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Short communication

Effect of leaf extract of *Pandanus amaryllifolius* (Roxb.) on growth of *Escherichia coli* and *Micrococcus* (*Staphylococcus*) *aureus*

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Abstract

Pandanus amaryllifolius leaves are used in food preparation in Asian countries as flavoring agent. It was primarily used for giving a particular fragrance to rice preparation. Later on it was used in bread preparation to give a typical nutty flavor. The aroma compound is 2-acetyl-1-pyrroline derived from amino acid - phenylalanine. In this investigation an attempt has been made to find out effect of the leaf extract on growth of 2 important microorganisms i.e. *E. coli* and *M . aureus* from the point of view of food safety. It was found that the leaf extracts in water and ethanol stimulates the growth of these microorganisms which is an alarming signal as both these organisms are capable of producing potent endotoxin and exotoxin. *M. aureus* which was previously known as *Staphylococcus aureus* produces heat stable exotoxins in rice preparations. The reasons for this could be due to the reducing sugar content of 17% (as glucose) in the leaves, which gets easily extracted during cooking.

Introduction

Pandanus amaryllifolius (Roxb.) is a tropical plant which is used mostly as a flavoring agent for certain rice and bread recipe. It is a type of screw pine. The plant bears no fruits as it is sterile and is to be propagated by vegetative parts. The leaves are to be used preferably fresh but can also be used in dried form. It gives a nutty flavor to bread. The flavor is due to a compound called as 2-acetyl-1-pyrroline (Wongpornchai *et al*., 2003). This compound is also naturally produced in rice too but in many varieties it is inhibited by the enzyme betaine aldehyde dehydrogenase (Niu *et al.*, 2008) and hence these varieties of rice are not aromatic. However, after the cooking the leaves are to be discarded. These are not be consumed. There are some reports that the leaves can be used as cockroach repellent against *Blattella germanica* (L.) (Li and Ho, 2003). MacLeod and Pieris (1982) found that this species of *Pandanus* leaves is rich in terpenes and sesquiterpene hydrocarbons (6 - 42%), and the major aroma component - 2-acetyl-1-pyrroline (2AP) (Yoshihashi, 2002).

Beside the aromatic compound, the leaves also have been reported to contain maltodextrin (Chaiseri and Cheetangdee, 2006). The total carbohydrate content has been reported to be as high as 17% in the leaves. Many a times the leaf extracts are spray dried and used as fortifying agent for carbohydrate supplements. There are certain diseases of humans which can be controlled, by the extracts of the leaves, like hair loss, darkening hair, dandruff; neurasthenia, aneroxia, arthritis; different types of sore pains, etc. The diuretic effect of the leaves has been reported by Ysrael *et al.* (1995). Likewise several such applications are reported in a scattered manner.

This investigation was aimed at trying to find out whether by adding such leaves to rice preparation could reduce the incidences food poisoning (especially staphylocoocal food poisoning) by inhibiting its growth in the food preparation. It is well known that staphylococcal exotoxin is a heat stable toxin capable of withstanding temperature of 80°C to 100°C when heated for about 5 minutes or less (which is more or less a common practice in rural or semi rural areas) (Ortega *et al*., 2010). The organism cannot with stand temperature more than 60°C for few seconds. The second most important characteristic of this organism is that it is capable of growing under osmophilic condition like 5% NaCl solution. Beside *Staphylococcus* (now *Micrococcus*) *aureus* the effect of this plant on endotoxin producing microorganism like *E. coli* was also checked. Though rice is not a good medium for the growth and multiplication of this organism, it serves as an indication for the sanitation and hygiene in processing food material. This is especially true for plain rice or mildly spiced cooked rice. The staphylococcal exotoxin production is further enhanced if there are pieces of meat present in the cooked rice. Moreover, these pieces of meat would also give protection against heat, to these organisms.

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Material and Methods

The plant material was collected locally from botanical gardens. Different extracts of the leaves of the plants were made in solvents like ethanol and distilled water. Two types of extracts were made, one by soxhlet method (Jensen, 2007) and the other by keeping it at steady condition at 25°C to 28°C (occasionally shaking). In soxhlet extraction 20 g of the leaves (cut in to small pieces) were used where refluxing was carried out using 100 ml solvents like distilled water only. Ethanol extracts was made under steady state conditions. Likewise in the steady state 20 g of the materials were immersed in 100 ml solvent for 2 hours at 25°C to 28°C after suitable homogenization. The objective of preparing these extracts was to locate the antibacterial properties in any of these extracts.

The microorganisms used were *Escherichia coli* NCIM 2832 and *Micrococcus aureus* NCIM 2654. The antimicrobial action was first observed using agar well diffusion method using nutrient agar as medium (Irshad *et al.*, 2012). This was followed by checking the effect of the extracts on growth pattern of these organisms. Here, 10 ml of the respective bacterial suspension (of 90 minutes old culture) in saline containing 5 x 10^8 cells /ml, was added to 100 ml of medium along with 10 ml of the respective extract. Similar set of experiments were set, but instead of extracts, the respective solvents were added. This was the solvent control set. The third set of flasks was used where 10 ml of extracts were added but no organism was inoculated. This set of flasks, was used for adjusting zero of the spectrophotometer. The flasks were incubated for 150 minutes on a rotary shaker at 120 r.p.m. The growth was monitored by measuring absorbance at 540 nm at every 30 minutes interval. The solvent extracts were also used for checking reducing sugar content by Dinitro Salicylic acid method (Miller, 1959) and total carbohydrate by phenol sulfuric acid method (Masuko *et al*., 2005).

Results and Discussion

At the very beginning, it should be noted that it was not possible to carry out soxhlet extraction using both the solvents. However, it was possible to carry out extraction at steady state condition. It can be seen from Figure 1 and 2 that in case of *E. coli*, stationary state extractions using ethanol and distilled water showed enhanced growth, and it was very prominent with ethanolic extract. The soxhlet extract in distilled water did not have any significant effect on growth. Figure 3 and 4 indicates that *Micrococcus aureus* showed significant growth enhancement with ethanolic extracts prepared under stationary conditions at 25°C to 28°C and soxhlet extract in distilled water. This is in agreement with the previous observation (Dumaoal *et al.*, 2010). The reducing sugar content of the extracts was 17 percent on an average as glucose and total carbohydrate content

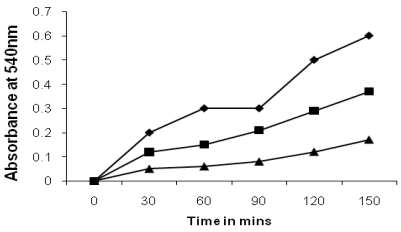

Figure 1. Growth pattern of *E. coli* in presence of aqueous extract of leaves of *Pandanus amaryllifolius*. (■) in presence of aqueous extract under stationary condition, (♦) growth without any extract and (▲)in presence of aqueous extract done in soxhlet.

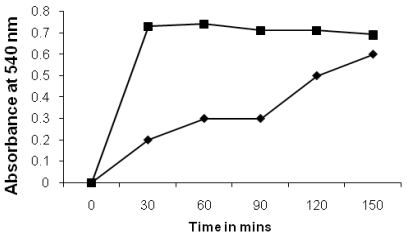

Figure 2. Growth pattern of *E. coli* in presence of ethanol extract of leaves of *Pandanus amaryllifolius.* (■) in presence of ethanol extract under stationary condition, (♦) growth without any extract.

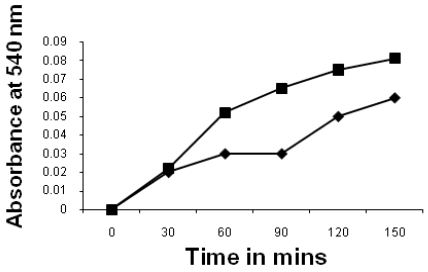

Figure 3. Growth pattern of *M. aureus* in presence of ethanol extract of leaves of *Pandanus amaryllifolius.* (■) in presence of ethanol extract under stationary condition, (♦) growth without any extract.

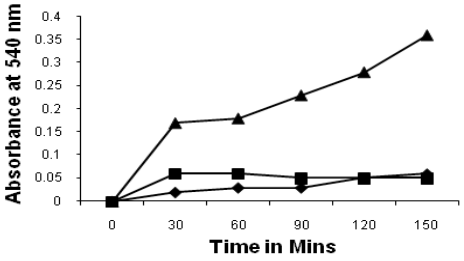

Figure 4. Growth pattern of *M. aureus* in presence of aqueous extract of leaves of *Pandanus amaryllifolius.* (▲) in presence of aqueous extract done in soxhlet, (♦) growth without any extract and (■) in presence of aqueous extract.

was 22%. This was independent of the solvent used in extraction procedures.This observation agrees with that of Chaiseri and Cheetangdee (2006).

There is very scanty information available on antimicrobial characteristics of the leaf extracts of this plant. Some researchers conducted experiments and found that the leaf extract (especially the aqueous extract) shows no inhibitory effect as seen by zone of inhibition by agar well method (Dumaol *et al*., 2010). This is inspite of reports that Pandan leaves contain certain alkaloids like Norpandamarilactonine-A,-B, Pandamarilactam-3x,-3y, Pandamarilactone-1, Pandamarilactonine-A,-B,-C, Pandamarine, Pandanamine (Lopez and Nonato, 2005). These alkaloids seems not to have any antimicrobial activity or anti bacterial activity. On the other hand, the enhanced growth of endotoxin producing *E. coli* and exotoxin producing *M. aureus* could be attributed to the presence of reducing sugar (estimated as glucose), could mean that food preparations, especially cooked rice preparations, if handled un hygienically and stored for sufficiently long period of time like overnight then it could lead to enhanced growth of potent heat stable exotoxin producers like *M. aureus*, increasing the probability of the incidence of staphylococcal food poisoning. It is well known that the mortality in such food poisoning cases is low, but it is high amongst elderly and infirm people and small children, especially with children below the age 10 years.

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