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SYNERGISTIC ANTIMICROBIAL EFFECT OF COW URINE AND AZADIRACHTA INDICA ON INFECTIOUS MICROBES

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ABSTRACT

The study is aimed towards studying synergistic antimicrobial effect of cow urine and *Azadirachta indica* against bacterial, fungal and yeast strains. Mother Nature is the best healer. Last few decades have seen tremendous development in pharma sector. Even though these industries have produced a large number of new drugs and new antibiotics and so does the resistance among microbes is growing. Bacterial strains used were *Streptomyces aureofaciens* (MTCC 325), *E.coli* (MTCC 448), *Streptococcus mutans* (MTCC 497) and *Pseudomonas aeruginosa* (MTCC 7093). Fungal strains used were *Candida albicans* (MTCC183) and yeasts like *Candida paraopsilosis* (MTCC 1965), *Candida tropicalis* (MTCC 184), and *Candida glabrata* (MTCC 3019). When equilibrated amount of cow urine is used with *Azadirachta indica* remarkable synergistic effects were observed in the case of *C. tropicalis, C. glabrata, P. aeruginosa, S. aureofaciens*.

INTRODUCTION: India is a holy land where each and every living organism and non living has something to offer and they been respected for that reason. And the things offered by Mother Nature are beyond the human limit to explain. Nature has always been the foremost source of medicinal valuable agents. Herbal medicine is the oldest and most reliable form of health care known to Homo sapiens. India provide habitat to rich medicinal plants flora with more than 7500 species [1].

One of the most important is *Azadirachta indica* or Neem; it is native plant of south Asia. Every study on Neem emphasizes antimicrobial activity of *Azadirachta indica*. *Azadirachta indica* shows significant lethal effect on Gram positive and Gram negative bacteria, yeasts and fungi which causes a wide array of human and animal diseases. It is also believed India has 30 per cent of the world's cattle.

There are 26 distinctive breeds of cow in India. The hump, long ears and bushy tail distinguish the Indian cow. In west though cows are just walking hamburgers but in Asia and especially in south Asia they are considered walking medicinal dispensary. Given in many Ancient manuscripts that cow's urine or Go-Muttra was taken for preventing diseases and hence to remain healthy. It is a treasurerous medicine and various ancient medicinal treatment practices use it to cure curable and incurable diseases [2].

Cow urine is used as a therapeutic agent. Like for example panchagavya, a blend of five compounds obtained from the cow, namely dung, urine, milk, ghee and curd is used as a medicine and sometimes as fertilizer for plants [3]. This study is focused at synergistic antimicrobial activity of Cow- urine and *Azadirachta indica.* Wide use of antibiotics in the treatment of microbial infection has led to the development of strains which are resistant. Ours

surrounding is full of microbes and human and animal body's temperature, nutrients present in the body provide a five star welcome to them. Human immune system protects body from their attack. Immune system comprises of the innate and acquired immunity. Innate immune system is non-specific with natural killer cells; complement system, antigen presenting cells, neutrophiles and macrophages as its main component which gets immediately activated in presence of foreign particles or pathogen or microbes but non-specifically.

If pathogens not treated of cleaned by the primary immune system, secondary or acquired (specific) immune system come in to activation. Acquired immune system comprises of humoral immunity and cell mediated immunity [4]. There are some other factors which are also responsible in altering the overall natural immunity. Immuno-stimulants stimulates while immune-suppressor suppress the immunity but non-specifically. And the use of *Azadirachta indica* and gou-Muttra as immune stimulants is well noted.

***Azadirachtra indica*:** In India, the tree is variously known as "Sacred Tree," "Heal all," "Nature's Drugstore," "Village Pharmacy" and "Panacea for all diseases. Neem tree was described as *Azadirachta indica* by De jussieu in 1830 and its taxonomic position was also given by him [5]. It is considered as a major component in Ayurvedic, homeopathic and Unani medicine and is having reputed place in the modern medicine, particularly prescribed for skin disease. Sanskrit name of Neem is "Arishtha" means reliever of sickness. Its value is recognized by U.S. National Academy of Science, considering it as a tree for solving global problems.

During the 20th century chemicals investigation of the products of Neem was done. It was Siddique who in 1942 isolated "Nimbin" the first compound isolated from Neem oil. Till now more than 135 compounds have been isolated from Neem [5]. Neem work is mainly focused on oral care which is critical issue in both developed countries as well as in developing countries where professional dental care is limited. With no doubt medicinal plants like Neem are the prime source of drugs in developed and developing countries as drugs and herbal extracts. Neem is the tree whose each part have some or other medicinal value from bark, leaves, seed oil and even wood.

Neem have antibacterial, anti-malarial, anti-fungal, anti-inflammatory, immune-modulatory, anti-tumor, anti-fertility, anti-ulcer effects, diuretic, anti-pyretic, anti-arthiritic, hypoglycemic, spermicidal and other activities. These activities of Neem are because of the presence of compounds like Nimbidin, Nimbin, Sodium nimbidate, Gedunin, Nembolide, Cyclic trisulphide, cyclic tetrasulphide, PolySaccharides, NB-II polypeptido glycan [5].

Cow urine or Gou-Muttra: Our Ayurveda has always focused on prevention of disease and maintaining the health and treatment of diseases. Cow urine acts like a magical potion for the treatment of the disease like cancer, asthma, chronic renal failure, hepatitis ABC, urological disorders, respiratory diseases and also plays its part as antimicrobial against disease like Eczema, Psoriasis, acne vulgaris, scabies and other various kinds of allergies [6].

Urine contains volatile salts which are beneficial to the human body because these salts destroy acidity and get rid of pain in kidney, intestine, and womb; furthermore urine, a natural tonic, eliminates giddiness, tension in nerves, lazy feeling, hemicrama, paralysis, common cold, diseases of brain, nerves, joints and leucorrhea (a whitish discharge from vagina resulting from inflammation or congestion of the mucous membrane) [7].

Cow urine contains a no. of components and some are volatile in nature. The components of cow urine are responsible for showing antimicrobial activity. Cow urine is rich in potassium, chloride, calcium, estrogen, phosphorous, urinary proteins[9]. Various research have also found different components like urea, uric acid, nitrogen, sulfur, copper, iron, sodium, other salts, carbolic acid, ammonia, sugar lactose, Vitamin-A,B,C,D,E, gonadotropin, phenols and also some anticancer substances [10].

MATERIALS AND METHOD:

Azadirachata indica: This study confined itself to the extract obtained from the fresh leaves of *Azadirachta*

indica during the monsoon season. Leaves are taken and washed completely with lukewarm water and then with boiling water to remove microbes present over the surface. They are air dried and grinded to juice with the pestle and mortar. For each 10 gm of leaves 5ml of water is used to ease the grinding. Juice is obtained and centrifuged to remove the solid leaf material. This is stored at 4 degree Celsius.

Cow urine: Fresh cow urine is taken from Indian breed cow. Cow urine used is photo-activated by keeping in a transparent bottle in sunlight for 48 hours. And then centrifuged for removing all debris and precipitated material and kept at 4 degree Celsius for further long time use. To be sure of cow urine is free of microbes it is inoculated in broth and also nutrient agar plate spreading is done. Photo activation and centrifugation make cow urine free from microbes. Cow urine contains a no of beneficial compounds.

Microbial cultures: Antimicrobial activity is tested against bacteria, fungi and yeasts. Micro-organisms uses are listed in **table 1**.

TABLE 1: LIST OF MICROBES AGAINST WHICH ANTIMICROBIAL ACTIVITY IS TESTED

S No.	Microbes type	MTCC No.
1	*C. albicans*	MTCC 183
2	*C. tropicalis*	MTCC 184
3	*C. glabrata*	MTCC 3019
4	*C. propsilosis*	MTCC 1965
5	*E. coli*	MTCC 448
6	*P. aeruginosa*	MTCC 7093
7	*S. mutans*	MTCC 497
8	*S. aureofaciens*	MTCC 325

Minimum Inhibitory Concentration: To find the minimum inhibitory concentration agar diffusion method and broth dilution method are followed. For agar diffusion method plates containing Muller Hinton agar (MHA) were made and then spreaded with each different strains and well of 12 mm was made at centre. Different volume of extract is added then to find at which minimum volume growth of microbe is inhibited.

Agar Diffusion Method: Agar diffusion method is employed to find antimicrobial activity of the *Azadirachta indica* extracts and Gou Muttra. Mueller Hinton agar (MHA) from Himedia is used. Agar is made as mentioned in the instructions. Sterilize it by autoclaving at 121 degree Celsius under 15 lb psi (per square inch) pressures. Sterilized agar poured and allowed to solidify in Petri plates in laminar flow. 100 micro liters of 48 hours old microbial culture is then spread on to it by sterile cotton buds. Wells are made at center which is then filled with 150 micro liters of cow urine and *Azadirachta indica* alone and also in combination to check out their antimicrobial activity.

Broth Dilution Method: In case of broth dilution method, different-different volume of mentioned antimicrobial substances were added in the Muller Hinton broth, microbes were inoculated into tubes containing culture broth and varying concentrations of the antimicrobial substances to be tested. The tubes are incubated to allow microbial growth. The interpretation of this MIC depends on the organism/antimicrobial pair being tested.

RESULTS: *Azadirachta indica* and Cow urine has shown remarkable antimicrobial effects. When equilibrated amount of cow urine is used with *Azadirachta indica* remarkable synergistic effects were observed against *C. tropicalis, C. glabrata, P. aeruginosa, S. aureofaciens.* Alone, extract of *Azadirachta indica* showed antimicrobial activity towards *C. albicans*, *C. glabrata, E. coli*, *P. aeruginosa* and *S. aureofaciens.* In case of Cow urine, strong anti microbial activity is noted towards *E. coli, S. aureofaciens* and *C. albicans.*

MIC Results for different volumes of *Azadirachta indica* extract against microbes: MIC, in case of Neem for *C. tropicalis and S. aureofaciens* was 70-110 micro liters, for *C. propsilosis* it was 100-110 micro liters, for *C. albicans, P. aeruginosa, E. coli, C. glabrata, S. mutans,* it was 110 micro liters. Similarly, in case of cow urine, MIC was 200 micro liters for *C. albicans, E. coli, S. aureofaciens.* All other were resistant up to 200 micro liters. And when synergistically Cow urine and *Azadirachta indica* are used MIC obtained in case of *C. tropicalis, E. coli, S. aureofaciens* 100-110 micro liters but in case of *C. albicans, C. glabrata, C. propsilosis, S. mutans and P. aeruginosa* MIC was 110 micro liters.

TABLE 2: INHIBITION RESULTS GOU MUTTRA (COW URINE =C.U.)

S. No.	Microbes type	MTCC No.	Extract used	Vol. of culture #	Vol. of extract#	Inhibition zone^
1	*C. albicans*	MTCC 183	C.U.	100	150	19mm
2	*C. tropicalis*	MTCC 184	C.U.	100	150	------
3	*C. glabrata*	MTCC 3019	C.U.	100	150	------
4	*C. propsilosis*	MTCC 1965	C.U.	100	150	------
5	*E. coli*	MTCC 448	C.U.	100	150	19mm
6	*P. aeruginosa*	MTCC 7093	C.U.	100	150	-------
7	*S. mutans*	MTCC 497	C.U.	100	150	-------
8	*S. aureofaciens*	MTCC 325	C.U.	100	150	14mm

TABLE 3: INHIBITION RESULTS FOR NEEM (*AZADARACTA INDICA* =A.I.)

S. No.	Microbes type	MTCC No.	Extract used	Vol. of culture#	Vol. of extract#	Inhibition zone^
1	*C. albicans*	MTCC 183	A.I.	100	150	25mm
2	*C. tropicalis*	MTCC 184	A.I.	100	150	-------
3	*C. glabrata*	MTCC 3019	A.I.	100	150	16mm
4	*C. propsilosis*	MTCC 1965	A.I.	100	150	-------
5	*E. coli*	MTCC 448	A.I.	100	150	18mm
6	*P. aeruginosa*	MTCC 7093	A.I.	100	150	12mm
7	*S. mutans*	MTCC 497	A.I.	100	150	--------
8	*S. aureofaciens*	MTCC 325	A.I.	100	150	34mm

TABLE 4: INHIBITION RESULTS FOR (*AZADARACTA INDICA* = A.I.) + GOU MUTTRA (COW URINE = C.U.)

S No.	Microbes type	MTCC No.	Extract used	Vol. of culture#	Vol. of extract #	Inhibition zone^
1	*C. albicans*	MTCC 183	A.I + C.U	100	150	------
2	*C. tropicalis*	MTCC 184	A.I + C.U	100	150	20mm
3	*C. glabrata*	MTCC 3019	A.I + C.U	100	150	12mm
4	*C. propsilosis*	MTCC 1965	A.I + C.U	100	150	------
5	*E. coli*	MTCC 448	A.I + C.U	100	150	------
6	*P. aeruginosa*	MTCC 7093	A.I + C.U	100	150	22mm
7	*S. mutans*	MTCC 497	A.I + C.U	100	150	------
8	*S. aureofaciens*	MTCC 325	A.I + C.U	100	150	20mm

^ Inhibition zone also includes the diameter of the well of 9mm. #volume in micro liters (10^{-6} liters)

DISCUSSION: This study proved the efficiency of natural products as strong antimicrobial agents. Cow urine on photo activation become antimicrobial. *Azadirachta indica* also has shown remarkable antimicrobial effects. When equilibrated amount of cow urine is used with *Azadirachta indica* remarkable synergistic effects were observed against *C. tropicalis, C. glabrata, P. aeruginosa, S. aureofaciens.*

Alone, extract of *Azadirachta indica* show antimicrobial activity towards *C.albicans*, *C.glabrata, E. coli*, *P.aeruginosa* and *S. aureofaciens.* In case of Cow urine strong anti microbial activity is noted towards *E.coli, S.aureofaciens* and *C.albicans*. Mother Nature is the best healer. Last few decades have seen tremendous development in pharma sector. Even though these industries have produced a large number of new drugs and new antibiotics and so does the resistance among microbes is growing[11]. Development of resistance towards medicine makes the future of antimicrobial drug uncertain. Plants and animals products have been valuable resource of nature for maintaining human health. Use of cow urine and phytoextracts can be of great importance for treating diseases, especially the microbial one. Numbers of researches is been conducted throughout the world to proof their efficiency.

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