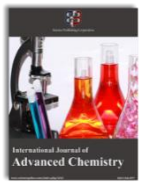
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Research paper



Antimicrobial activities of Desi Cow Dung Extracts against human pathogens and phytochemical analysis using chloroform extracts

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Abstract

The main objective is to examine the anti-microbial properties and Photochemical screening of the cow dung extracts which are useful to kill the pathogens in humans.Nowadays there is increasing microbial drug resistance problem, so new alternative to synthetics drugs are explored. Along with it antimicrobial activities of natural products are in search. Study of anti-microbial activities of desi cow dung extracts of chloroform against microorganisms namely E. coli and K. Pneumonia.Also the phytochemical analysis for the flavonoids, glycosides, steroids, tannins and phenol compounds. The antimicrobial sensitivity test by disk diffusion method and the disk made from the cow dung showed the zone of inhibition in the petri-plates of both the microorganisms namely E. coli and K. Pneumonia.The phytochemical analysis also showed colour change and precipitation except the phenols. As the microorganisms are becoming resistant to the antibiotics and synthetic drugs, new alternate natural drugs can be explored and used against several diseases caused by this microorganisms.

Keywords: *Antimicrobial; Cow dung; Microorganisms; Pneumonia: Synthetic Drugs*

1. Introduction

A mixture of dung and urine is called cow dung which generally is in the ratio of 3:1. It consist of crude fibre, crude protein, cellulose, hemicellulose and 24 types of minerals such as N, K, S, traces of P, Fe, Co, Mg, P, Cl, Mn, etc. For enhancing soil fertility, it is normally used as an organic fertilizer, also as a source of fuel, for dressing seeds, plastering cut ends of vegetatively propagated sugarcane, dressing plant wounds, sprinkling diluted suspension of CD on plant surface, etc. from ancient times. In India, cattle's rearing is a tradition in the country and intimately limited to the agricultural economy. In a number of ayurvedic formulations various products obtained from cow milk, ghee, curd, urine, and dung are widely used. Cow dung is traditionally used as organic fertilizer in Indian sub-continental farming for centuries. For increasing the mineral status of soil and enhancing the resistance of plant against pests and diseases the cow dung is added; it also stimulate plant growth and other beneficial activities such as sulpho oxidation and phosphorus solubilisation [1].
The Hindu Vedas say that the cow is holy and should be worshiped. Cows are very important animal resources and are highly useful in agriculture and dairy industry, in India [2]. Cow's urine, milk, ghee, curd and dung are five major substances obtained from cow and termed panchgavya. All the five products possess medicinal properties against many disorders. Cow dung can act as skin tonic for boils and heat rashes when mixed with crushed neem leaves and smeared on skin. Toothaches get removed when it is used as tooth-paste, so instead of toothpaste which is made of dead bones of the animals and chemicals, it is a good alternative. The fresh cow dung kills the germs of Malaria and T.B [3]. For the good health and well-being, panchgavya is considered as a gift from heaven. For the removal of the mental and physical disorders, consumption of panchgavya is considered very helpful. The different products of panchgavya are having some unique and specific properties that are why the individual product can be used for the healing and the treatment of the particular disease and its prevention.
The undigested residue of plant matter which has passed through the animal's gut is called cow dung. Cow dung is comprised of organic matter including fibrous material that passed through the cow's digestive system, among other liquid digest that has been left after the fermentation, absorption and filtration, then acidified, then absorbed again. Carbon, nitrogen, hydrogen, oxygen, phosphorus, etc. are the chemical composition which are mostly present in cow dung along with salts, cells sloughed off as the digest a went through the digestive tract, some urea, mucus, also the cellulose, lignin and hemicellulose. Antimicrobeis termed as an agent that kills microorganisms or inhibits their growth [4]. About 80% water and supports a matrix of undigested plant material that is rich in nutrients, micro-organisms, and their by-products is normally the composition found in the cow dung. Cow dung microflora contains an abundant number of lactobacilli and cocci and bacilli, someidentified and unidentified yeasts and fungi [5].

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Different uses of cow dung are: Fuel, fertilizer, heat source (cow dung is naturally hot -compost makes hotter put in glass house to heat glass house or run pipes thru it to get hot water), purifier (natural antiseptic qualities), floor coating, mud brick additive, skin tonic (good for boils and heat rash), smoke producer (smoldering cow patties keep away mosquitoes), brass polisher (tamarind removes oxidation), mud additive, mud brick additive (mud and lime becomes like cement), pond PH balancer (neutralizes acid), tooth polish.

Escherichia coli (known asE. coli) is a gram-negative, facultatively anaerobic, rod-shaped, bacterium of the genus Escherichia that is commonly found in the lower intestine of warm-bloodedorganisms (endotherms) [6]. Most E. coli strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination[7]. The normal flora of the gut contains harmless strains of E. coli, and by producing vitamin k2 benefits its host [8] and preventing colonization of the intestine with pathogenic bacteria [9].

Heat, chemical and pH are resistant to spores of E. coli. However the antibiotic sensitivities of different strains of Escherichia colivary widely. Many antibiotics that are effective against Gram-positive organisms were resistance to the Gram-negative organisms [10].

About 0.1% of gut flora constitutes of E. coli and other facultative anaerobes pathogenic strains of the bacterium cause disease mostly through the route of gut andfaecal–oral transmission. For a limited amount of time the cells are able to survive outside the body, which makes them potential indicator organisms to test environmental samples for faecal contamination[11].

An environmentally persistent E. coli which can survive for extended periods outside of a host has been examined by a growing body of research.Recently, towards the identification of potential sources of faecal contamination impacting waterways and beaches research efforts have been directing their research work. It is called microbial source tracking. E. coli can become "naturalized" to soil, and algae in tropical, sand, sediments, subtropical, and temperate environments has been found in resent research. The continuous use of this bacterium as an indicator of the faecal contamination this phenomenon concerns the raising issues [12].

Klebsiella pneumoniae is the Gram-negative, encapsulated,non-motile,, rod-shapedand lactose-fermenting, facultative anaerobicbacterium. On MacConkey agar it appears as a mucoid lactose fermenter. Although found in the normal flora of the skin, mouth, and intestines, if aspirated (inhaled) it can cause destructive changes to human and animal lungs, specifically to the alveoli (in the lungs) resulting in bloody sputum. Klebsiella bacteria is the most common condition caused outside the hospital is pneumonia, typically in the form of bronchitis and also bronchopneumonia. These patients have an increased tendency to develop cavitation, lung abscess, pleural adhesions and emphysema. Even with antimicrobial therapy, it has a death rate around 50%. The mortality rate can be nearly 100% for people with bacteraemia and alcoholism [13].

Klebsiella organisms are often resistant to multiple antibiotics. Plasmids as the primary source of the resistance genes is the whole evidence implicated [14].Klebsiella species are resistant to many classes of antibiotics, this species are having ability to produce extended-spectrum beta-lactamases (ESBL). The most frequent are resistance to the fluoroquinolones,
aminoglycosides, chloramphenicol,tetracyclines,and trimethoprim/sulfamethoxazole[15].

In addition to pneumonia, infections in the urinary tract, lower biliary tract, and surgical wound sites is also caused by Klebsiella. The range of clinical diseases includes pneumonia, urinary tract infection, thrombophlebitis, diarrhoea, cholecystitis, upper respiratory tract infection, wound infection,meningitis, bacteremia, osteomyelitis, and even septicemia. Contamination of the device becomes a risk for patients with an invasive device in their bodies; for example, neonatal ward devices, and urinary catheters, respiratory support equipment, which put patients at increased risk. Also, the use of antibiotics can be a factor that increases the risk of nosocomial infection with Klebsiella bacteria,entry of the bacteria into the blood can be followed by sepsis and septic shock.

For the urinary tract infections in older people, Klebsiella ranks second to E. coli. It is also an opportunistic pathogen for patients with enteric pathogenicity, chronic pulmonary disease, nasal mucosa atrophy, and rhinoscleroma.New antibioticresistant strains of K. pneumoniae are appearing [16]. With this view, this study focused on the antimicrobial activities of dung extracts of Indian desi cow against human pathogens. An organic compound with formula $CHCl_3$ is chloroform or trichloromethane. It is a dense liquid, colourless, sweet-smelling, which is precursor to PTFE and refrigerants, and is produced on a large scale but the latter application is declining.It is one of the four chloromethanes and a trihalomethane. Through the environment, the total global flux of chloroform is approximately 660000 tonnes per year [17].

About 90% of emissions are natural in origin. Fungi are believed to produce chloroform in soil and many kinds of seaweed produce chloroform [18].

From soil and surface water chloroform volatilizes readily and undergoes degradation in air to produce phosgene, formyl chloride, dichloromethane,carbon monoxide, hydrogen chloride and carbon dioxide. Its half-life ranges from 55 to 620 days in air. Biodegradation in soil and water is slow. In aquatic organisms chloroform does not significantly bio accumulate [19].

In hydrogen bonding, the hydrogen attached to carbon in chloroform participates [20]. Chloroform is also used worldwide in pesticide formulations, as a solvent for fats, waxes, gutta-percha, oils, and resins, rubber as a cleansing agent, grain fumigant, alkaloids, in fire extinguishers, and in the rubber industry [21]. $CDCl_3$ is a common solvent used in NMR spectroscopy.

Flavonoids are the largest, most varied, and most studied group of phytochemicals. More than 6,000 flavonoids that occur in plant foods have been described. Several phytochemicals that act as a protective mechanism against environmental stressors is produced by pants. The more environmental stressors, the more phytochemicals a plant produces. As a result, phytochemical content can vary with growing conditions.

The extraction, screening and identification of the medicinally active substances found in plants is referred to as phytochemical analysis. Flavonoids, alkaloids, steroids, glycosides, tannin, antioxidants and phenolic compounds are some of the bioactive substances that can be derived from plants. The use of plants and plant extracts to heal, relieve pain and promote good health dates back to before the beginnings of medical science, although the knowledge of how these substances provide medicinal value to humans reflects a relatively recent scientific understanding [22].

2. Materials and methods

2.1. Glass wares

Petri plates
Test tubes
Flask



Fig. 1:

Pipette
Sterile containers

2.2. Chemicals

Chloroform
10% NaOH
Concentrated H_2SO_4
10% Lead Acetate

2.3. Culture

Escherichia coli
Klebsiella pneumonia

2.4. Others

Burner
Antibiotic disc
Mueller Hinton Agar
Whatman No 1 filter paper
Empty disc

3. Method

3.1. Cow dung collection

The cow dung of desi cow was collected from the Panjaraporgaushala at Rajkot.

3.2. Culture collection

Two strains of gram negative human pathogens namely Escherichia coli and klebsiella pneumonia were collected from B. Voc (microbiology) department of Shree M and N Virani Science College.

3.3. Powdered cow dung

Some amount of cow dung from desi cow was collected and shadow dried for 5 days. The moisturecontent of cow dung was high. The dried cow dung was powdered manually.

3.4. Preparation of cow dung extracts

100 ml of chloroform was added in 10g ofpowdered of desi cow in a conical flask and it was kept in a rotary shaker for 3 days. Then the extract was filtered in to a smallconical flask by using whatman No 1 filter paper.



Fig. 2:

3.5. Preparation of the cow dung extract disk

The empty disk was impregnated with 50µl of the chloroform extract of cow dung from desi cow and dried in oven, until the disk was completely saturated with extract this process was repeatedly done. For the study of antimicrobial activity of desi cow dung.

3.6. Antibiotic sensitivity test

Kirby-Bauer method also known as disc diffusion antibiotic sensitivity testing is a test which uses antibiotic-impregnated wafers to test whether particular bacteria are susceptible to specific antibiotics. Based on the observation that the degree of inhibition of bacterial growth on agar medium surrounding an antimicrobial compound containing disc correlates with susceptibility to the agent. Whether organism is sensitive, resistant or intermediate toa particular antibiotic or the antimicrobial compound is determined by the zone of inhibition. Four to five similar colonies were taken from the pure bacterial culture slant of identified organism and then inoculate into the nutrient broth and incubated at 37`c for 24 hours. Initially 100 µl culture was spread with the spreader on the entire plate of the Mueller-Hinton agar for testing the antimicrobial activity of desi cow dung extract. One saturated disk containing desi cow dung and the other three commercially available antibiotic discs were gently pressed on to the microbe carpeted Muller-Hinton agar plate at the scientific distance from each other and the edge of the plate. The antibiotic discs were used to compare the zone of extract saturated disc and the antibiotic disc of Gram negative bacteria. The antibiotic discs which were used are:
Amikacin, Chloramphenicol, Gentamycin, Ofloxacin, Vancomycin, Methicillin and Penicillin-G. One plate of the Escherichia coli and one plate of Klebsiella pneumonia were done by this method. Then these plates were incubating at 37`c for 18-24 hours (overnight incubation). The diameter of the zone of bacterial growth inhibition around each disc was measured and the susceptibility or resistance to the agent in each disc was determined according to the standardized table provided by the Hi-media Laboratories, Mumbai.

3.7. Phytochemical screening of cow dung extract

The chloroform extracts of desi cow dung was used for phytochemical screening. Collect all the required materials like test-tubes, auto pipettes, sterile tip-box and racks to the working area.

3.8. Test for flavonoids

3.8.1. Lead acetate test

Take 0.5 ml of the cow dung extract with the help of auto pipette in the test-tube. Then few drops of lead acetate solution were added to the cow dung extract. The yellow colour precipitate were formed which indicates the presence of flavonoids in the cow dung.

3.9. Test for steroids

3.9.1. Salkowski test

Take 2 ml of cow dung extract using the auto pipette in the glass test-tube, and then add 2 ml of chloroform and 2 ml of concentrated H_2SO_4 and shaken it well. If the chloroform layer appeared red and acid layer showed greenish yellow fluorescence, it indicates the presence of steroids in the cow dung.

3.10. Test for tannins

Take 5 ml of the cow dung extract with the help of auto pipette in glass test-tube, then add 1 ml of 10% lead acetate solution to the extract. If there if formation of yellow precipitate, it indicates the presence of tannins in the cow dung.

3.11. Test for phenols

Take small amount of the cow dung extract in the glass test-tube. Later the extract was dissolved in 0.5 ml of 20% Sulphuric acid solution. Following it add few drops of 2% Sodium hydroxide solution, if it turned blue it indicates the presence of phenols in the cow dung.

3.12. Test for glycosides

Take small amount of the cow dung extract in the glass test-tube and then dissolve it in 1 ml of water, later add aqueous 10% Sodium hydroxide solution to the mixture of cow dung extract and water. The yellow colour precipitation were formed which indicated the presence of glycosides in the cow dung

4. Results

4.1. Antimicrobial sensitivity test

The experiment was conducted using the desi cow dung extracts of chloroform on the micro-organisms. Its results were compared with the standard readily available antibiotic disk. The CH antibiotic disk showed the zone of 12 mm and PC antibiotic disk showed the zone of 21 mm. This antibiotic disk showed sensitivity to the test organism named Escherichia coli. While the CF antibiotic disk showed resistance against the test organism named Escherichia coli. Similarly, the AK antibiotic disk showed the zone of 19 mm and PC antibiotic disk showed the zone of 17 mm and CL antibiotic disk showed the zone of 11 mm. This antibiotic disk showed sensitivity to the test organism named Klebsiella pneumonia. The desi cow dung disk prepared from the extraction showed sensitivity to all the test microorganisms.

4.2. Phytochemical analysis

Chloroform extract of the desi cow dung has shown colour change and positive test results with the flavonoids, glycosides, steroids, tannins test and has shown no colour change and negative test result with phenols test.

4.2.1. Flavonoids test results

4.2.2. Lead-acetate test

When the reaction mixture was prepared the colour change and the yellow precipitates was observed which indicates positive test results.
Glycosides test results
When the reaction mixture was prepared the colour change and the yellow precipitates was observed which indicates positive test results.

4.2.3. Steroids test results

4.2.4. Salkowski test

When the reaction mixture was prepared the upper layer appeared red and the lower layer showed greenish yellow precipitates which indicates positive test results.

4.2.5. Tannins test results

When the reaction mixture was prepared the colour change and the yellow precipitates was observed which indicates positive test results.

4.2.6. Phenols test results

The phenols test did not show blue precipitates and indicated negative results.

Table 1: Phytochemical Analysis of Chloroform Extract of DESI Cow Dung

Sr. No	Phytochemical	Extract chloroform(DESI cow)
01	Flavonoids	+
02	Glycosides	+
03	Steroids	+
04	Tannins	+
05	Phenols	-

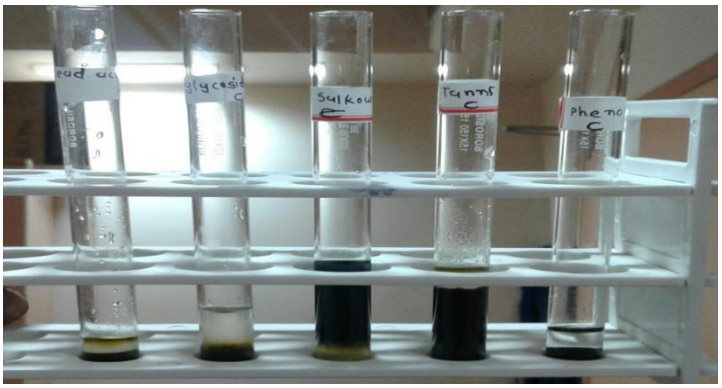


Fig. 3: Figure of Phytochemical Analysis.

Table 2: Phytochemical Analysis of Chloroform Extract of DESI Cow Dung

Sr. No	Phytochemical	Extract chloroform(DESI cow)
01	Flavonoids	+
02	Glycosides	+
03	Steroids	+
04	Tannins	+
05	Phenols	-

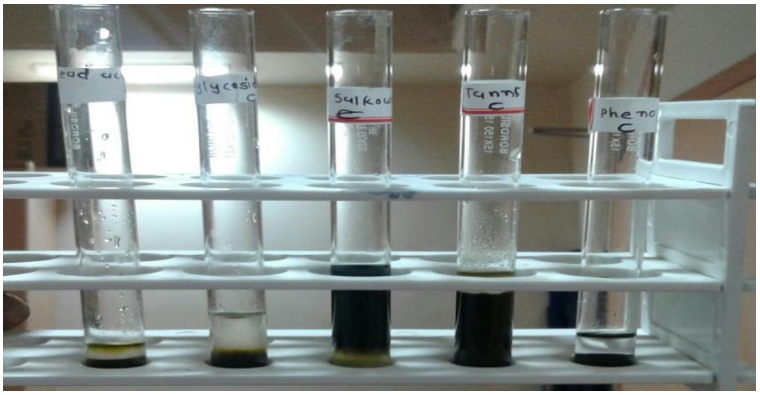


Fig. 4:

5. Discussion

In Indian Vedas, the cow is considered the most religious and vulnerable animal of Hindus. Cows are a very important animal in India, and useful in dairy and agriculture industry. In the production of biogas and in organic fertilizer the cow dung has been used since years. "Dalang" or "Dalam" is the evaporated extract of cow dung known in northeast Nigeria and in some part of Northern Cameroun and has been used as soup condiment and in the treatment of infections. Indian cow, jersey, Holstein and buffalo are the other different healthy cows. Cow dung was collected in the early morning from panjarapor gaushala Rajkot. Cow dung was then put for shed dried for 5 days. Later, the dried cow dung was powdered manually. Then powdered material 100 ml of chloroform was added in 10 g of powdered desi cow dungs in a conical flask and it was kept in rotary shaker for 3 days. The cow dung extract was then filtered using What man No 1 filter paper and stored in flask which was air tightly packed for future use.

Then cow dung extraction procedure was followed by the antimicrobial activities by disc diffusion technique at different cow dung extract against and E. coli.andK. pneumonia. Desi cow dung extracts was having the activity against both the pathogens namely and E. coli. andK. pneumonia.The Indian cow dung namely Jersey, Holsten, buffaloes etc dung extract was showing the inhibition zone of microorganisms.

The procedure was then followed by phytochemical analysisby desi cow dung extract prepared in chloroform solvent and it was found that flavonoids were present along with the glycosides, tannins, steroidsbut phenols was not found. These phytochemical compound are present the cow dung.

6. Conclusion

From the experiment that was conducted, it was concluded that the chloroform extracts of desi cow dung possessed superior antimicrobial property against human pathogens. The cow dung from desi cow had antimicrobial property against is Klebsiella pneumonia as well as Escherichia coli.

Besides the desi cow dung extracts possess antimicrobial activity against all the test microorganisms equally to that of the readily available antimicrobial disk. Since cow dung are abundant in nature, easily available as well as 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 the desi cow dung can be exploited to formulate drugs for several diseases caused by antibiotic resistant pathogenic microorganisms. Many different antiseptics and other drugs can be made by the use of cow dung.

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