The Pharma Innovation Journal 2019; 8(8): 307-310

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(8): 307-310

www.thepharmajournal.com
Received: 22-06-2019
Accepted: 24-07-2019

Dr. Kamal Kishor
M.V.Sc. Scholar COVS, A.H Jabalpur, Madhya Pradesh, India

Dr. Y.P. Sahni
Director Research Services COVS, A.H, NDVSU, Jabalpur, Madhya Pradesh, India

Dr. R.K. Sharma
Professor and Head I/C Dean COVS, A.H Jabalpur, Madhya Pradesh, India

Dr. Vidhi Gautam
Assistant Professor COVS & A.H Jabalpur, Madhya Pradesh, India

Dr. Anju Nayak
Associate Professor and I/C Head, COVS, A.H Jabalpur, Madhya Pradesh, India

Dr. Alka Sawarkar
Assistant Professor, Nagpur Veterinary College, Nagpur, Maharashtra, India

Correspondence
Dr. Kamal Kishor
M.V.Sc. Scholar COVS, A.H Jabalpur, Madhya Pradesh, India

In vitro antibacterial activity of Panchgavya, *Nigella sativa* and *Asparagus racemosus*

Dr. Kamal Kishor, Dr. Y.P. Sahni, Dr. R.K. Sharma, Dr. Vidhi Gautam, Dr. Anju Nayak and Dr. Alka Sawarkar

Abstract
In recent times, the scientific research has received a great concern about the indiscriminate use of antibiotics which leads to emergence of multiple drug resistance. Panchgavya, formulation of cow milk, curd, ghee, urine and dung has been claimed as antibacterial and growth promoter. Similarly indigenous herbs like *Nigella sativa* and *Asparagus racemosus* have been recommended as growth promoter and antibacterial. The present study was conducted to investigate *in vitro* antibacterial activity of Panchgavya, *Nigella sativa* and *Asparagus racemosus*. The results indicated that Panchgavya, *Nigella sativa, Asparagus racemosus* and their combinations exhibited *in vitro* antibacterial activity against *Staphylococcus aureus, Bacillus cereus, Escherichia coli* and *Salmonella* Typhimurium.

Keywords: Antibacterial, Panchgavya, *Nigella sativa, Asparagus racemosus*

Introduction
In recent years great concern has arisen about the use of antibiotics as supplement at sub-therapeutic level in poultry feed due to emergence of multiple drug resistant bacteria.The use of antibiotics as growth promoters is facing serious criticism. There are some important reasons that restrict the use of antibiotics such as the drug resistance in bacteria and the drug residues in meat. The utilization of growth promoters of natural origin become of an interest in recent years (Wray and Davies, 2000) [19]. Antibiotics can be replaced by alternatives such as prebiotics, probiotics and botanicals. Recently, Council for Scientific Industrial Research (CSIR), India has identified cow urine distillate for its antimicrobial and antifungal properties (Mathivanan *et al*., 2007) [12]. Panchgavya is one such formulation mentioned in Ayurveda, can be used as growth promoters in animals (Dhama *et al*., 2005) [3]. Panchgavya has been claimed to be useful against liver disorders, fever and inflammations and has hepatoprotective and immunostimulant activity in rats. The herb *Nigella sativa* is known as "Kalonji". *Nigella sativa* has been extensively studied for its biological activities and therapeutic potential and it possess wide spectrum of activities like antimicrobial, anthelmintic, analgesics, anti-inflammatory etc. Most of the therapeutic properties of this plant are due to the presence of thymoquinone, thymol and carvacol (Sarkar *et al*., 2015) [15]. Pharmacologically active constituents of *Nigella sativa* are thymoquinone, dithymoquinone, thymohydroquinone and thymol (Guler *et al*., 2006) [6]. The herb *Asparagus racemosus* is known as "Shatavari" or "Queen of herbs". *Asparagus racemosus* belongs to the family Aspragaceae traditionally used as anthelmintic, antiseptic, anti-diarrhoeal and anti-dysenteric. This plant is recommended in Ayurveda for prevention and treatment of gastric ulcers, dyspepsia and as a galactogogue. *Asparagus racemosus* has been successfully employed by some ayurvedic practitioners for inflammation, nervous disorder, liver diseases and certain infectious diseases (Sinha and Biswas 2011) [17].

Material and Methods
Plant material
Roots of *Asparagus racemosus* and seeds of *Nigella sativa* were procured from the Department of Aromatic and Medicinal Plants, Agriculture College, JNKVV, Jabalpur. Roots of *Asparagus racemosus* and seeds of *Nigella sativa* were dried, crushed and used for experimental purpose.

Preparation of Panchgavya
The Panchgavya was prepared as per the method described by Natarajan (2003) [14].

Sterility check
Panchgavya, seed powder of *Nigella sativa* and root powder of *Asparagus racemosus* were autoclaved at 121 °C for 15-20 minutes at 15 lbs. pressure to ensure their sterility.

Extraction of plant material
The seeds of *Nigella sativa* and roots of *Asparagus racemosus* were powdered with the help of grinder. The powdered samples were kept in an air tight container away from sun light until use. Extraction of powdered plant material was done through soxhlet extraction using ethanol as solvent in ratio of 1:10 g/ml (Sinha and Biswas, 2011) [17].

Table 1: List of procured culture from Hi media

S. No.	Bacteria	ATCC Catalogue No.
1.	*Escherichia coli*	25922
2.	*Salmonella* Typhimurium	13311
3.	*Bacillus cereus*	11778
4.	*Staphylococcus aureus*	6538

Preparation and sterilization of media
Mueller Hinton media (Hi Media) was prepared by adding agar into the distilled water. Hot plate was used for the proper mixing of media and autoclaved at 121 °C for 15-20 minutes at 15 lbs.

Preparation of Antibacterial disc
Different combinations of Panchgavya with *Nigella sativa* and *Asparagus racemosus* were prepared. Sterile discs were soaked with these combinations in a sterile petridish for 24 hours and dried in laminar air flow. Dried discs were used immediately for discs impregnation in the inoculated plates, as per the method described by Kirubaharan *et al*. (1999) [10] with slight modifications.

Preparation of bacterial broth
The known culture of *Escherichia coli*, *Salmonella* Typhimurium, *Staphylococcus aureusm* and *Bacillus cereus* was inoculated in brain heart Infusion broth and incubated at 37 °C for 2 to 6 hours. Bacterial concentration was determined as per the method described by Henric *et al.* (1956) [8].

Antibiotic sensitivity Test (AST)
Fresh, two to six hours bacterial culture of *Escherichia coli*, *Salmonella* Typhimurium, *Staphylococcus aureus* and *Bacillus cereus* was spread on sterile Mueller Hinton agar plate as per the method described by Bauer *et al*. (1969) [1]. Microbial growth inhibition effect was studied by the disc diffusion method. The dried discs inoculated with Panchgavya and plant samples were kept on each plate at a definite distance. A disc was also used for negative and positive controls. All the plates were incubated at 37 °C for 24 hour. The antibacterial activity was observed by the formation of zone of inhibition. Zone of inhibition was measured by antibiotic zone inhibition reader.

Results and Discussion
The antibacterial activity of Panchgavya, *Nigella sativa*, *Asparagus racemosus* and their combinations was evaluated against gram positive and gram negative pathogenic bacteria, namely *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* Typhimurium. Results were recorded in terms of zone of inhibition around the discs of Panchgavya, *Nigella sativa*, *Asparagus racemosus* and their combinations. The zone of inhibition around the disc indicated absence of bacterial growth.

Antibacterial sensitivity against gram positive bacteria
In-vitro antibacterial activities of Panchgavya, *Nigella sativa*, *Asparagus racemosus* and their combinations against different gram positive bacteria have been shown in table 02 and zone of inhibition shown in table 03. The Panchgavya and the ethanolic extract of *Nigella sativa* and *Asparagus racemosus* showed antibacterial activity by inhibiting the growth of *Staphylococcus aureus and Bacillus cereus.*

Table 2: *In-vitro* antibacterial activity of Panchgavya, *Nigella sativa*, *Asparagus racemosus* and their combinations against gram positive bacteria

S. No.	Combinations	***Staphylococcus aureus***	***Bacillus cereus***
1.	Panchgavya	S	S
2.	*Nigella sativa*	S	S
3.	*Asparagus racemosus*	S	S
4.	Panchgavya and *Nigella sativa*	S	S
5.	Panchgavya and *Asparagus racemosus*	S	S
6.	*Nigella sativa* and *Asparagus racemosus*	S	S
7.	Panchgavya, *Nigella sativa* and *Asparagus racemosus*	S	S

S-Sensitive

Table 3: Zone of inhibition (mm) exhibited by Panchgavya, *Nigella sativa*, *Asparagus racemosus* and their combinations against gram positive bacteria

S. No.	Combinations	Zone of inhibition (mm)	
		Staphylococcus aureus	***Bacillus cereus***
1.	Control (Standard Ciprofloxacin)	29	28
2.	Panchgavya	12	21
3.	*Nigella sativa*	18	19
4.	*Asparagus racemosus*	21	21
5.	Panchgavya and *Nigella sativa*	19	20
6.	Panchgavya and *Asparagus racemosus*	21	19
7.	*Nigella sativa* and *Asparagus racemosus*	20	18
8.	Panchgavya, *Nigella sativa* and *Asparagus racemosus*	18	19

Antibacterial sensitivity against gram negative bacteria

In-vitro antibacterial activities of Panchgavya, *Nigella sativa*, *Asparagus racemosus* and their combinations against different gram negative bacteria have been shown in table 04 and zone of inhibition shown in table 05. The Panchgavya and the ethanolic extract of *Nigella sativa* and *Asparagus racemosus* showed antibacterial activity by inhibiting the growth of *Salmonella* Typhimurium and *Escherichia coli*.

Table 4: *In-vitro* antibacterial activity of Panchgavya, *Nigella sativa*, *Asparagus racemosus* and their combinations against gram negative bacteria

S. No.	Combinations	***Salmonella* Typhimurium**	***Escherichia coli***
1.	Panchgavya	S	S
2.	*Nigella sativa*	S	S
3.	*Asparagus racemosus*	S	S
4.	Panchgavya and *Nigella sativa*	S	S
5.	Panchgavya and *Asparagus racemosus*	S	S
6.	*Nigella sativa* and *Asparagus racemosus*	S	S
7.	Panchgavya, *Nigella sativa* and *Asparagus racemosus*	S	S

S-Sensitive

Table 5: Zone of inhibition (mm) exhibited by Panchgavya, *Nigella sativa*, *Asparagus racemosus* and their combinations against gram negative bacteria

S. No.	Combinations	Zone of inhibition (mm)	
		***Salmonella* Typhimurium**	***Escherichia coli***
1.	Control (Standard Ciprofloxacin)	39	35
2.	Panchgavya	23	20
3.	*Nigella sativa*	20	17
4.	*Asparagus racemosus*	19	20
5.	Panchgavya and *Nigella sativa*	21	16
6.	Panchgavya and *Asparagus racemosus*	17	12
7.	*Nigella sativa* and *Asparagus racemosus*	18	14
8.	Panchgavya, *Nigella sativa* and *Asparagus racemosus*	19	16

The results clearly indicated that Panchgavya, *Nigella sativa* and *Asparagus racemosus* and their combinations exhibited antibacterial activity against gram positive bacteria viz *Staphylococcus aureus* and *Bacillus cereus.* Similarly gram negative bacteria such as *Escherichia coli* and *Salmonella* Typhimurium were also found sensitive against Panchgavya, *Nigella sativa*, *Asparagus racemosus* and their combinations. The maximum zone of inhibition around the discs treated with Panchgavya, *Nigella sativa* suggested there *in vitro* antibacterial activity against *Salmonella* Typhimurium and *Asparagus racemosus* against *Staphylococcus aureus* and *Bacillus cereus*.

The antibacterial activity of Panchgavya indicated that cow urine, a potential component of Panchgavya, produced antibacterial activity against various gram positive and gram negative bacteria (Jarald *et al*., 2008) [9]. The report of Gajbhiye *et al*. (2018) [5] also evaluated antibacterial activity of Panchgavya against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* Typhimurium and *Pseudomonas aeruginosa*. Among the four isolates of bacteria used in the study, Panchgavya produced maximum zone of inhibition against *Escherichia coli*, *Salmonella* Typhimurium and *Bacillus cereus* as 20, 23 and 21 mm, respectively. The similar study was conducted by Deepika *et al.* (2016) [2] who also observed considerable zone of inhibition of Panchgavya to the extent of 28 and 33 mm against *Escherichia coli* and *Staphylococcus aureus*, respectively.

In the present study, *Nigella sativa* also demonstrated considerable zone of inhibition against *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella* Typhimurium and *Escherichia coli* exhibiting 18, 19, 20 and 17 mm zone of inhibition, respectively. The antibacterial activity of *Nigella sativa* has also been demonstrated against various pathogenic bacteria by Khan and Kour (2016) [4] who reported that pathogenic bacteria *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella* Typhimurium are sensitive to ethanolic extract of *Nigella sativa*. Halawani (2009) [7] also reported antibacterial activity of *Nigella sativa* against *Escherichia coli*, *Salmonella* Typhimurium, *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacterial isolates. The similar work on antibacterial activity of *Nigella sativa* has

also been conducted by Mouwakeh *et al*. (2018) [13]. In the present study, ethanolic extract of *Nigella sativa* exhibited maximum zone of inhibition against *Salmonella* Typhimurium followed by *Bacillus cereus, Staphylococcus aureus* and *Escherichia coli* ranging from 20 to 17 mm.

Asparagus racemosus used in the study, exhibited antibacterial activity against *Staphylococcus aureus, Bacillus cereus, Salmonella* Typhimurium and *Escherichia coli* isolates of bacteria. The maximum zone of inhibition was observed against *Staphylococcus aureus* whereas minimum zone of inhibition against *Salmonella* Typhimurium. The reports of Mandal *et al*. (2000) [11] and Sinha and Biswas (2011) [17] also stated the antibacterial activity of *Asparagus racemosus* against gram positive and gram negative bacteria. Other coworker's viz. Shevale *et al*. (2015) and Uddin *et al*. (2012) [16, 18] also demonstrated *in vitro* antibacterial activity of extract of *Asparagus racemosus* against various pathogenic bacteria.

Several scientific reports stated above clearly indicated *in vitro* antibacterial activity of *Nigella sativa* and *Asparagus racemosus* against gram positive and gram negative bacteria which could be a source of evidence for accessing *Nigella sativa* and *Asparagus racemosus* as an antibacterial and an alternative of antibiotics.

Conclusions

Panchgavya, *Nigella sativa, Asparagus racemosus* and their combinations exhibited *in vitro* antibacterial activity against gram positive (*Staphylococcus aureus* and *Bacillus cereus*) and gram negative (*Salmonella* Typhimurium and *Escherichia coli*) bacteria. Panchgavya produced maximum zone of inhibition against *Salmonella* Typhimurium whereas *Asparagus racemosus* depicted maximum zone of inhibition against *Bacillus cereus* however *Nigella sativa* showed maximum zone of inhibition against *Salmonella* Typhimurium.

References

1. Bauer, AW, Kirby WMM, Sherris SC, Turk M. Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology. 1969*;* 45:493-496.
2. Deepika M, Nashima K, Rajeswari S. Antimicrobial activity of panchagavya against urinary tract infection. Internationat Journal of Current Pharmacological Research. 2016; 8(3):68-70.
3. Dhama K, Chauhan RS, Lokesh S. Anticancer activity of cow urine: current status and future directions. International Journal of Cow Science. 2005; 1:1-25.
4. Khan AR, Kour K. Wide spectrum antibacterial activity of *Nigella sativa* L. seeds. IOSR Journal of Pharmacy. 2016; 7(6):12-16.
5. Gajbhiye SP, More J, Kolte D. Antimicrobial activities (antibacterial and antifungal) effect of panchagavya alone prepared by fermentation method. Word Journal of Pharmacy and Pharmaceutical Science. 2018; 7(7):1336-1349.
6. Guler T, Dalkilic B, Ertas ON, Ciftci M. The effect of dietary black cumin seeds (*Nigella sativa* L.) on the performance of broilers. Asian-Australian Journal of Animal Science. 2006; 19:425-430.
7. Halawani E. Antibacterial activity of thymoquinone and thymohydroquinone of *Nigella sativa* L. and their interaction with some antibiotics. Advances in Biological Research. 2009; 3(5-6):148-152.
8. Henric J, Stafseth J, Stockton J, John O, Newman T. A laboratory manual of immunology. 3rd (Edn.), 1956, 4-6p.
9. Jarald E, Edwin S, Tiwari V, Garg R, Toppo E. Antioxidant and antimicrobial activities of cow urine. Global Journal Pharmacology. 2008; 2(2):20-22.
10. Kirubaharan JJ, Paliniswami KS, Anubukumar K, Mohan A. *In vitro* studies on antibacterial effect of crude garlic extract on *Escherichia coli.* Indian Veterinary Journal. 1999; 76:797-799.
11. Mandal SC, Nandy A, Pal M, Saha BP. Evaluation of antibacterial activity of *Asparagus racemosus* wild root phytotherapy research. An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2000; 14(2):118-119.
12. Mathivanan R, Edvin SC, Amutha R, Viswanathan K. Panchgavya and *Andrographis paniculata* as alternatives to antibiotic growth promoter on broiler production and carcass characteristics. International Journal of Poultry Science. 2007; 5:1144-1150.
13. Mouwakeh A, Telbisz A, Spengler G, Mohacsi-Farkas C, Kisko G. Antibacterial and resistance modifying activities of *Nigella sativa* essential oil and its active compounds against *Listeria monocytogenes*. *In Vivo*. 2018; 32:737-743.
14. Natarajan K. Panchgavya: a manual 1st (Edn.), 2003, 1-4p.
15. Sarkar CK, Mostofa M, Sikder MMH, Ansari WK, Ahamed MM, Parvej MS *et al*. Efficacy of kalojeera seeds (*Nigella sativa*) and Thankuni (*Centella asiatica*) supplementation on the growth performance of broiler chicken. International Journal. 2015; 1(4):196-200.
16. Shevale UL, Mundrawale AS, Yadav SR, Chavan JJ, Jamdade CB, Patil DB. Phytochemical and antimicrobial studies on *Asparagus racemosus*. World Journal of Pharmaceutical Research. 2015; 4(9):1805-1810.
17. Sinha, SN, Biswas M. Effect of extracts from *Asparagus racemosus* wild root against pathogenic bacteria. International Journal of Applied Biology and Pharmaceutical Technology. 2011; 2(3):312-314.
18. Uddin M, Ghufran MA, Idrees M, Irshad M, Jabeen S, Ahmad W *et al*. Antibacterial activity of methanolic root extracts of *Asparagus racemosus*. Journal of Public Health and Biological Sciences. 2012; 1:5-32.
19. Wray C, Davies RH. Competitive exclusion-an alternative to antibiotics. Veterinary Journal. 2000; 59:107-108.