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Antimicrobial and spermicidal activities of *Parthenium hysterophorus* Linn. and *Alstonia scholaris* Linn.

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Parthenium hysterophorus Linn. of Asteraceae family and *Alstonia scholaris* Linn. of Apocynaceae family are used as remedy for a wide variety of ailments. The extracts obtained from aerial parts of *P. hysterophorus* and bark of *A. scholaris* was evaluated for antimicrobial activity against three bacterial species (*Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli)* and three fungal species (*Aspergillus niger, Candida albicans* and *Fusarium oxysporum*). The extracts at various concentrations were also screened for antifertility activity against Holstein Friesian cattle sperms. The bark extract of *Alstonia scholaris* obtained with dichloromethane-ether-methanol (1:1:1) has shown maximum antimicrobial and spermicidal activity.

Keywords: Antimicrobial, Spermicidal, *Parthenium hysterophorus*, *Alstonia scholaris*, Bark, Aerial parts.

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Introduction

Parthenium hysterophorus Linn. (Family-Asteraceae), commonly known as Congress grass, Congress weed, Carrot weed and Wild feverfew. The "Scourge of India" is an exotic weed that was accidentally introduced in India in 1956 through imported food grains and is now considered as one of the most feared noxious weed[1]. The plant is used in the treatment of ulcerated sores, wounds, fever, anaemia and heart troubles. A decoction of the root finds use in treatment of dysentry[2] and the lower concentrations of extracts might find use as antifungal agent[3]. It is applied externally on skin disorders and decoction of the plant is often taken internally as a remedy for a wide variety of ailments[4]. It is also reported as promising remedy against hepatic amoebiasis[5]. *Alstonia scholaris* Linn. belongs to the family Apocynaceae and is commonly called Blackboard tree, Indian devil tree, Ditabark and Milkwood pine. The *Alstonia* species is rich in alkaloids, steroids and triterpenoids and these substances may be responsible for the toxicity[6]. The plant is used as a tonic, anthelmintic, stimulant, carminative and expectorant[7,8]. The bark extract of the powdered stem bark is a bitter tonic and febrifuge, useful for the treatment of malaria, diarrhoea and dysentry[7]. The bark decoction is also used to treat asthma, hypertension, lung cancer and pneumonia while the leaf infusion is used to treat fever[8]. Its bark extract showed significant antifertility effects in male rats[9]. A major alkaloid, alstonine has antitumor activity in YC8 lymphoma and Ehrlich ascites carcinoma cells[10]. The bacterial species, viz. *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* and Fungi *Aspergillus niger, Candida albicans* and *Fusarium oxysporium* were taken up as test microorganisms.

The objective of this paper is to ascertain the antibacterial, antifungal and spermicidal activity of extracts obtained from aerial parts of *Parthenium hysterophorus* (Plate 1A) and bark of *Alstonia scholaris* (Plate 1B).

Materials and Methods

Plant material

The aerial parts of *P. hysterophorus* and bark of *A. scholaris* were collected from Kala khet, Kota and identification was done with the help of Department of Botany, Govt. College, Kota.

Extraction

The air-dried and coarsely powdered aerial parts of *P. hysterophorus* (3 kg) were extracted with petroleum ether (b.p. 60-80º) over water bath for

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Plate 1—*Parthenium hysterophorus* (A) and *Alstonia scholaris* (B)

12 h × 6 times (extract 1). The resulting plant material was air dried and extracted with dichloromethane–ether-methanol (1:1:1) mixture at room temperature for 12 h × 2 times (extract 2). Extract 3 and 4 were obtained by repeating same procedure with bark of *A. scholaris*. The resulting extracts were concentrated in vacuum and left over night in the refrigerator. Concentrations of all the extracts at 200, 400, 600, 800 ppm were made by methanol for antimicrobial and by baker's yeast for spermicidal activity.

Microbes

S. aureus, *P. aeruginosa*, *E. coli* were procured from the medical college, Kota and Fungi *A. niger*, *C. albicans*, *F. oxysporium* were procured from Microbial Type Culture Collection Centre (MTCC), Chandigarh.

Sperms

The sperms of normal HF cattle in a frozen state were recovered from a straw after thowing and made available from Animal Husbandry Department, Kota. Only semen with normal sperm morphology and motility was used.

Antimicrobial Activity

Media: Different media used for activities are as follows:

Antibacterial activity: Nutrient agar (High media): Peptic digest of animal tissue, 5.0 g; NaCl, 5.0 g; Beef extract, 1.50 g; Yeast extract, 1.50 g; Agar, 15.0 g; Distilled water, 1 lt. 28 g of high media nutrient agar was suspended in 1 lt distilled water. It was boiled and pH was adjusted to 7.4. Media was sterilized by autoclaving at 15 lb. pressure for 15 minutes and plates were prepared.

Antifungal activity: (a) *Candida albicans*, yeast extract 3.0 g; peptone 10.0 g; dextrose, 20.0 g; media was sterilized by autoclaving at 15 lb. pressure for 15 minutes and plates were prepared; (b) *Fusarium oxysporum*, potatoes (scrubbed and diced), 200.0 g; Sucrose, 20.0 g; Agar, 20.0 g; Distilled water, 1.0 lt. Diced potatoes were boiled in water for 1 h. The pulp was squeezed through cheese cloth. After adding agar and sucrose it is boiled till dissolution. Media was sterilized by autoclaving at 15 lb pressure for 15 minutes and plates were prepared; (C*) Aspergillus niger*, Czapek concentrate, 10.0 ml; K_2HPO_4, 1.0 g; Yeast extract, 5.0 g; Sucrose, 30.0 g; Agar, 15.0 g; Distilled water, 1.0 l; $NaNO_3$, 30.0 g; KCl, 5.0 g. Plates were prepared by similar procedure.

Susceptibility test procedure

Pure cultures of all three bacteria under test were used as inoculums. Three to four similar colonies from each pure culture were selected, transferred into 5 ml of suitable broth and were incubated at 35ºC for 2-8 h separately. Sterile non-toxic cotton swab was rotated in the tube and entire agar surface of the petridish was streaked with swab for three times. For testing antifungal activity in the plates of above media respective seeds of the fungi were added. In these petridishes, discs (prepared from Whatman filter paper no.1) dipped in each concentration of every extract were applied using aseptic technique. The standards used were Gentamycin and Mycostatin for antibacterial and antifungal activity, respectively. The petridishes are then incubated at 37-38ºC for 18 h for antibacterial activity and for 48 h for antifungal activity. After the incubation period, diameter of zone of inhibition was measured.

Spermicidal activity

***Incubation of sperm with P. hysterophorus* and *A. scholaris* extract**

Incubation tubes were prepared in duplicate by adding 100 µl of sperm suspension and 100 µl of respective concentrations of extracts or control solution and incubated at 37ºC. Control experiments were performed in parallel using equivalent amounts of solvent without plant extracts. At the end of each incubation period, 10 µl of the sperm suspension was pipetted into center of a cleaned, prewarmed microscopic slide and covered with a 22 × 22 mm^2 coverslip. The slide was then transferred to heated microscope stage (37ºC) and photographed. Briefly the motility of sperms was observed under 40x magnification of motic digital microscope with image processing software and CCD colored camera attached to a computer assembly. Animal Husbandry Department, RDDC, Kota provided facility.

Results and Discussion

Antimicrobial activity

All the four extracts at 4 concentrations, viz. 200, 400, 600, 800 ppm were screened for antibacterial and antifungal activity against *S. aureus*, *P. aeruginosa*, *E. coli* and fungi *A. niger*, *C. albicans* and *F. oxysporum*, respectively. Wider the zone of inhibition most sensitive is the compound for a particular species of bacterial or fungal culture used.

Antibacterial study indicated that extract 1 showed wider zone of inhibition, viz. 9.5, 9.0 and 6.2 at 800 ppm concentration for bacteria *S. aureus*, *E. coli*, *P. aeruginosa* (Plate 2A) in comparison to extract 2 which showed 8.0, 8.2 and 5.2 mm zone of inhibition at same concentration as mentioned in Table 1.

Maximum zone of inhibition was measured for extract 4 of *A. scholaris* at 800 ppm concentration. It was measured 12.5 mm for *S. aureus*, 13.0 mm for *E. coli* and 13.0 mm for *P. aeruginosa* as mentioned in Table 2 and shown by Plate 2B while for extract 3 it was measured 10.0 mm for *S. aureus*, 10.5 mm for *E. coli*. and 11.5 mm for *P. aeruginosa* mentioned in Table 2.

Table 1—Antibacterial activity of *Parthenium hysterophorus* (Extract 1, 2)

		Mean value of area of inhibition in mm IZ (AI))		
Bacteria →		*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*
Gentamycin (Control) →		20	22	16
Extract	*Conc. (ppm)* ↓			
1	200	8.5 (0.43)	8.0 (0.36)	5.5 (0.34)
1	400	8.8 (0.44)	8.2 (0.37)	5.8 (0.36)
1	600	9.0 (0.45)	8.5 (0.38)	6.0 (0.38)
1	800	9.5 (0.48)	9.0 (0.40)	6.2 (0.39)
2	200	7.0 (0.35)	7.0 (0.31)	4.5 (0.28)
2	400	7.2 (0.36)	7.8 (0.35)	4.8 (0.30)
2	600	7.8 (0.39)	8.0 (0.36)	5.0 (0.31)
2	800	8.0 (0.40)	8.2 (0.37)	5.2 (0.33)

IZ= Inhibition area excluding diameter of disk
AI= Activity index= Inhibition area of sample/inhibition area of standard

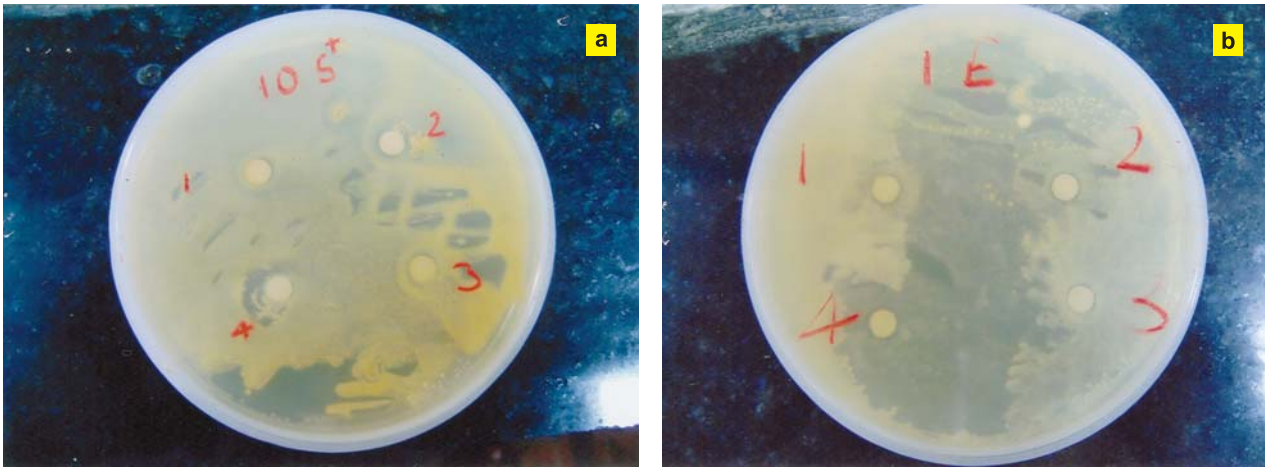

Plate 2—A. Antibacterial activity of extract 2 of *P. hysterophorus*; B. Antibacterial activity of extract 4 of *A. scholaris*

Antifungal study indicated that zone of inhibition of extract 1 was found to be more than extract 2 at 800 ppm concentration, viz. 9.2, 6.5 and 9.5 mm for fungi *C. albicans*, *A. niger*, *F. oxysporum* as given in Table 3 and Plate 3A while extract 2 showed 8.0, 6.0, 8.0 mm of zone of inhibition at same concentration as mentioned in Table 3.

Table 2—Antibacterial activity of *Alstonia scholaris* (Extract 3, 4)

		Mean value of area of inhibition in mm IZ (AI))		
Bacteria →		*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*
Gentamycin (Control) →		20	22	16
Extract	*Conc (ppm)* ↓			
3	200	9.0 (0.45)	9.0 (0.41)	10.0 (0.63)
3	400	9.2 (0.46)	9.8 (0.45)	10.5 (0.66)
3	600	9.7 (0.49)	10.0 (0.45)	11.0 (0.69)
3	800	10.0 (0.50)	10.5 (0.48)	11.5 (0.72)
4	200	11.0 (0.55)	11.0 (0.50)	12.0 (0.75)
4	400	11.5 (0.58)	11.5 (0.52)	12.5 (0.78)
4	600	12.0 (0.60)	12.6 (0.57)	12.8 (0.80)
4	800	12.5 (0.63)	13.0 (0.60)	13.0 (0.81)

IZ= Inhibition area excluding diameter of disk

AI= Activity index= Inhibition area of sample/Inhibition area of standard

Zone of inhibition was noticed maximum with extract 4 of *A. scholaris* at 800 ppm. It was calculated to be 15.0 mm for *C. albicans*, 16.0 mm for fungi *A. niger* and 14.0 mm for *F. oxysporum* as represented by Table 4 and Plate 3B. While for extract 3 it was measured 13.0 mm for *Candida albicans*, 14.0 mm for fungi *A. niger* and 12.0 mm for *F. oxysporum* at 800 ppm concentration as shown in Table 4.

Table 3—Antifungal activity of *Parthenium hysterophorus*. (Extract 1, 2)

		Mean value of area of inhibition in mm IZ (AI)		
Fungi →		*Candida albicans*	*Aspergillus niger*	*Fusarium oxysporum*
Mycostatin (Control) →		15	17	16
Extract	*Conc. (ppm)* ↓			
1	200	8.5 (0.57)	5.5 (0.32)	8.2 (0.51)
1	400	8.7 (0.58)	6.0 (0.35)	8.4 (0.53)
1	600	9.0 (0.6)	6.0 (0.35)	9.0 (0.56)
1	800	9.2 (0.61)	6.5 (0.38)	9.5 (0.60)
2	200	6.5 (0.43)	5.0 (0.29)	7.0 (0.44)
2	400	7.0 (0.46)	5.0 (0.29)	7.0 (0.44)
2	600	7.5 (0.50)	5.4 (0.32)	7.5 (0.47)
2	800	8.0 (0.53)	6.0 (0.35)	8.0 (0.50)

IZ= Inhibition area excluding diameter of disk

AI= Activity index= Inhibition area of sample/inhibition area of standard

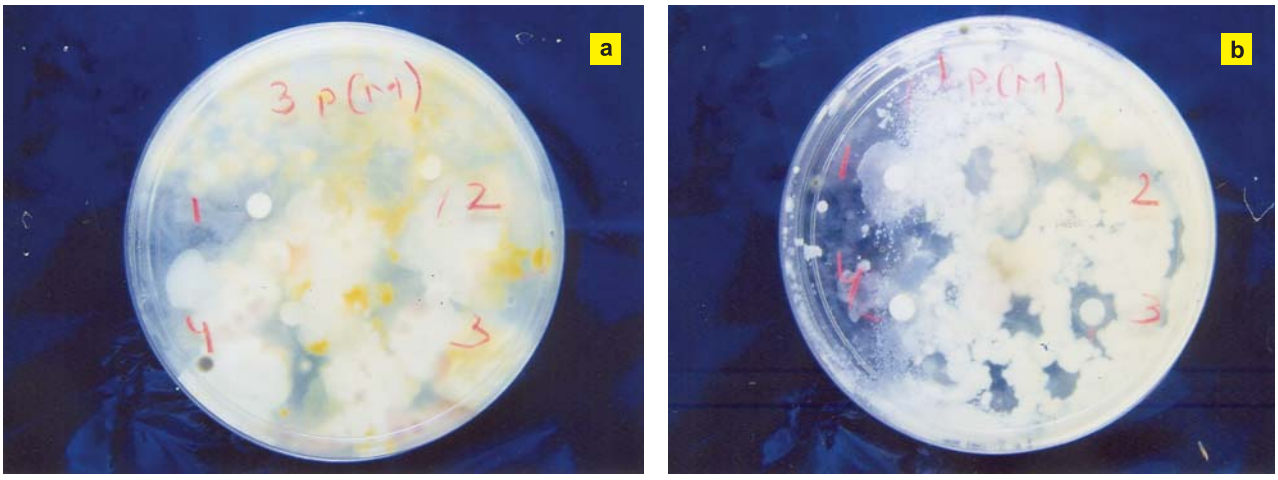

Plate 3—A. Antifungal activity of extract 2 of *P. hysterophorus*; B. Antifungal activity of extract 4 of *A. scholaris*

Table 4—Antifungal activity of *Alstonia scholaris* (Extract 3, 4)

Fungi →		Mean value of Area of inhibition in mm IZ (AI)		
		Candida albicans	*Aspergillus niger*	*Fusarium oxysporum*
Mycostatin (Control) →		15	17	16
Extract	*Conc. (ppm)* ↓			
3	200	11.0 (0.73)	11.5 (0.68)	9.8 (0.61)
3	400	12.0 (0.8)	12.0 (0.71)	10.0 (0.63)
3	600	12.5 (0.83)	13.0 (0.76)	11.2 (0.70)
3	800	13.0 (0.87)	14.0 (0.82)	12.0 (0.75)
4	200	12.0 (0.80)	15.0 (0.88)	12.0 (0.75)
4	400	13.5 (0.9)	15.5 (0.91)	13.0 (0.81)
4	600	14.0 (0.93)	16.0 (0.94)	13.5 (0.84)
4	800	15.0 (1.0)	16.0 (0.94)	14.0 (0.88)

IZ= Inhibition area excluding diameter of disk
AI= Activity index= Inhibition area of sample/inhibition area of standard

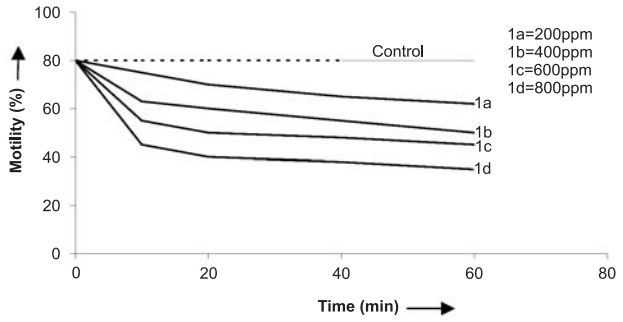

Fig. 1—Spermicidal activity of extract 1 of *Parthenium hysterophorus*

Spermicidal activity

Reduction in sperms motility was observed after adding the plant extract to semen and too it was dose and time dependent. It is recorded that with extract 1 (800 ppm) sperm motility has been reduced to 45% in 10 minutes. At 600 ppm, it was reduced to 55%. At 400 and 200 ppm concentration it was reduced to 63 and 75% in 10 minutes which is shown by Figure 1. Extract 2 at 800 ppm concentration, depressed sperm motility to 50% in 10 minutes. At 600 ppm concentration it reduces sperm motility to 60% in

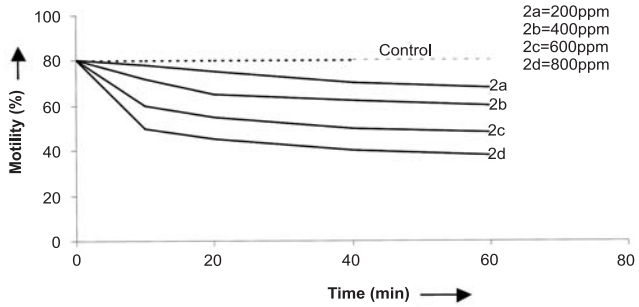

Fig.2—Spermicidal activity of extract 2 of *Parthenium hysterophorus*

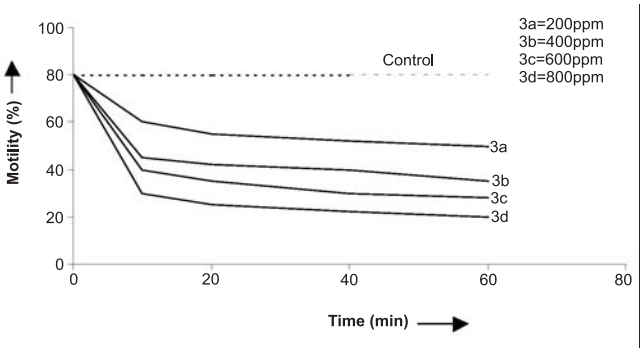

Fig. 3—Spermicidal activity of extract 3 of *Alstonia scholaris*

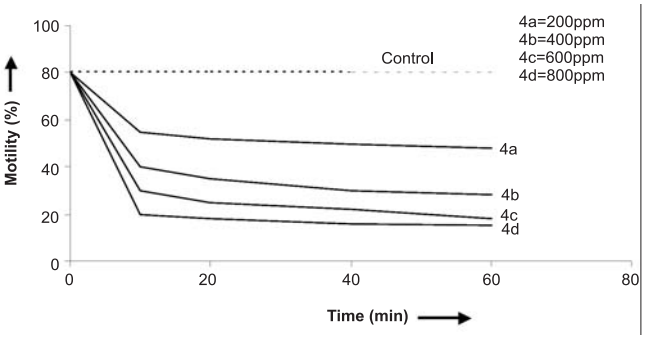

Fig. 4—Spermicidal activity of extract 4 of *Alstonia scholaris*

10 minutes. At 400 ppm concentration sperm motility was decreased to 72% and at 200 ppm concentration; it was reduced to 78%.

Both the extracts of *P. hysterophorus* after 60 min showed a further 5-10% decrease in sperm motility. In the same time extract 3 of *A. scholaris* at 800 ppm concentration reduces sperm motility to 30%. At 600, 400, 200 ppm concentration of above extract, sperm motility was reduced to 40, 45 and 60%, respectively in 10 minutes represented by Figure 2. Extract 4, at highest concentration (800 ppm) reduced sperm motility significantly to 20% in 10 minute. At 600, 400, 200 ppm concentration of above extract, sperm motility was reduced to 30, 40, 55% as represented by Figure 3 and Figure 4.

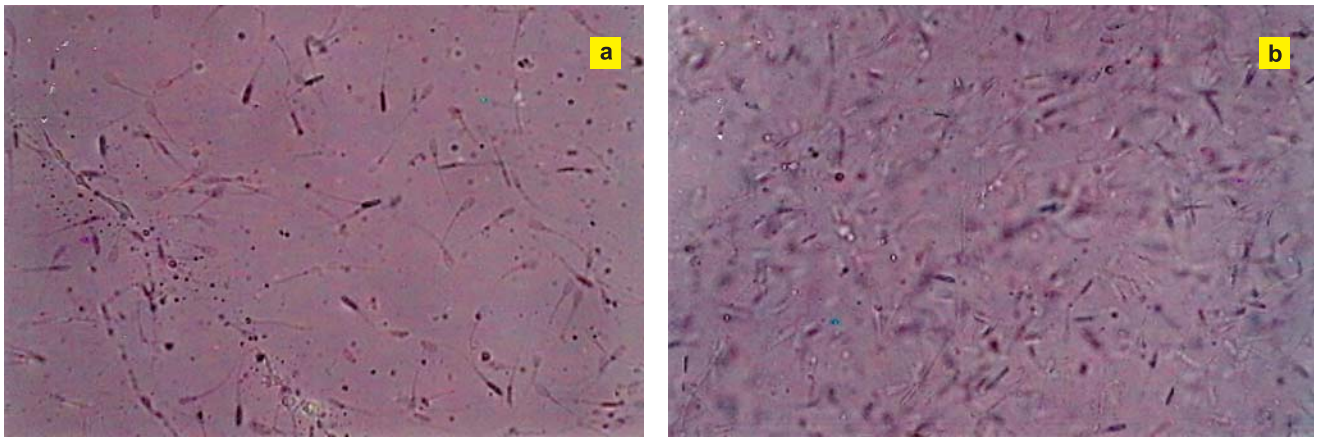

Plate 4—Spermicidal activity of *P. hysterophorus* (A) and *A. scholaris* (B)

Both the extracts of *A. scholaris* after 60 minutes showed a further 5-10% decrease in sperm motility. In HF cattle however, even the highest concentration of extracts obtained from aerial parts of *P. hysterophorus* and bark of *A. scholaris* with longest duration of exposure (60 min) was not found to be capable of completely suppressing sperms motility.

These results obtained in present study indicate that the extract 1 and 2 showed good activity against *S. aureus*, *E. coli* and fungi *C. albicans*, *F. oxysporum* as well. The extract 3 and 4 showed good activity against all bacteria and fungi used under test. It is also indicated that *A. scholaris* and *P. hysterophorus* are capable of inhibiting motility of HF cattle sperm. Extract 1 and 2 of *P. hysterophorus* reduces sperm motility to 35 and 40 %, respectively. Extract 3 and 4 of *A. scholaris* decreases sperm motility to 30 and 20%. Therefore, all the four extracts were capable of immobilizing sperms but to different degrees (Plate 4A & B).

Conclusion

The petroleum ether extract of *P. hysterophorus* and dichloromethane–ether-methanol (1:1:1) extract of *A. scholaris* showed good antimicrobial and spermicidal activity. Finally, in perusal of our study it is concluded that extracts obtained from bark of *A. scholaris* has shown maximum antimicrobial and spermicidal activity. The chemical constituents of the extract which is responsible for this effect would be investigated for these pharmacological activities in our phytochemical studies yet to be performed in future.

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