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Study of Antimicrobial Profile of Various Cow Dung Extracts Against *Escherichia coli* and *Staphylococcus* species

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ABSTRACT

Now a days due to increase in antimicrobial resistance, there is a quest for alternative to synthetic antimicrobial drugs. As cow dung is a natural product with reported antimicrobial activity. In the present study 30 cow dung samples from different cattle breeds in and around Bhubaneswar, Odisha were tested against the *Escherichia coli* and *Staphylococcus species* isolated from milk samples. Methanol, Ethanol and Aqueous extracts of cow dung were prepared and its antimicrobial properties were assessed as per Disk Diffusion method. From the studies conducted it was revealed that the Methanol extract of cow dung exhibits more antimicrobial activity than the Ethanol and Aqueous extracts of cow dung.

HIGHLIGHTS

- Cow dung has antimicrobial activity against *Escherichia coli* and *Staphylococcus* species.
- Methanol extracts of cow dung possess antimicrobial activity higher than ethanol and aqueous extracts.

Keywords: Antimicrobial resistance, Aqueous extract *Escherichia coli,* Ethanol extract, Methanol extract, Staphylococcus species

Mastitis is an inflammatory condition of mammary gland most often caused by bacterial intramammary infection. The principal pathogens causing mastitis are pathogenic *E. coli* and *Staphylococcus spp*. It adversely affects milk production and causes widespread economic losses. In the present study *E. coli* and *Staphylococcus spp.* were isolated from mastitis milk samples and were processed as per routine method of microbial isolation.

The use of antimicrobials over long period has triggered the development of multidrug resistant strains, which has resulted in the use of increasing dose of antimicrobials, causing the danger of increasing amount of drug residues in the milk, a potential biohazard. So, an alternative should be used in replacement of these antibiotics. In present study cow dung extracts were obtained by using solvents like ethanol, methanol and distilled water are used as an alternative to antibiotics. It was even reported that cow dung possess antimicrobial properties as per the reasearchers (Eja *et al*., 2011; Waziri and Suleiman, 2012; Omojowo and Omojasola, 2013; Patel *et al*., 2015; Rajeswari *et al*., 2016; Atnafie *et al*., 2017; Munshi *et al*., 2019; Manishimwe *et al*., 2021).

MATERIALS AND METHODS

Sample Collection

Total of 30 number of cow dung samples were collected

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from different breeds of cattle present in and around Bhubaneswar, Odisha.

Escherichia coli and *Staphylococcus spp*. were isolated from the milk samples collected from the cows with a history of mastitis as per the method of Mallik (1967) by using EMB agar and MSA agar. All the media and antibiotic discs used in the present study were obtained from HI-Media Laboratories, Mumbai.

Preparation of cow dung extracts (Methanol, Ethanol and Aqueous extracts) and discs

100 ml of Methanol, Ethanol and Distilled water were added to 10 g of powdered cow dung in different conical flasks and were kept in a rotary shaker for 3 days. Then the extract was filtered in to a small conical flask by using Whatman No 1 filter paper. The empty disks were impregnated with 50 µl of the Methanol, Ethanol and Aqueous extract of cow dung and were dried in oven, until the disks are completely saturated with extracts, this process was repeatedly done (Rajeswari *et al*., 2016).

Antibiotic susceptibility test

Antibiotic susceptibility test was carried out as per Kirby-Bauer (1996) disc diffusion method using MHA agar and different antibiotic disks obtained from Hi-media laboratories, Mumbai.

RESULTS AND DISCUSSION

On microbial investigation and analysis of antimicrobial action of different types of cow dung extracts on *E. coli* and *Staphylococcus sp*ecies, methanol extracts were found to exhibit high antimicrobial activity as compared to ethanol, aqueous extracts and some antibiotics like Gentamycin, ciprofloxacin, cefuroxime, nitrofurantoin and oxytetracycline were shown in (Table 2). Analysis of 30 cow dung samples collected from desi cattle (15), Jersey(3), HF(6) and HF cross(6) in and around Bhubaneswar, Odisha were shown in the (Table 1).

Methanol extracts of *desi* cattle dung has shown high zone of inhibition followed by HF, Jersey and HF cross against *E. coli*. Methanol extracts of *desi* cattle (21.6±1.226) was statistically ($p<0.05$) higher as compared to the HF (13.67±), Jersey (10.67±0.667) and HF cross (7.67±0.882). Methanol extracts of HF has significant ($p<0.05$) difference when compared to *desi* cattle and HF cross. Methanol extracts of Jersey cattle has significant ($p<0.05$) difference when compared to *desi* cattle. Methanol extracts of HF cross has significant ($p<0.05$) difference when compared to *desi* cattle and HF. Methanol extracts of *desi* cattle dung has shown high zone of inhibition followed by HF, Jersey and HF cross against *Staphylococcus sp.* Methanol extracts of *desi* cattle (20.87±0.975) has significant ($p<0.05$) difference when compared to HF (15.83±0.477), Jersey (14.0±0.00) and HF cross (10.83±1.138). Methanol

Table 1: Depicts the zone of inhibition of various cow dung extracts against *E. coli* and *Staphylococcus* sp.

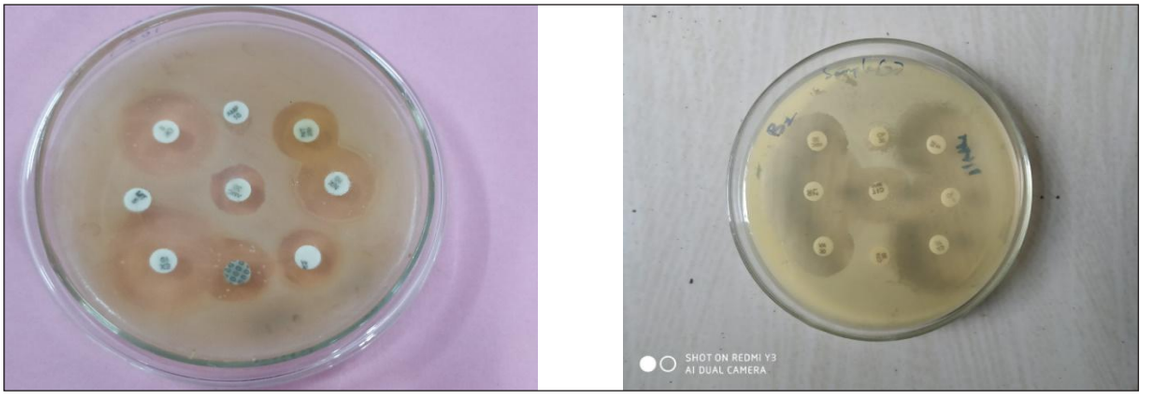
Sl. No.	Name of the breed	No. of samples collected (n)	Type of extract	Mean ± standard error values of zone of inhibitions	
				Against *E. coli*	Against *Staphylococcus sp.*
1	Desi cattle	15	Ethanol	5.6±0.559	5.2 ±.0763
			Aqueous	2.53±0.559	3.73±.483
			Methanol	$21.6±1.226^{bcd}$	$20.87±0.975^{bcd}$
2	HF	6	Ethanol	5.5±1.118	6.33±1.706
			Aqueous	1.5±0.719	4.0±1.183
			Methanol	$13.67±0.558^{ad}$	$15.83±0.477^{ad}$
3	Jersey	3	Ethanol	3.0±0.557	9.0±0.577
			Aqueous	2.67±0.333	5.0±0.577
			Methanol	$10.67±0.667^{a}$	$14.0±0.00^{a}$
4	HF cross	6	Ethanol	5.5±0.847	4.67±1.145
			Aqueous	3.0±1.155	4.0±1.211
			Methanol	$7.67±0.882^{ab}$	$10.83±1.138^{ab}$

Values having different superscript differ significantly ($p<0.05$).

Table 2: Depicts the comparative analysis of mean diameter of zone of inhibition of antibiotics with cow dung extracts

Sl. No.	Name of the breed	No. of samples collected (n)	Type of extract	Mean ± standard error values of zone of inhibitions	
				Against *E. coli*	Against *Staphylococcus sp.*
1	Desi cattle	15	Methanol	21.6 ± 1.226^{bcd}	20.87 ± 0.975^{bcd}
			Antibiotics	15.93 ± 0.825^{bd}	17.93 ± 1.560
2	HF	6	Methanol	13.67 ± 0.558^{ad}	15.83 ± 0.477^{ad}
			Antibiotics	11.83 ± 1.721^{ad}	13.83 ± 1.851
3	Jersey	3	Methanol	10.67 ± 0.667^{a}	14.0 ± 0.00^{a}
			Antibiotics	16.67 ± 3.667^{d}	14.33 ± 2.33
4	HF cross	6	Methanol	7.67 ± 0.882^{ab}	10.83 ± 1.138^{ab}
			Antibiotics	6.0 ± 0.966^{abc}	14.5 ± 2.50

Values having different superscript differ significantly ($p<0.05$)



(A) **(B)**

Fig. 1: Zone of inhibition against *E. coli* **(A)** and *Staphylococcus sp.* **(B)** using different antibiotic discs

(A) Aqueous extracts **(B)** Methanol extracts **(C)** Ethanol extracts

Fig. 2: Zone of inhibition against *E. coli* using cow dung extracts

(A) Aqueous extracts **(B)** Methanol extracts **(C)** Ethanol extracts

Fig. 3: Zone of inhibition against *Staphylococcus* sp. using cow dung extracts

extracts of HF has significant ($p<0.05$) difference when compared to *desi* cattle and HF cross. Methanol extracts of Jersey cattle has significant ($p<0.05$) difference when compared to *desi* cattle. Methanol extracts of HF cross has significant ($p<0.05$) difference when compared to *desi* cattle and HF.

CONCLUSION

From the present study it was revealed that methanol extracts was found to be highly effective against *E. coli* and *Staphylococcus spp.* than ethanol and aqueous extracts. Methanol extracts were showing high antimicrobial activity when compared to some antibiotics also. So it was concluded that methanol extracts of cow dung were used controlling *E. coli* and *Staphylococcus* sp. infections.

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