Biological Forum – An International Journal **11(1): 231-235(2019)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Comparing antibacterial activity of "cow urine–*Acacia* stem bark extract" against pathogenic bacteria *E. coli*

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(Received 08 February 2019, Accepted 18 April, 2019)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: In India, Cow has been considered as a holy animal. The use of cow urine for curing many diseases has been known from ancient times. Similarly, the plants are worshiped in India. Also, the various plant parts are used in treatment of incurable diseases and the *Acacia* is one of them. Each part of *Acacia* like leaves, stem, etc. used to treat a number of diseases. The present study was undertaken to determine the antibacterial activity against pathogenic bacteria *E. coli* at different concentrations with different organic and cow urine extract of *Acacia* using well diffusion method. The cow urine extract of *Acacia* shows the maximum antibacterial activity which is may be due to the presence of inhibitory components in both of them. Thus the combination of both of them can be used to treat many diseases caused by pathogenic bacteria *E. coli.*

Keywords: *Acacia*, well diffusion method, antibacterial activity.

How to cite this article: Sharma, Kajal, Singh, Deepinderpal, Kaur, Sandeep, Kaur, Amanpreet and Kaur, Navjot (2019). Comparing antibacterial activity of "*Acacia* stem bark – cow urine extract" and various organic extracts against pathogenic bacteria *E. coli*. *Biological Forum – An International Journal*, **11**(1): 231-235.

INTRODUCTION

'Vagbhata' an ancient rishi wrote a book named "Astanga Hrdayam" around 3500 years ago. He mentioned the miraculous benefits of cow urine as it contains all the important components required for a healthy person, without causing any disease. According to ancient literatures distillate of cow urine was the one to be used mainly and the distillate was found to exhibit antoxidant effect (Krishnamurthi *et al*., 2004). Medical council of India and All India Institute of Medical Sciences have also tested and found that cow urine alone can cure near about 48 diseases in a complete way. As per all diseases of our body comes from imbalance of vata, pitta and kapha known as "tridosha". Cow urine maintains the balance between the three in our body and cures even so called incurable diseases (Kritikar and Basu, 2003). Cow urine treatment and research center, Indore has proved that various diseases viz; diabetes, asthma, migraine, thyroid, cancer, AIDS, arthritis, gynecological problems can not only be cure by urine therapy but it improve the quality of life also (Jain *et al*., 2010). In the present status, *Acacia* stem bark is used to study antibacterial activity by extraction of it in cow urine. The bark of this plant is strong antioxidant, astringent, anti inflammatory, antibacterial and antifungal in nature.

The extract of this plant is used to treat sore throats and diarrhea, also useful in high blood pressure, dysentery, gastric problems, bronchial asthma, cough, leucorrhoea and leprosy. It is used as mouthwash for mouth, gum, sore throat, gingivitis, dental and oral infections. The extract of *Acacia* species is found to stimulate the synthesis and release of prolactin in the female rate and may be given a better result for lactating women (Lompo *et al*., 2004). Today, the exploration of naturally derived antimicrobials are in great demand to reduce the several side effects caused by chemically synthesized antibiotics or antimicrobials.

MATERIALS AND METHODS

A. Sample Collection

Collection of cow urine: Fresh cow urine is collected in a sterile bottle in morning time and it is then filtered through an ordinary filter paper. Chemoprofiling confirmed the presence of protein, urea, uric acid, creatinine, phenol, aromatic acids, enzymes like acid phosphatase, alkaline phosphatase, amylase and vitamins (Gowenlock and McMurray, 1988).

Selection of plant part: The stem bark of *Acacia* spp. is collected from local area and kept for drying in a room under sunlight exposure for 5-6 days so that no or less constituents changes takes place. It was then powdered by using a grinder or mixer.

Fig. 1. Fresh cow urine.

Fig. 2. *Acacia* spp. stem bark powder.

B. Extract preparation

Cow urine extract: 10 gram of bark powder is mixed with 200 ml of fresh cow urine and sonicated for 5-6 hrs. The supernatant of extract was collected and stored in refrigerated for further use. The fresh cow urine is used rather than its distillate.

Organic extracts: 10 g of *Acacia* stem bark powder was weighed and successively extracted with 100 ml of sterile distilled water, chloroform, methanol and placed in a sonicator at temperature 37°C for 4.05 hours. The extracts were collected and the solvents were completely removed using vacuum and stored in refrigerated for further use.

Bacterial culture: The bacterial culture of *E. coli* MTCC 0165 was purchased from IMTECH Chandigarh, it was screened for the antibacterial activity of *Acacia* spp. and cow urine. The purity of bacterial culture was maintained to prevent it from any contamination.

Antibacterial assay: The comparison of antibacterial activity for various extracts of stem bark of *Acacia* spp. and its cow urine extract was tested by using Well Diffusion Method (Perez *et al.*,1990). The wells of 3 mm were made by using the hollow tube which was pressed above the semisolid Mueller Hinton agar medium that was inoculated in Petri plate.

One or two wells are made for testing the antibacterial activity.

Inoculums and incubation: 0.1ml of bacterial broth was inoculated to the agar plate and spread by using the sterile spreader. After that, the wells were made for testing the antimicrobial activity of extracts. The extracts were inoculated in wells and incubated at 37°C for 24-48 days in an incubator.

Procedure for testing the antimicrobial activity: Petri plates with Muller – Hinton agar were lawned with the inocula of 0.1 ml taken from the nutrient broth of *E. coli*. The wells of 3 mm were then applied to the lawned plates. The wells were then impregnated with 1ml of crude extracts respectively in each plate. Examination of inhibitory zone around each well was done, after an incubation period of 24-48 hours. The diameter of the inhibitory zone around each well was measured in mm was indicated as antibacterial activity (Kumari *et al*., 2018). Each extract of *Acacia* stem bark was tested thrice against the test organisms in triplicates along with cow urine control.

RESULTS AND DISCUSSION

It is discernible from the Table 1 that the treatments prepared using different concentration of extract showed antimicrobial activity.

Table 1: Antimicrobial activity of various extracts of *Acacia* spp. and cow urine against *E. coli*.

Name of extract	Conc. of the fraction in µL/zone of inhibition in mm		
	600 µL	800 µL	1000 µL
Cow urine (control) (T_1)	6±0.04	9.5±0.06	12±0.08
Aqueous extract (T_2)	4±0.05	6.2±0.03	10±0.04
Cow urine extract (T_3)	5±0.02	12±0.05	18±0.06
Methanol extract (T_4)	3.5±0.06	7.5 ±0.04	11.6±0.03
Chloroform extract (T_5)	Nil	5.8±0.02	8.6±0.04

Data presented as means ± SD (n=3). [ab] Means within rows with different uppercase superscript are significantly different ($p<0.05$) from each other.

T_1 = Cow urine (control), T_2 = Aqueous extract, T_3 = Cow urine extract, T_4 = Methanol extract, T_5 = Chloroform extract

Different treatments with the extracts prepared from *Acacia* spp. stem bark using fresh cow urine and different solvents showed varying degree of antimicrobial activity against pathogenic bacteria *E. coli*. Among these treatments, cow urine extract was found to be more effective against *E. coli* followed by methanol, aqueous extracts while chloroform extract was found least effective ($p<0.05$). All the extracts were found to be effective against the pathogenic organism. Maximum activity with respect to the zone of inhibition was recorded for treatment T_3 containing cow urine and *Acacia* spp. extract (18±0.06 mm at 1000 µL conc.) followed by treatment T_4 containing methanol extract (11.6±0.03 mm at 1000 µL conc.), treatment T_2 containing aqueous extract (10±0.04 mm at 1000 µL conc.) and treatment T_5 containing chloroform extract (8.6±0.04 mm at 1000 µL conc.).

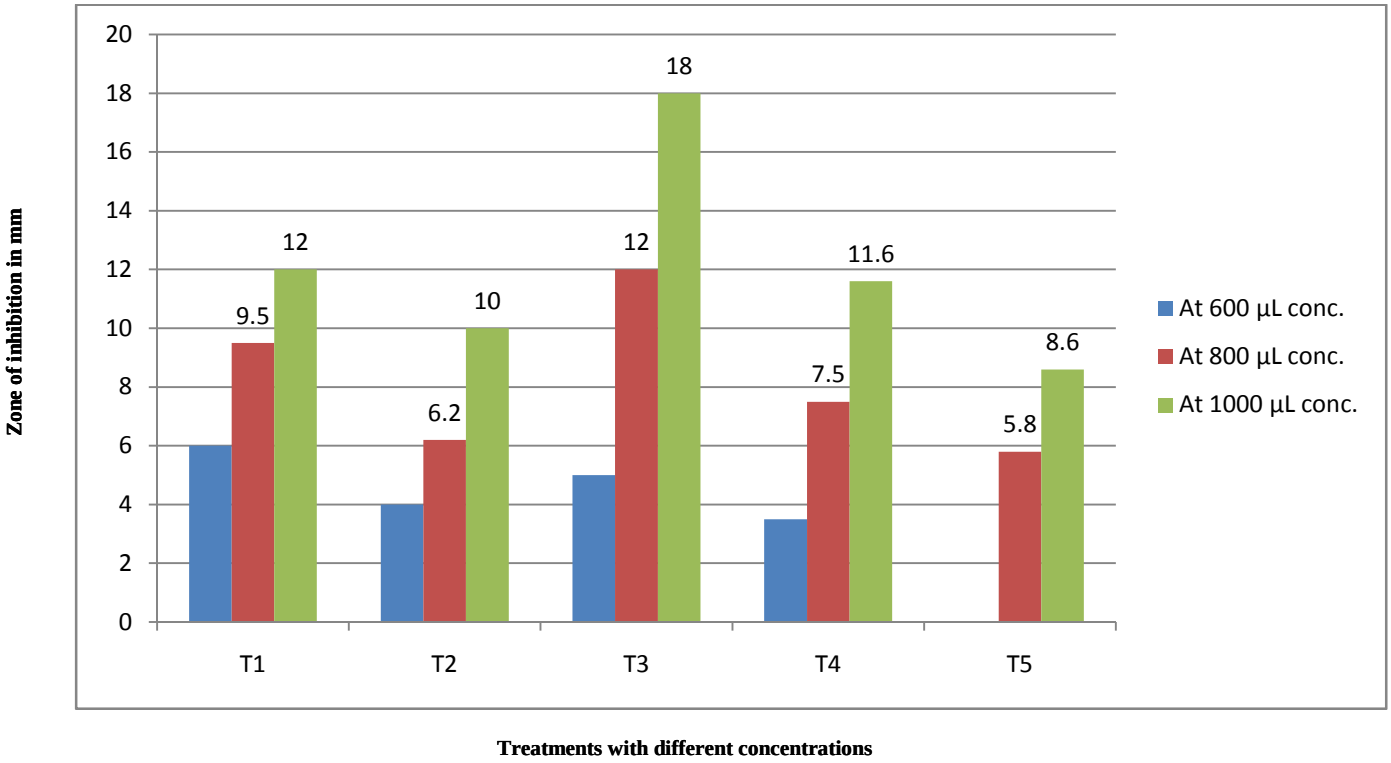

Fig. 3. Inhibitory action of different extracts of *Acacia* spp. against *E. coli.*

Fig. 4. Zone of inhibition at 600µL conc. showing by treatment T_3.

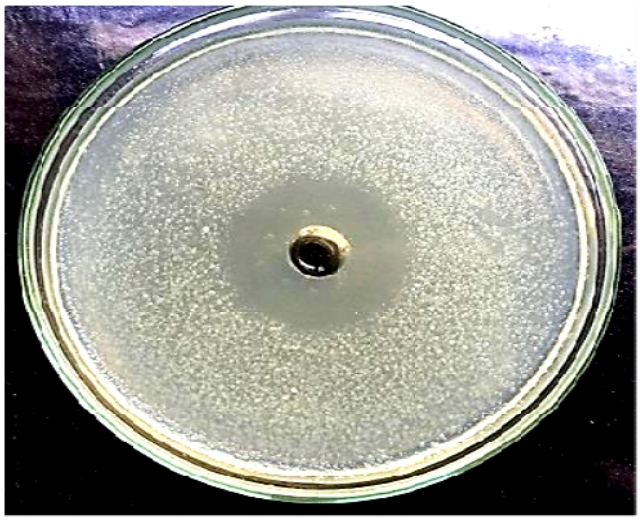

Fig. 5. Zone of inhibition at 800µL conc. showing by treatment T_3.

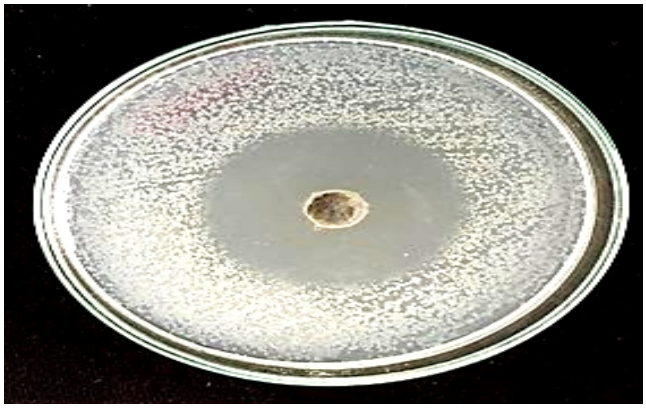

Fig. 6. Zone of inhibition at 1000µL conc. showing by treatment T_3.

It has been shown that cow urine extract of *Acacia* spp. has been reported to possess remarked antibacterial activity against pathogenic bacteria *E. coli*. This extract was found more effective at concentration of 1000 µg and produced 5-18 mm zone of inhibition. This is then followed by methanol, aqueous and chloroform fractions.

From last decades, this plant was used in treated many diseases, today it makes an important media for treatment of number of diseases like hypoglycemic (Wadood *et al.,* 1989), anti-aggregation platelet effect (Shah *et al*., 1997), antigen toxic and antimicrobial (Boubaker *et al.,* 2012), antihypertensive, antispasmodic (Gilani *et al.,* 1999, Arias *et al.,* 2004) and anti-inflammatory activity (Dafallah and Mustapha, 1996). The antimicrobial potency of plants is believed to be due to tannins, saponins, phenolic compounds, essential oils and flavonoids (Aboaba and Efuwape, 2001).

Tannins inhibit the microbial growth by causing the bacterial colonies to disintegrate by interfering with the bacterial cell wall (Erasto *et al*., 2004). Flavonoids also show antimicrobial activity by inhibiting the synthesis of nucleic acid or by inhibition of cytoplasmic membrane functioning and energy metabolism (Cushnie and Lamb, 2005). On the other hand phenols present in cow urine are effective bactericidal agents against a broad spectrum of gram negative and gram positive bacteria (Linton and Dick, 1990). It has been reported that presence of polyphenols and flavonoids in extracts are related to bactericidal activity (Acharyya *et al.,* 2009). Moreover it possesses antimicrobial activity due to the presence of urinary peptides, volatile and non-volatile substances and amino acids. Furthermore, the presence of more amount of phenol in cow urine compare to distillate may be the reason for its better activity (Edwin Jarald *et al.,* 2008). So the antimicrobial activity of *Acacia*-cow urine extract is due to the presence of diversity of major and minor compound with varying complexity. The present study reveal that the cow urine with *Acacia* spp. show efficient activity to reduce the growth of pathogenic bacteria.

CONCLUSION

From the above study it can be concluded that extract of cow urine and *Acacia* stem bark powder showed maximum zone of inhibition while the organic and aqueous extracts does not show such high antibacterial activity. Thus the plant based formulation of cow urine can be used for curing many diseases caused by *E. coli.* It is also an inventive step for the development of new drugs.

ACKNOWLEDGEMENT

At the outset, we are greatly indebted to the Department of Life Sciences, RIMT University, Mandi Gobindgarh, for allowing us to undertake the present study and permitting us to use the institute facilities.

CONFLICT OF INTEREST STATEMENT

All the authors declare that there is no conflict of interest.

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