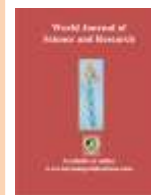




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Research Article

Zoology

ANTIMICROBIAL ACTIVITY OF ANIMAL WASTE (JERSEY COW DUNG)

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ABSTRACT

In the present study to investigate the antimicrobial activity in animal waste (Cow dung). Antibacterial activity showed about methanol extracts (7.50mm), hydro alcohol extracts (12.00mm), aqueous extracts (6.50mm) and Std. (14.25mm) zone against the test organisms of *Escherichia coli*. Methanol extracts (5.75mm), hydro alcohol extracts (4.50mm), aqueous extracts (5.50mm) and Std. (7.75mm) zone against the test organisms of *Staphylococcus aureus*. Methanol extracts (6.00mm), hydro alcohol extracts (5.50mm), aqueous extracts (4.50mm) and Std. (7.50mm) zone against the test organisms of *Bacillus subtilis*. Methanol extracts (6.75mm), hydro alcohol extracts (6.25mm), aqueous extracts (5.50mm) and Std. (10.75mm) zone against the test organisms of *Pseudomonas aeruginos*. Antifungal activity showed about methanol extracts (10.75mm), hydro alcohol extracts (10.75mm), aqueous extracts (11.50mm) and Std. (8.75mm) zone against the test organisms of *Candida albicans*. Methanol extracts (5.50mm), hydro alcohol extracts (4.12mm), aqueous extracts (5.25mm) and Std. (5.25mm) zone against the test organisms of *Aspergillus flavus*. Methanol extracts (4.50mm), hydro alcohol extracts (5.25mm), aqueous extracts (4.75mm) and Std. (5.75mm) zone against the test organisms of *Aspergillus niger*

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INTRODUCTION

In India cattle's rearing is a tradition in the country and intimately limited to the agricultural economy. Different products obtained from cow milk, ghee, curd, urine, and dung are used widely in a number of Ayurveda formulations. Cow dung is traditionally used as organic fertilizer in Indian sub-continental farming for centuries. The addition of cow dung increases the mineral status of soil, enhances the resistance of plant against pests and diseases; stimulate plant growth and other beneficial activities such as sulpho oxidation and phosphorus solubilization (Naskar Sethuraman and Ray, 2003).

The Hindu Vedas say that the cow is holy and should be worshiped. In India, cows are very important animal resources and are highly useful in agriculture and dairy industry (Jonker and Kohn, 2001). Panchagavya is a term used to describe five major substances, obtained from cow, which include cow's urine, milk, ghee, curd and dung. All the five products possess medicinal properties against many disorders. This kind of treatment is called Panchagavya therapy or cowpats. Cowpathy is an old system of medicine mentioned in ancient Indian literature (Ayurveda) as Panchagavya Chikitsa. The Ayurveda medicines of animal origin are mainly prepared from Panchagavya which boost up the body immune system and makes the body refractory to various diseases (Chauhan and Lokesh Singhal, 2006). Although some Indian literature mentioned the medicinal property of cow excretion, only a few were proved. Several useful properties of cow urine got confirmed by researchers patent also. But there is no report available on antimicrobial activity of cow dung (Rajeswar *et al.*, 2016).

Indian cow dung had possessed superior antimicrobial activity than cow dung. All the test microorganisms were sensitive to the Indian cow dung. Ethanol extract of the Indian cow dung had shown antimicrobial activity against the entire test organism. In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new antimicrobial substances from natural sources including animal waste.

MATERIALS AND METHODS

Collection of plant materials

The Jersey Cow waste were collected in December 2017 from Mattaiyanpatty, Pudukkottai district, Tamil Nadu, India. The Sheep waste makes a fine powder using grinder mixture. The powder materials were used for further studies.

Determination of antimicrobial activity

Antibiogram was done by disc diffusion method (NCCLS, 1993; Awoyinka *et al.*, 2007) using plant extracts. Petri plates were prepared by pouring 30 ml of NA /PDA medium for bacteria/fungi. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mins. The surfaces of media were inoculated with bacteria/fungi from a broth culture. A sterile cotton swab is dipped into a standardized bacterial/ fungi test suspension and used to evenly inoculate the entire surface of the Nutrient agar/PDA plate. Briefly, inoculums containing bacteria specie were spread on Nutrient agar plates and fungus strains were spread on potato dextrose agar. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the methanol, hydro alcohol and aqueous extracts (50 μ l) were laid down on the surface of inoculated agar plate. The plates were incubated at 37°C for 24 h for the bacteria and at room temperature (30 \pm 1) for 24-48 hr. for yeasts strains. Each sample was tested in triplicate.

Statistical analysis

The results were presented as mean \pm SD. Data was statistically analyzed using student "t" test. P. values set as lower than 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Antibacterial activate

The different extract of Jersey Cow waste was screened against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginos* species of bacteria were evaluated using the standard agar disc diffusion method is used to detect the antibacterial activity of Jersey Cow waste. The solidified Nutrient agar plates were swapped with the test organism and the samples were impregnated. After the incubation the zone was measured. The antimicrobial activity of sheep waste extracts was detected by the indication of zone around the disc. The *in vitro* antibacterial activity of the Jersey Cow waste extract against these bacteria were qualitatively assessed by the presence of inhibition zones represented in the photographic plate 1. The inhibitory activities in culture media of the bacteria reported in Table 1 were comparable with standard antimicrobial viz. chloramphenicol.

After 24 hours of incubation, the inhibitory effect of Jersey Cow waste extracts (methanol, hydro alcohol and aqueous) were significant as compared to standard chloramphenicol. Zone of inhibition (ZoI) was used

as a measure for comparing bactericidal activity of these Jersey Cow waste extracts showed about methanol extracts (7.50mm), hydro alcohol extracts (12.00mm), aqueous extracts (6.50mm) and Std. (14.25mm) zone against the test organisms of *Escherichia coli*. Methanol extracts (5.75mm), hydro alcohol extracts (4.50mm), aqueous extracts (5.50mm) and Std. (7.75mm) zone against the test organisms of *Staphylococcus aureus*. Methanol extracts (6.00mm), hydro alcohol extracts

(5.50mm), aqueous extracts (4.50mm) and Std. (7.50mm) zone against the test organisms of *Bacillus subtilis*. Methanol extracts (6.75mm), hydro alcohol extracts (6.25mm), aqueous extracts (5.50mm) and Std. (10.75mm) zone against the test organisms of *Pseudomonas aeruginos*. Considering the advantage of the microbicidal activities of the sample. Among the various extract, methanolic extract of cow waste possess potential activity against gram positive and negative bacteria.

Table 1: Antibacterial activity of Jersey Cow waste extracts

Samples	<i>Escherichia coli</i> (mm)	<i>Staphylococcus aureus</i> (mm)	<i>Bacillus subtilis</i> (mm)	<i>Pseudomonas aeruginos</i> (mm)
Methanol extracts (50µl)	7.50±0.52	5.75±0.40	6.00±0.42	6.75±0.47
Hydro alcohol extracts (50µl)	12.00±0.84	4.50±0.31	5.50±0.38	6.25±0.43
Aqueous extracts (50µl)	6.50±0.45	5.50±0.38	4.50±0.31	5.50±0.38
Standard (30µl)	14.25±0.99	7.75±0.54	7.50±0.52	10.75±0.75

Values were expressed as Mean ± SD
Bacterial standard : Chloramphenicol



Escherichia coli *Staphylococcus aureus* *Bacillus subtilis* *Pseudomonas aeruginos*

Plate 1: Antibacterial activity of Jersey Cow waste extracts

A relatively limited number of reports exist on the presence of antagonistic activity amongst cow dung microorganisms and antimicrobial activity of cow dung as a whole. Cow dung possesses antiseptic and prophylactic or disease preventive properties. It destroys the microorganism that causes disease and putrefaction. Medicinal properties of five products collectively known as panchgavya obtained from cow namely milk, ghee, curd, dung and urine are supported by their use in the preparation of various herbal medicines (Pathak and Kumar 2003; Jarald *et al.* 2008).

Panchgavya therapy utilises these five products singly or in combination with herbal or mineral drugs for the treatment of many diseases like flu, allergies, colds, cough, asthma, renal disorders, gastrointestinal tract disorders, acidity, ulcer, wound healing, heart diseases, skin

infections, tuberculosis, chickenpox, hepatitis, leprosy and several other bacterial and viral infections. Panchgavya also seems to be beneficial even for the diseases such as cancer, acquired immunodeficiency syndrome (AIDS) and diabetes. Immunostimulatory, immunomodulatory and antiinflammatory effects of panchagavya are also being mentioned in Ayurveda (Jain *et al.* 2010; Sathasivam *et al.* 2010; Girija *et al.* 2013; Dhama *et al.* 2013). Recently, central nervous system action of panchgavya on spontaneous motor activity, muscle tone and pain has been determined in albino rats (Paliwal *et al.* 2013).

Antifungal activity

The different extract of Jersey Cow waste was screened against *Candida albicans*, *Aspergillus flavus* and *Aspergillus niger* species of fungal were evaluated using the standard agar disc diffusion method. The disc diffusion method is used to

detect the antimicrobial activity of Jersey Cow waste. The solidified Potato dextrose agar plates were swamped with the test organism and the samples were impregnated. After the incubation the zone was measured. The antifungal activity of Jersey Cow waste extracts was detected by the indication of zone around the disc. The *in vitro* antifungal activity of the Jersey Cow waste extract against these bacteria were qualitatively assessed by the presence of inhibition zones represented in the photographic plate 2. The inhibitory activities in culture media of the bacteria reported in Table 2 were comparable with standard antimicrobiotic viz. Fluconazole.

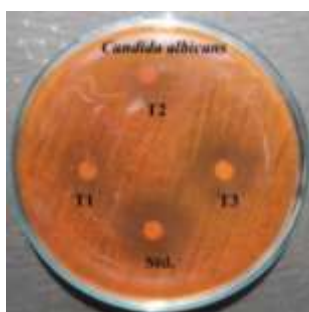
After 24 hours of incubation, the inhibitory effect of Jersey Cow waste extracts (methanol, hydro alcohol and aqueous) were significant as compared to standard Fluconazole.

Zone of inhibition (ZoI) was used as a measure for comparing fungal activity of these Jersey Cow waste extracts showed about methanol extracts (10.75mm), hydro alcohol extracts (10.75mm), aqueous extracts (11.50mm) and Std. (8.75mm) zone against the test organisms of *Candida albicans*. Methanol extracts (5.50mm), hydro alcohol extracts (4.12mm), aqueous extracts (5.25mm) and Std. (5.25mm) zone against the test organisms of *Aspergillus flavus*. Methanol extracts (4.50mm), hydro alcohol extracts (5.25mm), aqueous extracts (4.75mm) and Std. (5.75mm) zone against the test organisms of *Aspergillus niger*. Considering the advantage of the microbicidal activities of the sample. Among the various extract, methanolic extract of cow waste possess greater activity against fungus.

Table 1: Antifungal activity of Jersey Cow waste extracts

Samples	<i>Candida albicans</i> (mm)	<i>Aspergillus flavus</i> (mm)	<i>Aspergillus niger</i> (mm)
Methanol extracts (50µl)	10.75±0.75	5.50±0.38	5.50±0.31
Hydro alcohol extracts (50µl)	10.75±0.75	4.12±0.28	5.25±0.36
Aqueous extracts (50µl)	11.50±0.80	5.25±0.36	4.75±0.33
Standard (30µl)	8.75±0.61	5.25±0.36	5.75±0.40

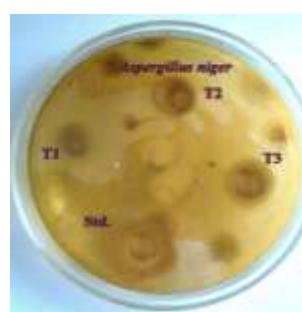
Values were expressed as Mean ± SD
Fungal standard : Fluconazole



Candida albicans



Aspergillus flavus



Aspergillus niger

Plate 2: Antifungal activity of Jersey Cow waste extracts

Cow dung has antifungal substance that inhibits the growth of coprophilous fungi (Dhama *et al.*, 2005b; Joseph and Sankarganesh 2011; Dhama *et al.*, 2013). *Eupenicillium bovisomum* present in cow dung produces patulodine-like compounds viz. CK2108A and CK2801B that possess significant antifungal activity. Detected

considerable numbers of enterococci in cow dung water with antilisterial effect. One isolated strain *Enterococcus facialis* V24 was found to produce a heat stable, largely hydrophobic antimicrobial substance with significant antimicrobial activity against pathogenic Gram-negative bacteria. Possible applications of cow dung microorganisms

in pharmaceutical industry has been indicated by (Teo and Teoh, 2011) and it was shown that isolate K4 possessed antibacterial activity against *E. coli*. Research has also been conducted on water, ethanol and n-Hexane extract of whole cow dung against *Candida*, *E. coli*, *Pseudomonas* and *Staphylococcus aureus* by (Shrivastava *et al.*, 2014) revealing their antimicrobial properties.

CONCLUSION

From the experiment conducted it was concluded that the various extracts of cow dung possessed antimicrobial property against human pathogens. The cow dung had potential antimicrobial and antifungal property. Besides the cow dung methanol extract possess superior antimicrobial activity than other extract and that shown antimicrobial property against all the test microorganisms. Since cow dung are abundant in nature, cost effective and easy to be processed, they are a promising solution for a variety of health problems in the near future. The medicinal properties of these cow dung can be exploited to formulate drugs for several diseases caused by antibiotic resistant pathogenic microorganisms. In this way, suggested that cow dung may be considered as an easily available bioresource for antimicrobial agent.

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