

## Assessment of the antibacterial activity and genetic diversity of three black pepper varieties (*Piper nigrum* Linn.)

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### Introduction

*Piper nigrum* (black pepper) is an important spice to enhance flavor, color, aroma and taste of food. Black pepper is also considered as a medicinal plant species which is used to treat asthma, chronic indigestion, obesity, sinus, congestion and fever (Ravindran, 2000). It has an antibacterial activity further highlighting its medicinal importance (Dorman and Dean, 2000). In Sri Lanka, black pepper is considered as one of the important export agricultural crops. However, neither medicinal properties nor the antibacterial effect of pepper in Sri Lankan pepper germplasm has been studied in detail. In neighboring India, there were reports on the antibacterial activity of pepper on *Bacillus cereus* and *Bacillus subtilis* (Perez and Anesini, 1994). According to Chaudhry and Tariq, (2006) “piperine, ([1-[5-[1, 3-benzodioxol-5-yl]-1-oxo-2, 4, pentadienyl]piperidine), a pungent alkaloid present in black pepper enhances the bioavailability of various structurally and therapeutically diverse drugs”. The genetic diversity of pepper germplasm has been studied using Inter-Simple Sequence Repeat (ISSR) markers, microsatellite markers and Randomly Amplified Polymorphic DNA (RAPD) markers. The present study was conducted to assess the antibacterial activity of pepper oleoresin (an extract from pepper) from three varieties of *P. nigrum* in Sri Lanka and also to assess their genetic diversity using RAPD markers.

### Methodology

*P. nigrum* samples: three *P. nigrum* varieties, Panniyur-1, MB12 and GK49 were used for the analysis. Seeds were collected from these three varieties to extract oleoresin and young leaves were picked to extract DNA. The samples were collected from Central Research Station, Department of Export Agriculture, Matale, Sri Lanka.

Preparation of oleoresin: pepper seeds were ground into a fine powder using a mechanical grinder. Ten grams of powder was weighed and filled into a thimble. The thimble was placed in a Soxhelt apparatus and was exposed to several cycles of distillation. The concentrated solution was rotary evaporated to extract the oleoresin.

Evaluation of the antibacterial activity: the antibacterial activity of pepper oleoresin against *Escherichia coli* (Ingerson-Mahar and Reid, 2011) and *Staphylococcus aureus* using agar well diffusion method. Mueller Hinton agar plates were prepared. The two strains of microbial solution were prepared with compared to the 0.5 McFarland solution and then they were spreaded on the agar surfaces. Wells were cut by using sterile cork borer and bottom of the wells was sealed with a little bit of medium. Pepper oleoresins were loaded in to the wells by

using a micropipette and petri dishes were finally incubated at 37 C for overnight in an incubator. The diameter of the zones of inhibition (DZI) was measured in cm (Figure 1).

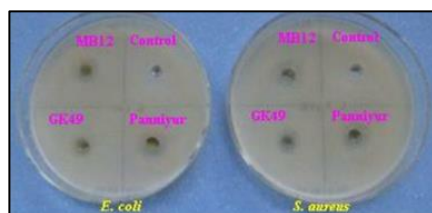


Figure 1. Antibacterial activity of three pepper varieties on *E. coli* and *S. aureus*.

Genomic DNA extraction: DNA was isolated by using Cetyl Trimethyl Ammonium Bromide (CTAB) method.

Polymerase Chain Reaction (PCR): Standard 15 µl PCR amplifications was performed using the thermal cycler (Takara, Japan) 94 C for 3min for initial denaturation followed by 30 cycles at 94°C for 45s, 36 C for 30 s and 72 C for 1.30 min and a final extension step at 72 C for 10 min. PCR products was size separated by using 1% agarose gel electrophoresis. PCR was performed for 15 RAPD primers (OPA1→14 and OPA18) (Operon Technologies, USA).

Data analysis: the DZI data of antibacterial activity were analyzed using General Linear Model Procedure in Statistical Package SAS 9.1 and DNA band data were analyzed for genetic diversity using the Statistical Package Minitab 14.

## Results and Discussion

Antibacterial activity: the oleoresins based on three pepper varieties, GK49, MB12 and Panniyur-1, showed significantly different antibacterial activity on *E. coli* and *S. aureus* in terms of DZI. As shown in Table 1, Mean DZI on all two bacterial species is 0.60 cm compared that of control test; DZI=0.00 cm ( $p>0.05$ ). However, *E. coli* (mean DZI was 0.40 cm) was significantly affected more than *S. aureus* (mean DZI was 0.49 cm) by the pepper oleoresins ( $p>0.05$ ).

Table 1. Antibacterial activity of oleoresins extracted from three varieties of *P. nigrum*.

<i>P. nigrum</i> variety	Diameter zone of inhibition (cm)
Control test	0.00b
GK49	0.58a
MB12	0.58a
Panniyur-1	0.62a

Means denoted by same letters are not significantly different at  $p>0.05$ .

The antibacterial effect of oleoresins of three pepper varieties on each of the bacterial species showed that all three oleoresins exhibited similar activity on *E. coli* (DZI=0.53 cm) whereas the varieties MB14 and GK49 had slightly higher antibacterial activity on *S. aureus* (DZI=0.63 cm). The oleoresin from the pepper variety Panniyur-1 conferred the highest recorded activity on *S. aureus* (DZI=0.70 cm). The variety Panniyur-1 was therefore considered as the pepper variety with highest antibacterial activity. The antibacterial effects of pepper reported by Karsha, and Lakshmi (2010) were consistent with these results.

Genetic Diversity: out of the 15 RAPD markers tested, six markers generated no bands and nine markers {OPA (1, 2, 9, 10, 11, 12, 13, 14 and 18)} generated polymorphic bands. The dendrogram constructed based on the polymorphic band data revealed that the three pepper

varieties were genetically diverse and the variety GK49 was the most distant variety from the rest (Figure 2). However, in order to comment on the genetic diversity of three varieties of pepper and any correlation of the diversity to antibacterial activity, more makers must be tested and that is one of our main focuses in the future.

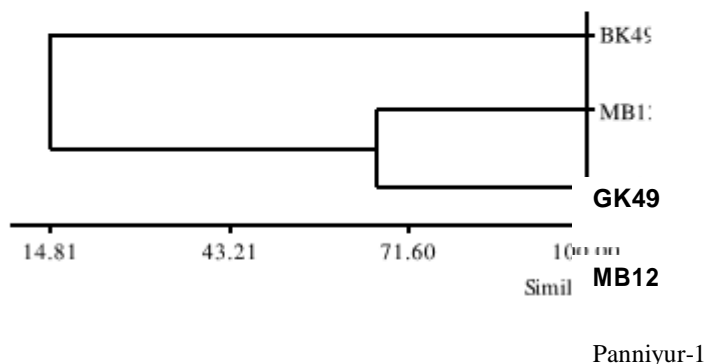


Figure 2. Dendrogram constructed based on the polymorphic RAPD band data using the algorithms of Single Linkage and Euclidean Distance in Minitab 14.

### Conclusions

The oleoresin extracted from pepper seeds has an antibacterial activity on *E. coli* and *S. aureus*. The pepper varieties GK49 and MB12 have similar levels of antibacterial activity but which is lower than that of Panniyur-1. *S. aureus* is affected more by pepper oleoresin compared to *E. coli* and Panniyur-1 exhibits the highest level of antibacterial activity. RAPD maker analysis revealed that the pepper varieties are genetically diverse. Such diversity must be further exploited and utilized to improve pepper as a successful cash crop with both enhanced medicinal and pungent properties.

### References

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