IN VITRO ANTI-PASTEURELLOSIS ACTIVITY OF DIFFERENT EXTRACTS OF POLYALTHIA LONGIFOLIA LEAVES

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ABSTRACT

The outbreaks of duck pasteurellosis which occur during monsoon period, caused by avian strains of Pasteurella multocida, is a serious problem with high mortality and morbidity affecting younger age groups. Polyalthia longifolia (P. longifolia) leaves which had antibacterial action, was tested for its efficacy against P. multocida by in vitro methods. The successive extracts of the plant materials were tested for its antibacterial effect using microtitre plate technique (to estimate the minimum inhibitory concentration, MIC) and disc diffusion method was performed to estimate the zone of inhibition. Microtitre plate technique was assayed by dissolving the plant extracts in sterile DMSO except the aqueous extracts which were dissolved in sterile distilled water. Various concentrations, 200 µg, 500 µg and 1 mg/well, of successive extracts of P. longifolia were used to find out the MIC. Disc diffusion method was performed with successive extracts of P. longifolia leaves at 200 µg, 500 µg and 1 mg concentrations. The results from the above in vitro assay revealed the efficacy of acetic extract of P. longifolia leaves against duck pasteurellosis. The acetic extract was further subjected to spectrophotometric, thin layer chromatography and high performance thin layer chromatographic analysis. The results of the present study could describe the anti-pasteurellosis activity of P. longifolia leaves.

Keywords – Pasteurella multocida, Polyalthia longifolia, Microtitre plate dilution method, Disc diffusion method, Phytochemical analysis

INTRODUCTION

Duck pasteurellosis caused by avian strains of Pasteurella multocida (P. multocida) with high mortality and morbidity has become a major menace in the burgeoning of the poultry industry. The disease outbreaks occur more frequently during monsoon period affecting younger age groups (Devi et al., 2000). The indiscriminate use and side effects of once effective antibiotics along with the multiple drug resistance by the pathogenic organisms has created the necessity for an alternative for its treatment. Thus, curbing the disease by herbal treatment could be chosen as a substitute for antibiotic resistance. Moreover, the demand for plant based therapeutics is increasing in both developed and developing countries due to recognition that they are natural products, non-narcotic, easily biodegradable producing minimum environment hazards (Ghosh et al., 2008). Polyalthia longifolia (Sonn.) Thwaites (Order: Magnoliales; Family Annonaceae) commonly known as Ashok tree, is an evergreen ornamental plant. The ethnopharmacological claims for P. longifolia include the use of its bark as a febrifuge. The antibacterial potentiality of P. longifolia was reported against some reference bacteria (Faizi et al., 2003, Murthy et al., 2005, Nair et al., 2005). The antifungal properties (Nair et al., 2006, Shivpuri et al., 1997) of P. longifolia are also well documented. But the anti-pasteurellosis activity of this plant has not been analysed. Hence the present study was aimed at testing the efficacy of P. longifolia leaves against P. multocida by in vitro methods.

MATERIALS AND METHODS

Chemicals: Tryptone soya agar and reagents for biochemical tests were purchased from Hi-media,

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Mumbai. 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl-2H-tetrazoliumbromide (MTT) was obtained from Fischer Scientific Worldwide Company, Hong Kong, and all other reagents were purchased from Merck India Ltd, Mumbai. The standard reference drug, Co-trimoxazole (sulpha trimethoprim combination, 25 ìg/disc), was purchased from Pathoteq Biological Laboratories, Gujarat, India.

**Bacterial strains:** *P. multocida A:* 1 strain (DP1) isolated from Niranam Duck Farm (Pathanamthitta district), serotyped at IVRI, Izatnagar and maintained in the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy was used for the entire study. Purity of the isolate was checked based on morphology, cultural and biochemical characteristics as described by Barrow and Feltham (1993).

**Preparation of the extracts:** *P. longifolia* leaves obtained from College of Veterinary and Animal Sciences, Mannuthy campus, were air-dried at room temperature, coarsely powdered using an electrical pulverizer and successive extractions were carried out with petroleum benzine, chloroform, acetone and methanol using Soxhlet apparatus. The extracts were evaporated in a rotary vacuum flash evaporator. The aqueous extract was prepared by taking the 100 g of the powdered leaves in five litres of water subjected to boiling with constant stirring. The extracts were filtered through a muslin cloth. All the extracts were kept under refrigeration for the complete evaporation of the solvents. The yield of the extracts was 3.898, 6.438, 2.084, 7.503, 4.906 per cent respectively.

**Antibacterial activity:** Antibacterial activity was determined using microtitre plate dilution method and disc diffusion method.

**Microtitre plate dilution method:** Microtitre plate dilution technique was performed as per the method of Sheena et al. (2003). The inhibition of the growth was detected as colourless wells and the lowest concentration of the extracts that completely inhibited the bacterial growth was assumed as minimum inhibitory concentration (MIC).

**Disc diffusion method:** Disc diffusion method was performed to determine the zone of inhibition as per the method of Bauer et al. (1966). The antibiotic sensitivity was assayed from the diameter of the clear zone of inhibition (in mm).

**PHYTOCHEMICAL ANALYSIS**

**Preliminary qualitative analysis of the plant extracts:** The plant extracts obtained with petroleum benzine, chloroform, acetone, methanol and water were tested for the presence of various active chemical constituents namely steroids, alkaloids, tannins, phenolic compounds, flavonoids, glycosides, diterpenes, triterpenes and saponins as per the procedure quoted by Harborne (1991). Based on the above results of *in vitro* assay, the acetonic extract of *P. longifolia* leaves were quantitatively analysed using spectrophotometric method. Thin layer chromatography and High Performance Thin Layer Chromatography were also performed (Srivastava, 2011).

**RESULTS AND DISCUSSION**

**BACTERIAL STRAINS:** The isolate, after 18 to 24 h of incubation, produced typical colonies on blood agar which were smooth, convex, translucent and butyrous and one to three millimetres in diameter. Gram’s staining revealed gram negative cocccobacillary organisms arranged singly or in pairs. The isolate grew aerobically and anaerobically, did not grow on Mac Conkey’s agar, was non-haemolytic on blood agar and non motile. The reactions given by the *Pasteurella* culture were in accordance with findings of Barrow and Feltham (1993), Buxton and Fraser (1977) and Heddleston (1976).

**Antibacterial activity:** All the successive extracts of *P. longifolia* leaves showed inhibition of bacterial growth in microtitre plate dilution method. The minimum inhibitory concentration (MIC) of petroleum benzene, chloroform, acetone and aqueous extracts were 200 ìg/well. However, the methanolic extract showed minimum inhibitory concentration of 500 ìg/well. Acetonic extract of *P. longifolia* leaves showed antipasteurellosis activity and the extract showed a measureable zone of inhibition at 1 mg and 500 ìg concentrations in disc diffusion method. The zones of inhibition of the extract at 1 mg and 500 ìg concentrations were 12.00 and 9.00 mm respectively. All the other successive extracts did not show any zone of inhibition. The standard drug (25 ìg/disc) showed zone of inhibition at 33.2 mm. Sulpha trimethoprim combination has been used in the treatment of duck
pasteurellosis in the field conditions with better results than any other antibiotics. Hence this drug was chosen as the standard reference drug in in vitro studies. The zone of inhibition shown by the acetonic extract was not up to the level of standard drug meanwhile it exhibits some antipasteurellosis activity by in vitro analysis. This necessitates further in vivo study of these plant extracts. Faizi et al. (2003) have reported that the bioassay guided fractionation of the ethanolic extract of *P. longifolia* showed promising antibacterial activity against thirteen gram-positive and nine gram-negative organisms.

**PHYTOCHEMICAL ANALYSIS:** Petroleum benzene, chloroform and acetonic extracts showed the presence of only flavonoids. The methanolic extracts indicated the presence of tannins, flavonoids, diterpenes, triterpenes and saponins while the aqueous extracts showed the presence of steroids, phenolic compounds, tannins, diterpenes and saponins. Spectrometric analysis of the acetonic extract of *P. longifolia* yielded 10.50% flavonoids. Other active principles in the plant extract were not analysed since they were not detected in the qualitative analysis. The absorbance values of the acetonic extract were 2.153 (235 nm), 0.854 (306 nm) and 0.877 (323 nm). Phytochemical analysis of the acetonic extract of *P. longifolia* leaves showed the presence of flavonoids. Diterpenes, identified in *P. longifolia* leaves by thin layer chromatographic method, appeared as a spot with Ethyl acetate : Methanol : Water (7:2:1) solvent system. The HPTLC profile also indicated the presence of diterpenes.

**CONCLUSION**

The objective of the present study was to evaluate the antipasteurellosis activity of different extracts of *P. longifolia* leaves. The acetonic extract of *P. longifolia* leaves showed more antipasteurellosis activity as evident from microtitre plate dilution technique and disc diffusion method. Other successive extracts also showed antibacterial property but only in microtitre plate dilution technique. The antipasteurellosis activity of the acetonic extract could be attributed to the presence of diterpenes.

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**REFERENCES**


