of new anti-infective agents.⁸⁻¹⁰

Asparagus racemosus, *Andrographis paniculata* and *Discorea villosa* are traditionally used as herbal medicines and belong to family Asparagaceae, Acanthaceae and Discoreaceae respectively. All the plants are rich in phytochemicals like flavonoids, steroids and saponins and shares some common medicinal properties like antioxidant, hepatoprotective, immunomodulatory etc. Based on the above findings, the present work was used to explore their anthelmintic and antimicrobial potential as in individual and also in combination.

MATERIALS AND METHODS

Plant material

The authenticated plants were collected and confirmed at Botany Department, Dr. H. S. Gour University, Sagar (M.P.).

Chemicals and drugs

The following drugs and chemicals were used: Sodium chloride (HIMEDIA), Ethanol (RANKEM), Piperazine citrate, a gift obtained from R. K. Pharmacy, Sagar, Gentamycin, Griseofulvin, Muller Hilton's Agar media (HIMEDIA), Potato Dextrose Agar media, 10% Tween 80.

Extract preparation

Dried and powdered plant materials were extracted with 70% ethanol. The extracts were concentrated and dried at 68°C and kept at 4°C for further studies.

Phytochemical test

Phytochemicals screening were performed to detect the presence or absence of various compounds such as tannins, flavonoids, alkaloids etc. as per standard methods.¹¹

Antimicrobial Activity

Test microorganisms: Following identified microbial cultures were obtained from IMTEC, Chandigarh, India.

Bacteria: *Escherichia coli* (MTCC 723), *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 736), *Pseudomonas sp* (MTCC 3365), *Staphylococcus xylossus* (MTCC 6149) and *Micrococcus sp.* (MTCC 8125).

Fungi: *Aspergillus niger* (MTCC 9933), *Aspergillus flavus* (MTCC 9167) and *Candida albicans* (MTCC 1637).



Antimicrobial Assay: For antibacterial assay¹², initially the stock cultures of bacteria were revived by inoculating in broth media and grown at 37°C for 18 h. The agar plates of the Muller Hilton's Agar media were prepared. Each plate was inoculated with an aliquot (0.1 ml) of the bacterial suspension (10^5 - 10^6 colony forming unit "CFU"/ml), which was spread evenly on the plate. After 20 min, antimicrobial disc soaked with the respective extracts of different concentration were placed on the plates. The positive and negative control wells were filled with Gentamycin (Standard drug) and 10% Tween 80 respectively. All the plates were incubated at 37°C for 24 h and then the diameter of zone of inhibition was noted.

Similarly, for the antifungal activity¹³, the stock cultures of fungi were revived by inoculating in broth media and grown at 27°C for 72 h. The agar plates of the Potato Dextrose Agar media were prepared. Each plate was inoculated with an aliquot (0.1 ml) of the fungal suspension (10^3 spores/ml), which was spread evenly on the plate. After 20 min, antimicrobial disc soaked with the respective extracts of different concentration were placed on the plates. The positive and negative control plates with Griseofulvin (standard drug) and 10% Tween 80 were also prepared. All the plates were incubated at 27°C for 72 h and then the diameter of zone of inhibition was noted.

Anthelmintic activity

Experimental model: The earthworms show physiological and anatomical resemblance with intestinal parasites and round worms¹⁴ and were used for the anthelmintic assay. Indian adult earthworms (*Pheretima posthuma*, 5-7cm in length) were freshly collected from organic farm of local earthworm breeding centre (N.H. 26, Sagar).

Experiment: The experiments were carried out by the method described by Singh *et al.*, 2009¹⁵. All the three extracts were dissolved in normal saline and diluted to get final concentrations of 20 and 40 mg/ml. Piperazine citrate (20 mg/ml) was used as a standard drug. Nine groups, each containing six earthworms were taken for the experiment. Extracts and piperazine citrate were dissolved separately in their respective concentration in 10ml normal saline. For combinational study, the different extracts were taken in the concentration ratio of 1:1:1. Time taken for paralysis and death of individual earthworm was noted. The paralysis occurs when the worms were placed in normal saline and were not able to move. The death was confirmed when the earthworms lost their motility and fading off their body colors.¹⁶

RESULTS

In the present investigation, the phytochemical screening showed the presence of flavonoids, carbohydrate, cardiac glycosides, saponin and steroids in all the plant extracts. Apart from this, alkaloids were present only in the extract of *Andrographis paniculata* and *Asparagus racemosus* while, glycosides were present only in *A. paniculata* extract (table 1). For antimicrobial screening,

hydroalcoholic extracts of *A. paniculata*, *A. racemosus* and *D. villosa* were tested against 6 bacterial and 3 fungal strains. The observations were compared with that of standard antibiotic drugs in terms of diameter of zone of inhibition. The combination of all three extracts was found most potent at a concentration of 16 mg/ml against *E. coli* (inhibition zone, 8 mm), *S. xylosum* (inhibition zone, 15 mm) and *Micrococcus* (inhibition zone, 15 mm). While, *A. racemosus* was found to be most effective against *S. aureus* and *P. Stringii* with 15 and 10 mm inhibition zone respectively. *A. paniculata* was most effective against *B. Subtilis* in comparison with all the three extracts. *D. villosa* was found to be most potent against *C. albicans* (9 mm zone of inhibition) while the combination was effective against *A. flavus* (6 mm zone of inhibition). *A. paniculata* showed moderate results against all three tested fungi (table 2 and 3).

Table 1: Phytochemical analysis of hydroalcoholic extract of *A. paniculata*, *A. racemosus* and *D. villosa* extracts

Phytochemical/Test	<i>A. paniculata</i>	<i>A. racemosus</i>	<i>D. villosa</i>
Alkaloids	+	+	-
Flavonoids	+	+	+
Tannins	+	-	+
Terpenoids	+	-	+
Steroids	+	+	+
Glycosides	+	-	-
Carbohydrates	+	+	+
Saponins	+	+	+
Cardiac glycoside	+	+	+
Phenolic compounds	+	-	+

+ : Present; - : Absent.

Table 2: Antibacterial activity of *A. paniculata*, *D. villosa* and *A. racemosus*

Extract	Conc. (mg/ml)	Zone of Inhibition (mm)					
		<i>E. coli</i>	<i>S. aureus</i>	<i>B. subtilis</i>	<i>S. xylosum</i>	<i>Micrococcus sp.</i>	<i>P. stringii</i>
AP	4.0	3	3	5	-	3	-
	8.0	5	4	6	-	3	-
	16	6	4	6	-	4	-
DV	4.0	3	-	-	3	3	-
	8.0	3	-	3	4	3	-
	16	4	2	4	5	4	3
AR	4.0	3	1	-	8	4	3
	8.0	3	5	-	10	4	5
	16	3	15	-	14	5	10
AP+DV+AR (1:1:1)	16	8	9	4	15	15	4
Gentamycin (Standard drug)	1.0	19	20	28	18	18	18

AP = *Andrographis paniculata*; DV = *Discorea villosa*; AR = *Asparagus racemosus*; - = No zone of inhibition



Table 3: Antifungal activity of *A. paniculata*, *D. villosa* and *A. racemosus*

Extract	Concentration (mg/ml)	Zone of Inhibition (mm)		
		<i>A. niger</i>	<i>A. flavus</i>	<i>C. albicans</i>
AP	4.0	3	3	4
	8.0	3	4	4
	16	4	4	5
DV	4.0	4	4	3
	8.0	4	5	4
	16	5	5	9
AR	4.0	3	3	3
	8.0	3	3	3
	16	4	4	4
AP+DV+AR (1:1:1)	16	-	6	8
Griseofulvin (Standard drug)	1.0	13	18	13

AP = *Andrographis paniculata*; DV = *Discorea villosa*; AR = *Asparagus racemosus*; - = No zone of inhibition

All the three plants analyzed for their anthelmintic potential showed a concentration depended activity. *A. paniculata* extract showed better activity by way of causing the paralysis of the worms at 3.33 min at 40 mg/ml and 5.33 min at 20 mg/ml and death in 5.16 min at 40 mg/ml and 7.50 min at 20 mg/ml. Combination of all three extracts (1:1:1) was found most potent and caused paralysis of the worm at 2.83 min and death at 4.33 min. The time taken by the standard drug (20 mg/ml) for the paralysis and death of the worms was 7.0 and 14.83 min respectively. The combination showed synergistic effect for anthelmintic activity (Table 4).

Table 4: Anthelmintic activity of *A. paniculata*, *D. villosa* and *A. racemosus*

Extract/Drug	Concentration (mg/ml)	Paralysis time	Death time
		Min.	
AP	20	5.33 ± 0.40	7.50 ± 0.20
	40	3.33 ± 0.18	5.16 ± 0.14
DV	20	10.23 ± 0.23	15.16 ± 0.43
	40	4.83 ± 0.14	6.50 ± 0.31
AR	20	12 ± 0.23	16.50 ± 0.20
	40	5.83 ± 0.14	7.33 ± 0.18
AP+DV+AR (1:1:1)	40	2.83 ± 0.14	4.33 ± 0.18
Piperazine citrate	20	7 ± 0.33	14.83 ± 0.36
Normal Saline (Control)	-	-	-

DISCUSSION

In the present investigation, all the extracts and their combination showed activity against some bacteria and fungi. This activity is mainly due to the presence of phytochemicals like tannins, flavonoids, alkaloids and glycosides. Tannins and flavonoids were known to possess antimicrobial potential against bacteria and fungi. Antimicrobial agents can damage pathogens in several ways. The major modes of actions are interference with cell wall synthesis, inhibition of protein synthesis,

interference with nucleic acid synthesis, and inhibition of a metabolic pathway.¹⁷⁻¹⁹ The most selective antimicrobial agents are those that interfere with the synthesis of bacterial cell walls e.g. penicillin, cephalosporin etc. these drugs have high therapeutic index because bacterial cell walls possess a unique structure, not found in eukaryotic cells. All three extracts may possess the similar mechanisms of action against the microbes.

Synthetic anthelmintic drugs like piperazine citrate, is known to cause paralysis of worms so that they are expelled in the feaces of men and animals. The extracts in the present study could cause paralysis as well as the death of the worms. These drugs may reach the target site in worms either orally or by diffusion and/or uptake through the cuticle, however the major uptake of the drug is through cuticle.²⁰ All the three extracts when taken in combination, generally showed synergistically better activity as compared to individual effect. The variation in activity of the plant extract might be due to the difference in the proportion of the active compounds responsible for the anthelmintic property.²¹ The active constituents may be the phenolics such as flavonoids and tannins present in the plant extracts.

CONCLUSION

As the search for new antimicrobial and anthelmintic agent is in demand, plant extracts may provide attractive alternate sources of antimicrobial and anthelmintic drug against various microbial and helminthes diseases. The present study provides the evidence of antimicrobial and anthelmintic property of *A. paniculata*, *A. racemosus* and *D. villosa*. Also, the synergistic effect shown by the plant extracts when taken in combination gave a better idea of working on a polyherbal combination which may give better result as compared to individual effect.

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