Assessment of Antimicrobial and Enzymatic Activity using Cow based products

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ABSTRACT

Cow dung and cow urine have potency towards antimicrobial activity. Various extracts of cow dung possessed partial antimicrobial activity against human pathogens. Enzymes play a vital role in commercial purposes. The enzymes isolated from panchagavya and the cow products have active bio-compounds which were used for commercial purposes. In antimicrobial activity, cow dung and cow urine are used together and panchagavya alone to know the activity was studied. In this Enzyme analysis, cow products are used such as panchagavya (T1), Cow dung (T2), and cow Urine (T3) used against some human and fish pathogens. Enzymes are identified and isolated from panchagavya and cow products. In this experiment, the antibacterial activity was evaluated from cow urine, cow dung, and Panchagavya. The fermented cow product of panchagavya shows superior towards the antimicrobial activity.

INTRODUCTION

Microorganisms are mainly responsible for infection in human beings, animals, fruits, and vegetables. The cow is a mobile medical dispensary and cow urine is a panacea of all diseases. Cow dung and cow urine have superior antimicrobial activity. Various extracts of cow dung possessed partial antimicrobial activity against human pathogens (**Rajeswari et al.2016**). The cow waste can be used as an antimicrobial agent in many ways. The antibacterial activity of panchagavya shows a variety of microorganisms. The importance of antimicrobial characteristics is very valuable because it has many economic credits. (**Joseph and Sankarganesh 2011**).

Cow urine has been practiced from time immemorial, to verify the ethnomedicinal value of cow urine and to find the novel potencies hidden on it (**Lanjhiyanar et al. 2020**). Cow dung is the undigested residue of plant matter which has passed through the animal gut. The chemical composition is mostly carbon, nitrogen, hydrogen, oxygen, phosphorus, etc with salts. Cell sloughed off as the digest went through the digestive tract, some urea, mucous, as well as cellulose, lignin and hemicelluloses.

Microbial enzymes are known to be superior enzymes obtained from different microorganisms, particularly for application in industries on commercial scales (**Nigam 2013**). These enzymes have been globally studied for the biosynthesis of economically viable preparations for various commercial applications. The use of enzymes for bioconversion of such by-products into valuable products. Enzyme bioconversion is an achievable alternative to value-added agro-industrial waste.

The application of organic fertilizers significantly increases the phosphatase dehydrogenase, cellulase and urease activity (**Phate et al. 2014**). Nowadays hundreds of enzymes are used industrially, half of the enzyme sources are from fungi and bacteria one-third are from bacteria and the remaining are available in plants and animals. Considerable variety of species and environments are screened for cellulolytic enzymes that possess improved characteristics, a process for which reliable detection and quantification of cellulose activity is a prerequisite (**Jhonsen et al. 2014**). Proteases are the enzymes that hydrolyze proteins. These enzymes are of great commercial importance contributing to more than 40% of the world's commercially produced enzymes. Approximately 50% of the enzymes used in industrial processes are proteolytic enzymes (**Goyal *et al*. 2018**). Due to the vast usefulness of these liquid organic fertilizers, this study aims to isolate the antibacterial and antifungal activity of Panchagavya, cow dung, and cow urine, and the enzyme activity was evaluated. In the present study, 16 bacteria and fungi were used to analyse the antimicrobial properties of the organic liquid.

Materials and Methods

The preparation of organic liquid fertilizers such as panchagavya was already discussed in the previous article (**Selvakumar et al. 2022**).

Materials for Antimicrobial activity

The paper disk plate method is the most commonly used technique for determining the susceptibility of microorganisms to chemotherapeutic agents. Small paper disks impregnated with known amounts of chemotherapeutic agents are placed upon the surface of an inoculated plate. After incubation, the plates are observed for any zone inhibition surrounding the disk. A zone of inhibition around the disk indicates that the organisms were inhibited by a drug that diffuses into the agar from the disk. This is a highly standardized technique. After the susceptibility test is performed in conformity with FDA procedure one can correlate the size of the zone of inhibition.

Antibacterial activity

Petri dishes with nutrient agar were inoculated with four different bacterial species. The sample is sterilized by passing each through a 0.22gm Millipore GV filter round paper discs with a radius of 0.8cm were dipped into the sample and placed in the centre on inoculated petri dishes. Bacterial colonies were allowed to grow overnight at 37˚C, and the inhibition zone around the disc was measured.

Methods for Enzyme analysis

The liquid sample was centrifuged at 10,000 rpm for 10 minutes at 4˚C and used as a crude enzyme. The crude sample was used for protease assay by (**Anson 1938**). The crude enzyme sample was subjected to CMCase screening. Carboxymethyl cellulase activity was measured as described by (**Ghose 1987**). Total protein estimation was carried out as described by (**Lowry et al. 1951**). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was carried out to determine the molecular weight of the purified keratinase as described by (**Laemmli 1970**). In enzyme analysis, there are three treatments are used such as Panchagavya Treatment (T1), Cow dung Treatment (T2) and Cow Urine Treatment (T3)

Statistical analysis

The data was subjected to be expressed as Mean±SE. One Way Analysis of Variance was known to calculate significance using SPSS 21.0.

Results

In this study, we are evaluating the antimicrobial activity of cow-based products such as Cow dung, Cow urine, and Panchagavya. The data revealed that the panchagavya shows maximum activity. In this Cow Dung, Cow urine and Panchagavya were cultured and a total of 40 bacteria were isolated. Out of these 40 isolates, 7 show activity against pathogens in cow dung and cow urine. The isolates were named A, B, C, D, E, F, G, and P Positive control Streptomycin was used to determine antimicrobial activity. The potential isolates A, B, C, D, E, F, and G showed antagonistic activity against selected strains were chosen. The culture supernatant (filtrate) of the isolate has significant antibacterial activity against D strains with the zone of incubation of 25mm against *Escherichia coli, and* 21mm *Vibrio* spp the data was tabulated in the table in 2. It also showed antibacterial activity against E strains 18mm zone of inhibition against *Aeromonas hydrophila* 15mm and 21 mm against *Vibrio* spp zone of inhibition. All values are the mean of three independent experiments. In this, the bacteria G showed maximum zone of inhibition towards four bacterial strains. These bacteria isolated from cow dung, and cow urine showed maximum in *Vibrio spp*. The culture supernatant (filtrate) of the isolate showed significant activity.

Table1.Antibacterial activity againstselected pathogens using Cow dung and Cowurine

Pathogens	A	B	C	D	E	F	G	P
Escherichia coli	15	12	-	25				24
Klebsilla pneumonia	-	-	6	-	-	-	-	18
Pseudomonas aeruginosa	-	-	-	7	-	-	-	15
Enterobacteraerogenes	-	-	-	-	-	-	-	20
Salmonella typhi	18	10	10	-	10	10	10	20
Vibrio spp	-	-	18	-	21	20	20	22
Aeromonashydrophila	7	15	-	-	18	18	14	16
Staphylococcus aureus	-	-	14	-	14	12	12	-

Out of these 40 isolates, 15 show activity against pathogens in Panchagavya. The isolates were named as 1 to 15 were used to determine antimicrobial activity. The potential isolate 1 to 15 showed antagonistic activity against selected strains. The culture supernatant (filtrate) of the isolate has significant antibacterial activity against strains. The zone of incubation was maximum in bacteria no 2 in 35mm against *Enterobacter aerogenes* and 30 mm *Aeromonashydrophilla*21mm *Vibrio* spp. The data was presented in the table no. 2. It also showed activity against a 30mm zone of inhibition against *Enterobacter aerogenes* and 22 mm in *Aeromonas hydrophila.* The minimum zone of inhibition was observed in *Klebsiella pneumonia* and *Staphylococcus aureus*. All values are the mean of three independent experiments.

Table2. Panchagavya selected bacteria using against some fish pathogen

Bacteria No	*K. pneumonia*	*E. aerogenes*	*B. subtilis*	*S. aureus*	*V. Spp.*	*A. hydrophilla*	*S. marcescens*
1	-	14	-	-	-	-	-
2	-	35	-	-	5	30	8
3	-	30	-	-	14	22	12
4	-	-	14	-	-	17	7
5	-	-	-	-	5	-	6
6	-	20	-	-	6	-	6
7	-	-	14	-	12	13	8
8	-	20	-	8	-	6	-
9	-	-	-	-	-	7	-
10		20	25	9	-	25	-
11	-	-	-	-	-	-	6

12	-	-	14	-	-	15	5
13	-	-	-	16	11	20	6
14	-	15	-	-	13	16	6
15	10	25	10	-	21	22	10

The screening on the proteolytic and cellulolytic microorganisms showed a clear zone around the colonies indicating that these liquid fertilizers had protease and cellulase activity. The data pertaining to the activity of proteolytic and cellulolytic microorganisms in the liquid fertilizer was tabulated in Tab 3. In protease, the (T1) sample shows maximum level of inhibition (16.4 ± 0.74). The minimum zone of inhibition was identified in (T2) 12.0 ± 0.70. In cellulolytic microorganisms, the maximum inhibition was observed in (T1) 12.2 ± 0.37, and the minimum inhibition was recorded in (T3) 9.6 ± 0.51.

Table: 3Proteolytic and cellulolytic bacteria activity in cow products

Treatment	Protease	Cellulase
Treatment (T_1)	16.4±0.74	12.2 ±0.37
Treatment (T_2)	12.0 ±0.70	12.0±0.47
Treatment(T_3)	14.0±0.31	9.6 ±0.51

Mean ± SE; Significant at P < 0.05

Based on the cultural characteristics as well as the intensity of clear zones produced, then the enzyme assay was carried out to isolate the two distinct enzymes such as protease and cellulase. The enzymatic activity was tabulated in the table. 4. In protease the sample (T1) (754U/ml) was significantly increased activity and the lowest protease was recorded in (T2) (412U/ml). In cellulase, the sample (T1) (12.4U/ml) showed significant variation. The lowest cellulase activity was observed in T3 (3.8U/ml).

Table4. Enzymatic activities in Cow products

Treatment	Protease (U/ml)	Cellulase (U/ml)
Treatment (T_1)	754	12.4
Treatment (T_2)	412	10.3
Treatment(T_3)	433	3.8

The crude enzyme was analyzed by SDS-PAGE, and the result was observed in a single polypeptide chain of high molecular weight protein with an apparent molecular weight in the lane. The protein content of the liquid fertilizer was tabulated in Tab 7. The protein content was higher in (T1) 94.7mg/ml, and the lowest protein content was recorded in (T3) 37mg/ml. This is insignificant at P≤0.05

Table5. Protein content of the Cow products

Treatment	Protease
Treatment (T_1)	94.7
Treatment (T_2)	37
Treatment(T_3)	82

Mean ± SE; Significant at P < 0.05

DISCUSSION

Agricultural wastes offer huge potential for reducing the production cost and increasing the use of enzyme for industrial purposes. Effective microorganisms (EMO'$_s$) in Panchagavya predominantly and lactic acid, bacteria, yeast, actinomycetes photosynthetic bacteria and certain fungi besides beneficial and proven fertilizers such as *Acetobacter, Azospirillum* and *Phosphobacterium* were detected which have the beneficial effect, especially in improving soil quality, growth and yield of crops (**Selvaraj 2007**). The cow urine rich in uric acid, a source of nitrogen was readily soluble and liquid form, one of the important compounds in Panchagavya and Jeevamurtha which was readily available to the plants directly influencing the nitrogen content of leaves. The result is in accordance with the findings of (**Punitha** ***et al.*****2014**) who reported that the enzymes released by bacteria convert the complex insoluble organic compounds to simpler soluble form. According to (**Sangeetha**, ***et al*****. 2010**) who revealed that the seedlings grown in soil preparation amended with traditional Panchagavya marginally increased the levels of the enzyme in all pulses. Our result was supported by the work of (**Vajanthaet al.2015**) who observed that increase in enzymatic activity with soil application of Panchagavya @15% may be due to stimulation of microorganism's population because of the addition of substrate and growth-promoting substance that are present in panchagavya. Studies conducted by (**Mallesha and Rao 2017**) reported that phosphatase activity was significantly higher with the application of compost + Vermicompost + Panchagavya and it was on par with the rest of all treatments except compost. According to (**Phate et al. 2014**) who revealed that sanjeevak 5 and panchagavya are the best formulations of liquid manure showing the highest microbial count and presence of the high number of extracellular enzymes. This synchronized the growth of sunflowers and sustained their productivity. Our result supports the work of (**Sutar** et al. **2018**) who observed that the auxin content in Panchagavya upon its application leads to the activation of cell division and cell elongation in the auxiliary buds which had a promising effect in an increased number of branches, leaves and leaf area. This conforms with the finding of (**Sreenivasa et al. 2009**) who revealed that the presence of such beneficial microbial biomass and nutrient status might have resulted in improved seed germination, seeding length, and seed vigour in soybean indicating beejamrutha as an efficient plant growth stimulant. (**Vallimayil and Sekar 2012**) reported that panchagavya-treated plants showed better growth than water-treated control plants. The results of the present work are in agreement with the report of (**Sarkar et al. 2014; Ali et al. 2011; Anandan et al. 2016), and (Mohan Kumar et al. 2017; Radha et al. 2014**) who revealed that an increase in fruit weight might be due to better utilization of plants accompanied with the enhancement of photosynthesis, other metabolic activity and greater division of food material to fruits.

CONCLUSION

Cow urine and cow dung have a fair amount of antimicrobial activity but when combined and fermented with supportive ingredients. Panchagavya has good antimicrobial, and enzymatic activity and the quantitative parameters show significant results when compared to the control.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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