

DETERMINATION OF MINIMAL INHIBITORY CONCENTRATION OF 70% ALCOHOLIC HOT EXTRACT OF *Piper nigrum* FRUIT EXTRACT AGAINST SOME PATHOGENIC BACTERIA *

M.K.PATIL, A.A.DESHMUKH¹, G.D.RANVIR², A.G.KARPE³ AND P.V.PATIL⁴

*Department of Veterinary Pharmacology and Toxicology,
College of Veterinary and Animal Science, Udgir-413517
Dist. Latur, Maharashtra (India)*

Abstract : The study was carried out to assess effective antibacterial concentration of 70% alcoholic hot extract of *Piper nigrum* fruit against pathogenic strains of *E.coli* (MTCC 96), *Staphylococcus aureus* (MTCC 96), *Streptococcus pyogenes* (MTCC 442), *Proteus mirabilis* (MTCC 1429) by Pelczar method. The present study revealed that *E.coli* and *Proteus mirabilis* are highly susceptible to the 70% alcoholic hot extract of *Piper nigrum* fruit as compared to other bacteria viz. *Staphylococcus aureus*, *Streptococcus pyogenes*.

Keywords : *Piper nigrum* (Piperaceae), Antibacterial effect.

Introduction

The dried fruit of *Piper nigrum* (Piperaceae) is categorized under spices and condiments. It possess antiperiodic, rubifacient, stimulant, aphrodisiac, anti-inflammatory, anthelmintic, antimalarial, antidiarrhoeal, resolvent, antipyretic and carminative properties and are useful in the treatment of cholera, dyspepsia, flatulence, asthma, biliousness, diseases of throat, nightblindness, piles, insomnia, toothache, diseases of spleen, chronic fever, leucoderma, lumbago, paraplegia, diarrhoea and various gastric ailments (**Anonymous, 1998; Anjaria, 2002 and Nadkarni, 1976**).

When it was screened for the antibacterial activity using different hot and cold

extracts viz. aqueous, 70% alcohol, acetone, chloroform and petroleum ether by Disc diffusion and Tube dilution method, it is found that 70% alcoholic hot extract showed potent antibacterial activity. Therefore 70% alcoholic hot extract was subjected for determination of minimal inhibitory concentration against some pathogenic bacteria.

Materials and Method

a) Collection of Plant material and Preparation of the Extracts

The Panniure-1 variety of *Piper nigrum* fruit was procured from Horticulture Division of Balasaheb Sawant Kokan Krishi Vidyapeeth, Dapoli, Dist.Ratnagiri (M.S.) for the investigation. The fine powder of *Piper nigrum* fruit was subjected for preparation of different

* Part of the M.V.Sc thesis submitted to MAFSU, Nagpur (Maharashtra)

1 Head, Deptt. of Veterinary Pharmacology and Toxicology, COVAS, Udgir, Dist. Latur (Maharashtra)

2. Associate Professor, Deptt. of Veterinary Pharmacology and Toxicology, PGIVAS, Akola (Maharashtra)

3. Associate Professor, Deptt. of Veterinary Microbiocology, COVAS, Parbhani (Maharashtra)

4. Veterinary Officer, RajaramBapu Milk Unit, Islampur, Tal.Walwa, Dist. Sangali (Maharashtra)

cold and hot extracts namely aqueous, 70% alcohol, acetone, chloroform and petroleum ether and extractability percentage for each extract was determined as per the method suggested by **Rosenthaler (1930)**.

b) Preparation of extract impregnated disc

The sterile blank disc was obtained from M/S, Hi-media Laboratory Ltd, Mumbai. Extracts impregnated discs were prepared using dissolved extracts in the respective solvents and impregnated on to the disc, until the discs get fully saturated and was air dried. The extract impregnated discs were collectively weighed before and after impregnation of the extract. The amount of the extract actually got absorbed on to the disc were recorded.

c) Test Organisms

The typed pathogenic bacterial culture of *Escherichia coli* (MTCC 723), *Staphylococcus aureus* (MTCC 96), *Streptococcus pyogenes* (MTCC 442), and *Proteus mirabilis* (MTCC 1429) were obtained from Microbial Typed Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh (Punjab). The pathogenic bacterial culture was sub-cultured and maintained on nutrient agar (MM 012) and in nutrient broth (M 088).

On using different hot and cold extracts *viz.* aqueous, 70% alcohol, acetone, chloroform and petroleum ether of *Piper nigrum* fruit by Disc diffusion and Tube dilution method, it is found that 70% alcoholic hot extract showed potent antibacterial activity as compared to other extracts.

Therefore effective antibacterial concentration (MIC) of 70% alcoholic hot

extract of *Piper nigrum* fruit was determined as per method suggested by **Pelczar *et al.* 1986**.

Minimum Inhibition Concentration

A set of cultural tube containing broth medium inoculated with test organism was placed with increasing amount of 70% alcoholic hot extract (2, 4, 6, 8 and 10 mg./ml). Two tubes in each set were kept as control i.e. one as bacterial control and other for extract control. The smallest amount of the extract that inhibits the growth of bacteria *in vitro* is referred as MIC.

All cultured tube treated with 70% alcoholic hot extract were incubated at 37°C for 24 hours. The concentration of 70% alcoholic hot extract of *Piper nigrum* fruit required to inhibit the growth of the organism was assessed spectrophotometrically by recording optical density (O.D.) and observing absence of growth or change in turbidity.

The data of this investigation were statistically analyzed by Student's t-test (**James, Crossland, 1980; Snedecor and Cochran, 1968**).

Results and Discussion

Among the 70% hot alcohol, hot chloroform and cold petroleum ether extracts of *Piper nigrum* fruit, the 70% alcoholic hot extract revealed better antibacterial activity as observe from Disc diffusion and Tube dilution method. Therefore the minimal inhibitory concentration of 70% alcoholic hot extract against the four bacteria was determined.

Table 1 depicts the minimal inhibitory concentration in terms of the mean optical density values of the four bacteria in extract control, bacterial control and extract treated

Table 1. Minimal inhibitory concentration (MIC) of 70% alcoholic hot extract of *Piper nigrum* fruit.

	Mean Optical Density \pm SE ^a			
	<i>E. coli</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus pyogenes</i>	<i>Proteus mirabilis</i>
Extract Control [No bacteria]	0.127 \pm 0.003	0.127 \pm 0.003	0.127 \pm 0.003	0.127 \pm 0.003
Bacterial control [No extract]	0.680 \pm 0.002	0.435 \pm 0.007	0.543 \pm 0.006	0.492 \pm 0.007
Extracts (mg/ml)				
2 mg/ml	0.588d \pm 0.003	0.398 \pm 0.05	0.503 \pm 0.031	0.487d \pm 0.003
4 mg/ml	0.617 \pm 0.036	0.407 \pm 0.103	0.500 \pm 0.031	0.491 \pm 0.031
6 mg/ml	0.650 \pm 0.259	0.213 \pm 0.040	0.467d \pm 0.014	0.489 \pm 0.007
8 mg/ml	0.598 \pm 0.014	0.437 \pm 0.003	0.510 \pm 0.023	0.488 \pm 0.057
10 mg/ml	0.660 \pm 0.026	0.149d \pm 0.027	0.518 \pm 0.013	0.502 \pm 0.116

d - Minimal Inhibitory Concentration

a - Mean of three observations

tubes (2,4,6,8 and 10 mg/ml). The lowest amount of the extract required to inhibit bacterial growth *in vitro* is considered as MIC value of the extract. It is evident from the results that the minimal inhibitory concentration of 70% alcoholic hot extract of *Piper nigrum* fruit was 2, 10, 6 and 2 (mg/ml) against *E.coli*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Proteus mirabilis* respectively. Hence it indicates much higher susceptibility of *E.coli* and *Proteus mirabilis* to the 70% alcoholic hot extract of *Piper nigrum* fruit as compared to the bacteria *Staphylococcus aureus* and *Streptococcus pyogenes*.

Acknowledgement

The authors are grateful to Horticulture Division of Balasaheb Sawant Kokan Krishi Vidyapeeth, Dapoli, Dist.Ratnagiri (M.S.) for of *Piper nigrum* for this study.

References

1. **Anonymous** : *Pashudhan*. 13 (4): 4 (1998).
2. **Anjaria, J.:** *Inventory of Traditional Veterinary Medicinal Practices in India*. Government of India, Ministry of Agriculture, 461 (2002).
3. **James, Cross-land.:** *Lewis's Pharmacology*. 5th ed. pp. 115-119, Churchill Livingstone Edinburgh London Melbourne and New York (1980).
4. **Nadkarni, K.M and Nadkarni, A.K. :** *Indian Materia Medica*, Vol.1, Popular Prakashan Private Ltd. Bombay, India. pp. 969 - 972 (1976).
5. **Pelczar, M.J., Chan, E.C.S. and Krige, N.R. :** *Microbiology*. 5th ed., pp. 535. McGraw Hill Book, Singapore (1986).
6. **Rosenthaler, L.:** *The chemical investigation of plants*. 1st ed., Bell and Sons, London: 36 (1930).
7. **Snedecor, G.W. and Cochran, W.G.:** *Statistical Methods*. pp. 534. Oxford and IBH, Calcutta (1968).

Address for correspondence:

Dr. (Miss) M.K.Patil, COVAS, Udgir,Dist. Latur (M.S.) E-mail. drpunam1@yahoo.com