

Study the antibacterial activity of cow dung extract against pathogenic microorganisms

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ABSTRACT:

In India Cow is called as Gomata by Hindu people because of their importance. Different product has been obtained from cow and all the product have some medicinal effect. Cow dung is the excreted undigested waste part of cow. Cow dung possess antimicrobial activity against pathogenic microorganism because of the secretion of the antimicrobial metabolite by the microflora present in the cow dung. For this study four cow dung samples were collected from different area of Guwahati. All the samples shows partial antimicrobial activity but Indian cow dung shows highest antimicrobial activity against *E.coli* and *Klebshiella sp.* The result of the study reveals that cow dung extract have beneficial antimicrobial activity which will help to formulate new drugs in near future.

Keyword : Cow dung , Microflora, *E.coli*

INTRODUCTION:

In India Cow is called as Gomata by Hindu people because of their importance. Panchgavya, is organic product composed by five major substances obtained from cow: urine, milk, ghee, curd and dung. All the five products have some medicinal properties. The treatment of this five product is known as panchagavya therapy or cow pathy. Cowpathy is an old system of medicine mentioned in ancient Indian literature (Ayurveda) as Panchagavya Chikitsa[1]. Cow dung is one the product which is traditionally used as organic fertilizer in agricultural area. Cow dung have different properties which increases the mineral status of soil, inhibit the growth of pathogenic microbes and stimulate the plant growth. It is used as a mosquito repellent through smoke generated from the burnt cow dung and subsequently ashes are applied for cleaning kitchen utensils from ancient time [4]. Cow dung can act as a raw material in biogas generation as well as a co product in agriculture, such as manure, biofertilizer, bio pesticide, pest repellent and as a source of energy [4].

Cow dung is the excreted part of the cow which contain undigested residue of plant matter which has passed through the gastrointestinal part of the cow . Cow is a herbivores' so cow dung is comprised of organic matter including fibrous material and also contain carbon, nitrogen, hydrogen, oxygen, phosphorus, etc. Because of these mineral compound increases the fertility of the soil. Cow dung also contains abundant number microflora like *bacilli*, *lactobacilli*, *Enterococcus* and some identified and unidentified fungi and yeasts. According to Ware et al. (1988), lower part of the gut of the cow contains various microorganisms,

including *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus acidophilus*, *B. subtilis*, *Enterococcus diacetylactis*, *Bifidobacterium* and yeasts (commonly *Saccharomyces cerevisiae*) having probiotic activity [3]. From various study reveals that the fresh cow dung has antimicrobial activity because of the secretion of anti microbial metabolite which is secreted by different microflora present in the cow dung.

With this view the aim of these study to determine the antibacterial activity of cow dung against pathogenic organism.

METHODOLOGY :-

Sample Collection :

Different cow dung (Indian cow, *Holstein*) were collected in and around Beltola, Bashishtha and Borbari region of Guwahati.

Culture collection :

Two pathogenic strains namely *Escherichia coli* and *Klebsiella pneumonia* samples were collected from down town hospital, Guwahati..

Powdered cow dung :

- 1000g of cow dung from Indian cow was collected and shadow dried for 5 day. The dried cow dung was powdered and had a net weight of 250g.
- 1000g of cow dung from *Holstein* was taken and shadow dried for 5 day. The dried cow dung was then powdered. The powdered material had a net weight of 250g.

Preparation of dung Extracts :

Acetone Extract

100 ml of acetone was added in 10 g of powdered different cow dungs (Indian cow, *Holstein*) in a conical flask and it was kept in a shaker incubator for 3 day. The extract was then filtered using Whatman No 1 filter paper and stored in a vial for future use.

Ethanol extract

100 ml of ethanol was added in 10 g of powdered different cow dungs (Indian cow, *Holstein*) in a conical flask and it was kept in a shaker incubator for 3 day. The extract was then filtered using Whatman No 1 filter paper and stored in a vial for future use.

Preparation of the disc containing cow dung extract :

The empty discs were impregnated with acetone and ethanol extracts of cow dung from Indian cow and Holstein cow dung separately and dried for 1 minute. This process was repeated until the disc was completely saturated with the extract was taken in the Petri dish. The disc was then used to study the antimicrobial activity of cow dung extracts against the bacterial and fungal strain

Study of effect of selected commercial Antibiotics against test organism

Antibiotic sensitivity of test organism used in the study was carried out by disc diffusion method. The antibiotics used for the study were Amikacin, Ofloxacin, Penicillin G, Vancomycin. The collected bacterial and fungal strains were received in nutrient broth. 18hrs old received test organisms were used for this study. 20ml of Muller Hinton Agar medium were poured in sterile Petri plates and allowed to solidify. The bacterial sample was spread over the medium with the help of the L shaped spreader. The antibiotic discs were dispensed with the help of a sterilized fore shape. Then the plates were incubated at 37 C. for 24 hours. After incubation period the zone of inhibition were observed and measured.

Antibacterial plate assay of cow dung extracts :

Antibacterial plate assay of acetone extract of cow dung

The inoculums of the test microorganisms were received in nutrient broth. 18hrs old received test organisms were used for this study. 20ml of Muller Hinton Agar medium were poured in sterilized petri plates and allowed to solidify. The bacterial sample was spread over the medium with the help of the L shaped spreader. The antibiotic discs containing acetone extract were dispensed with the help of a sterilized foreshape. Then the plates were incubated at 37 C. for 24 hours. After incubation period the zone of inhibition were observed and measured.

Antibacterial plate assay of ethanol extract of cow dung

The inoculums of the test microorganisms were received in nutrient broth. 18hrs old received test organisms were used for this study. 20ml of Muller Hinton Agar medium were poured in sterilized petri plates and allowed to solidify. The bacterial sample was spread over the medium with the help of the L shaped spreader. The antibiotic discs containing ethanol extract were dispensed with the help of a sterilized fore shape. Then the plates were incubated

at 37 C. for 24 hours. After incubation period the zone of inhibition were observed and measured.

RESULTS

Antibacterial plate assay of Indoor and Outdoor grazing Cow Dung individually :

Indoor grazing Cow dung

The study of antibacterial activity of ethanolic and methanolic extract of indoor grazing cow dung were carried out by disc diffusion method against different test collected bacterial strains. The ethanolic and acetone extracts of cow dungs showed zone of inhibition against different test bacterial strain to varying degrees.

The ethanolic extract of holstein cow showed highest zone of inhibition against E.coli (16mm). The acetone extract of indian cow dung showed lowest zone of inhibition against E.coli.

Table1 : Antibacterial activity of ethanolic and acetone extract of Indoor Grazing cow dung

Extracts	Klebsiella. Spp against Indian Cow Dung	Klebsiella. Spp against Holstein Cow Dung	E.coli against Indian Cow Dung	E.coli against Holstein Cow Dung
Ethanol extract	5mm	10mm	14mm	16mm
Acetone extract	8mm	11mm	5mm	6mm

Outdoor grazing cow dung

The study of antibacterial activity of ethanolic and methanolic extract of outdoor grazing cow dung were carried out by disc diffusion method against different test collected bacterial

strains. The ethanolic and acetone extracts of cow dungs showed zone of inhibition against different test bacterial strain to varying degrees .

The ethanolic extract of Indian cow dung showed highest zone of inhibition against Klebsiella spp. (17mm). The acetone extract of indian cow dung and holstein cow dung showed no zone of inhibition against E.coli.

Table 2: Antibacterial activity of ethanolic and acetone extract of outdoor grazing cow dung

Extracts	Klebsiella. Spp against Indian Cow Dung	Klebsiella. Spp against Holstein Cow Dung	E.coli against Indian Cow Dung	E.coli against Holstein Cow Dung
Ethanol extract	17mm	13mm	10mm	5mm
Acetone extract	10mm	NZ	NZ	NZ

Key : NZ- No zone

Antibacterial activity of ethanol extract of outdoor and indoor grazing cow dung :



E. coli



Klebsiella spp.

Figure 1: Antibacterial activity of ethanolic extract of indian and holestien cow dung (Outdoor grazing)against test bacterial strains



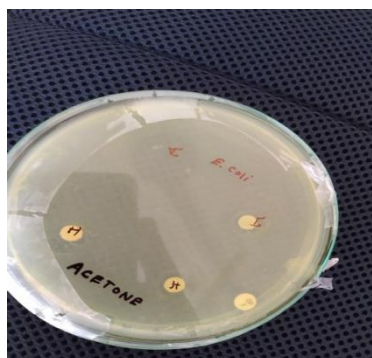
E.coli



Klebsiella spp.

Figure 2: Antibacterial activity of ethanolic extract of Indian and Holstein cow dung (Indoor grazing) against test bacterial strains.

Antibacterial activity of Acetone extract of outdoor and indoor grazing cow dung :



E. coli



Klebsiella spp.

Figure 3: Antibacterial activity of acetone extract of Indian and Holstein cow dung (outdoor grazing) against test bacterial strains



E. coli



Klebsiella spp.

Figure 4: Antibacterial activity of acetone extract of Indian and Holstein cow dung (indoor grazing) against test bacterial strains

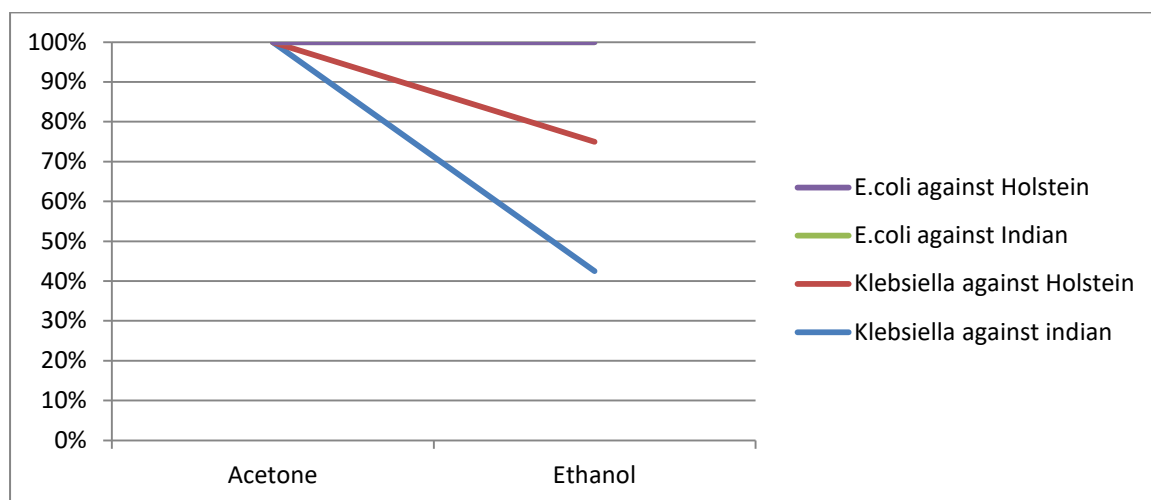


Figure 5 : Antibiotic sensitivity test of outdoor grazing cow dung extracts

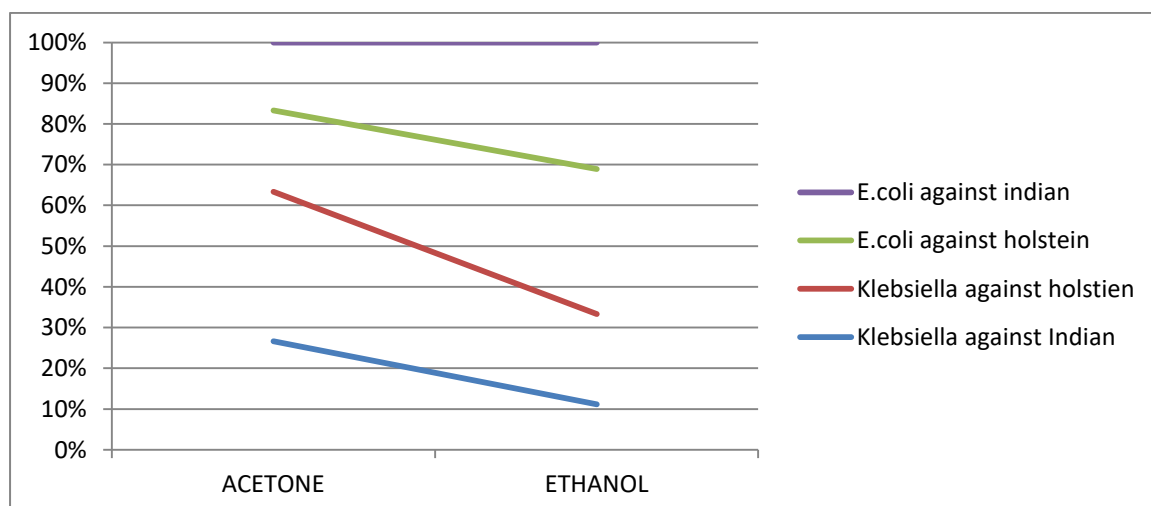


Figure6 : Antibiotic sensitivity test of indoor or grazing cow dung extracts

Discussion :

The present study was conducted to determine the antimicrobial activity of cow dung extract against pathogenic organism. The sample Indian cow and Holstein Cow dung was collected in the early morning from different area of Guwahati. Cow dung was collected kept in shed area for dried. After drying cow dung powder extract was prepared by adding two solvent such as Acetone and ethanol and it was kept in rotary shaker for 3 days. Extract was filtered using what man no.1 filtered paper and stored for further use. The antimicrobial activity of different cow dung extract was determine by using disc diffusion method against *Klebsiella sp* and *E.coli*. In this study shows Indian cow dung extract had more antimicrobial activity

than Holstein. Similar result observed in Rajeswari et.al research where Indian cow dung extract had superior antimicrobial activity than other cow dung extract sample.

CONCLUSIONS :

The findings of this study concluded that that all the cow dung samples shows partial antimicrobial activity against *E.coli and Klebsiella sp.* But Indian cow dung extracts possess superior antimicrobial activity than Holstein cow dung and that shown antimicrobial property against both the test microorganisms . In future from cow dung extract, drugs can be formulated which will have potential effect of several disease.

REFERENCES :

1. Rajeswari S, Poongothai E, Hemalatha N. Antimicrobial activities of cow dung extracts against human pathogens . *International Journal of Current Pharmaceutical Research* 2016;8(4):9-12.
2. Waziri M and. Suleiman JS. Analysis of Some Elements and Antimicrobial Activity of Evaporated Extract of Cow Dung Against Some Pathogens. *Journal of Scientific Research* ,2013;5(1):135-141
3. Girjia D, Deepa K, Xavier F, Antony I, Shidhi PR. Analysis of cow dung microbiota– A metagenomic approach. *International Journal of Biotechnology* ,2013;12:372-378
4. Munshi SK, Roy J and Noor R. Microbiological investigation and determination of the antimicrobial potential of cow dung samples. *Stamford Journal of Microbiology*, 2018;8 (1):34-37
5. Sharma B and Singh M. Isolation and characterization of bacteria from cow dung of desi cow breed on different morpho-biochemical parameters in Dehradun, Uttarakhand , India. *International Journal of Advances in Pharmacy , Biology and Chemistry*, 2015;4(2):276-280
6. Shrivastava S., Mishra A, Pal A. Cow dung: A boon for antimicrobial activity. *Life Science Leaflets*. 2014; 55: 152.
7. Hao L, Wang X, Zhang K, Xu Y, Zhou L, Li G. 2014. Identification and nematicidal activity of bacteria isolated from cow dung. *Ann Microbiol.* 64: 407-411. 33
8. Nargis A, Begam MF, Alam S, Shah Alam (2006); Inhibitory effect of different plant extract , Cow dung & cow urine on conidial germination of *Bipolariss orokiniana* .*Journal of Bio science*, 2006;14:87-92.

9. Garg SK, Anita Bhatnagar. Effect of different doses of organic fertilizer (cow dung) on pond productivity and fish biomass in still water ponds. *Journal of Applied Ichthyology* 1999;15:10-8.
10. Garg SK, Anita Bhatnagar. Effect of different doses of organic fertilizer (cow dung) on pond productivity and fish biomass in still water ponds. *Journal of Applied Ichthyology* 1999;15:10-8.
11. Gupta KK, Rana D. 2016. Isolation and evaluation of cow dung bacteria for their antimicrobial potential. *Biotechnology International*, 2016;9(2): 47-54.
12. Dharma K, Rathod R., Chauhan R.S, Tommar S. Panchgavya (Cow Pathy.) : An Overview. *International Journal of Cow Science*, 2005; 1(2), 26-29
13. Achilya GS, Kotagale NR, Wadodkar SG, Dorle AK. Hepatoprotective activity of Panchagavya Ghrita against CCl₄ induced hepatotoxicity in rats. *Indian Journal of Pharmacology* 2003; 35: 308-311
14. Sathasivam,AK, Muthuselvam M. 2010. Antimicrobial activities of cow urine distillate against some clinical pathogens. *Global J. Pharmacol.* 4 (1): 41-44. 32.