Molecular and Antibacterial Profile of Edible Oyster Mushrooms Pleurotus sajor-caju

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Abstract

Pleurotus varieties cultivated in Mauritius include 3 strains: *Pleurotus sajor-caju* strain CC114, *Pleurotus sajor-caju* strain CC200 and *Pleurotus sajor-caju* strain CC201. In this study the chemical composition, antimicrobial properties and genetic variation of the three *Pleurotus* strains were explored. Chemical screening of crude extracts of the *Pleurotus* strains revealed the presence of terpenes, phenols, alkaloids, saponins and hydrolysable tannins. Flavonols were however identified only in *Pleurotus* strain CC200 extracts and leucoanthocyanins were detected only in the extracts of *Pleurotus* CC114 strain. Antimicrobial activity was tested against *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus*. *Pleurotus sajor-caju* strain CC200 had more significant antimicrobial effect than *Pleurotus sajor-caju* strain CC114 and *Pleurotus sajor-caju* strain CC201 which both demonstrated nearly similar antimicrobial activity. Genomic DNA extraction was successfully carried out using the Phenol/Chloroform DNA extraction protocol and the DNA was purified using an RNAse treatment. Genetic relatedness among the three strains of *Pleurotus sajor-caju* was assessed using the RAPD technique. Out of the 50 primers used,

maximum polymorphism was observed using 8 Operon primers. Out of the 73 amplification products obtained with all three *Pleurotus* species, there was 28.8 % polymorphism which was observed. Maximum polymorphism was obtained following amplification using OPL 05. The fact that *Pleurotus sajor-caju* strain CC200 was least related to *Pleurotus sajor-caju* strain CC114 and *Pleurotus sajor-caju* strain CC201 could possibly explain the differences in the bioactivity of these mushrooms.

Keywords: Antimicrobial activity, Flavonoids, Pleurotus, Genetic diversity

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1. INTRODUCTION

Most of our medicines come from natural resources and scientists are still exploring the organisms of tropical rain forest for potentially valuable medical products. As the infectious microorganisms evolve and develop resistance to existing pharmaceuticals, bioprospecting for novel sources of medicines against bacterial, viral, fungal and parasitic diseases is now focusing on other lead sources including drugs of fungal origin.

Mushrooms have a long history of use as a source of food but also for their medicinal properties. The nutritive value of mushrooms is attributed to their high content of important amino acids, vitamins, minerals and low lipid content. The genus *Pleurotus* is one of the most diverse groups of cultivated mushrooms with high nutritional value, therapeutic properties and endowed with various environmental and biotechnological applications. *Pleurotus* are cultured on a wide variety of agro forestry products for the production of feed, enzymes, and medicinal products (Andrew *et al.* 2007).

Several medicinal properties have been reported in extracts of *Pleurotus* species. *Pleurotus* species have been used by human cultures all over the world for their nutritional value, medicinal properties and other beneficial effects. Oyster mushrooms are a good source of dietary fibre and other valuable nutrients. They

also contain a number of biologically active compounds with therapeutic activities. Oyster mushrooms modulate the immune system, inhibit tumour growth and inflammation, have hypoglycaemic and antithrombotic activities, lower blood lipid concentrations, prevent high blood pressure and atherosclerosis, and have antimicrobial and other activities (Gunde-Cimerman, 1999). Recent studies on various *Pleurotus* species have shown a number of therapeutic activities, such as antitumour, immunomodulatory, antigenotoxic, antioxidant, anti-inflammatory, hypocholesterolaemic, antihypertensive, antiplatelet-aggregating, antihyperglycaemic, antimicrobial and antiviral activities. Crude extracts of *Pleurotus* species have also been reported to have antitumour activities. Methanol extracts of *P. florida* and *P. pulmonarius* fruiting bodies significantly reduced solid tumours in mice (Jose & Janardhanan, 2000; 2002). It has been suggested that the bioactive compounds in the extract were water-soluble proteins, polypeptides and polysaccharides (Wasser, 2002). Methanol extracts of oyster mushroom have been shown to possess moderate antioxidant activities The antioxidant activity was positively correlated with total polyphenol content (Dubost *et al.* 2007; Elmastas *et al*. 2007). These activities have been reported for various extracts and isolated compounds, such as polysaccharides, polysaccharide- protein complexes, proteoglycans, proteins and DNA from oyster mushroom fermentation broth, mycelia or fruiting bodies. Crude extracts of *P. ostreatus* have been proved to possess antimicrobial activities against various microorganisms including gram-positive and gram-negative bacteria namely *Aspergillus niger, Fusarium oxysporum*, *Mycosphaerella arachidicola*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Gerasimenya *et al.* 2002). Antifungal activity of *P. eryngii* has been described against *Fusarium oxysporum* and *Mycosphaerella arachidicola (*Wang *et al*. 2004). *P. sajor-caju* extracts have been portrayed antimicrobial against *Fusarium oxysporum*, *Mycosphaerella arachidicola*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Ngai & Ng, 2006).

Oyster mushroom cultivation for the food industry is well established and its market has been steadily expanding in the recent years. The *Pleurotus* fruiting body is a cap-convex at first, expanding to broadly convex, eventually flat or up-turned and often wavy in age. It varies from 5-20 cm in diameter (Stamets, 1993)

and is white to greyish white in colour. The stalk of the mushroom is generally white, short and eccentric; that is not centrally attached. The gills are also white and overall, the mushroom is fragile relative to other types of mushrooms. *Pleurotus sajor-caju* is the most commercially cultivated mushroom and it exists as three strains in Mauritius: *Pleurotus sajor-caju* strain CC114, *Pleurotus sajor-caju* strain CC200 and *Pleurotus sajor-caju* strain CC201. These three strains of *Pleurotus* are morphologically similar except for the colour of their fruiting body. *Pleurotus sajor-caju* strain CC114 is white to cream in colour while *Pleurotus sajor-caju* strain CC200 is grey in colour and *Pleurotus sajor-caju* strain CC201 is grey to cream in colour (Table 1).

Table 1. Characteristics of the cultivated *Pleurotus sajor-caju* mushroom strains

	***Pleurotus sajor-caju* (CC114)**	***Pleurotus sajor-caju* (CC200)**	***Pleurotus sajor-caju* (CC201)**
Fruiting bag colonisation/ (week)	3-4	3-5	2-4
Susceptibility to contamination	Average	Average	Low
Initiation of pinhead formation/ (days)	7-10	6-12	4-7
Average yield/ fruiting bag of 3kg	750 g	710-745 g	760-815 g
Average number of fruits/ flush	1-10	3-7	10-20
Cap colour (At pinhead stage)	Cream	Grey black	Bluish grey
Cap colour (At maturity stage)	White to cream	Grey	Grey to cream

There are significant problems in classifying *Pleurotus* isolates using only morphological characters which are often unreliable due to the large influence exerted by environmental factors (Zervakis *et al.* 2001). Moreover, considering that the starting point in the cultivation of an edible mushroom is usually a pure culture or spores, the use of molecular tools is almost essential to ensure that the inoculum used is from the correct species.

Genotypic characterization is imperative to make suitable biological analysis about population, structure and evolutionary ways within and among species (Mahmood *et al*. 2009). Kumar (1999) and Astarini *et al*. (2004) have described the expression of particular gene as a collective outcome of environment and genetic makeup of a species/strain. Biochemical markers can be a source to reflect the genetic variability because they are direct product of genes. Molecular fingerprinting constitutes one of the efficient tools of plant biotechnology used for the assessment of genetic diversity (Mehmood *et al*. 2008). Several DNA markers along with morphological traits have been used for the determination of genetic variations at molecular level (Sajida *et al*. 2009). The genetic diversity of mushrooms has been revealed using molecular markers especially random amplified polymorphic DNA (RAPD) (Staniaszek *et al*. 2002; Stajic *et al*. 2005; Ravash *et al*. 2009). Molecular tools provide more accurate methods for identification than the few characters afforded by traditional morphological features (Fonseca *et al.* 2008). The Random Amplified Polymorphic DNA technique (RAPD) (Williams *et al*. 1990; Welsh & Mclelland, 1990; Manaf *et al*. 2006) has some advantages, as the efficiency to generate a large number of markers for genomic mapping without any previous knowledge about the organism genetics, the requirement of little amount of DNA, the quickness, simplicity and reproducibility in the data acquisition, the low cost and accessibility of this technology.

Singh *et al*. (2006) characterized several mushroom accessions using DNA fingerprinting and ribosomal rRNA gene sequencing and reported the presence of genetic diversity. Hyeon-Su *et al.* (2007) used RAPD markers to study the diversity of *Pleurotus eryngii* and its sequence analysis and reported that, grouping based on physiological parameters is closely related to RAPD based grouping. Stajic *et al.* (2005) used randomly amplified polymorphic DNA technique to assess the genetic diversity among 37 *Pleurotus* species of mushrooms. Stajic *et al.* (2005) highlighted that RAPD- polymerase chain reaction (PCR) amplification was better than morphological analysis to evaluate the genetic diversity among 45 *Pleurotus* strains.

Proper standardization of natural medicines and their formulations is dependent on consistent identification and quantification of active constituents in the plant material. The WHO has emphasized the need to ensure the quality of natural medicinal product using modern controlled techniques and applying suitable standards (WHO 1990; Chaudhury, 1992). The presence or absence of chemical constituent has been found useful in the placement of the plant in taxonomic categories. Edible mushrooms have been categorized as functional food due to their rich nutritional value and therapeutic properties described by several researchers, but regulation is permitted only after scientific validation of healthy physiological effects. To be considered as functional food, conditions of use and nutritional value, chemical composition or molecular characterization or the product formulation must be validated. Keeping in view the usefulness of morphological, chemical and molecular markers, the present study was planned to investigate the genetic diversity among different strains of cultivated mushroom and to validate their antimicrobial properties. *Pleurotus* cultivation has grown to a commercial scale in Mauritius and it is important for Mauritians to know the beneficial effects of eating these mushrooms. In this study, we focus primarily on the on aspects of the chemical composition and pharmacological properties of these mushrooms in an attempt to characterize them as functional foods and hence allow consumers to make a better choice.

2. MATERIALS AND METHODS

Mushroom collection: The three different *Pleurotus* strains were obtained from the Mushroom Unit of the Agricultural Research and Extension Unit (AREU), La-Brasserie, Mauritius. The species were authenticated with an accession number given by the Mushroom Unit of AREU itself (Table 1). The fungal strains are available in the culture collection of AREU and are referred as *Pleurotus sajor-caju* strain CC114, CC200, CC20

Molecular study

Sample preparation: The different strains of *Pleurotus* were obtained in test-tubes. The tubes were brought into the laboratory and each strain was sub-

cultured in ten petri-dishes containing Potato Dextrose Agar (PDA) as agar medium.

DNA extraction protocol: Genomic DNA extraction was carried out using a modification of the method used by Ranghoo *et al.* (1999, 2001). 0.1 g of fresh and young mycelium was ground using liquid nitrogen, dispensed in 500µl of extraction buffer (200 Mm Tris-HCl (pH 8.0), 500 Mm NaCl, 25 Mm EDTA (pH 8.0) and 0.5% SDS) in a 1.5ml eppendorf tube. 350µl of phenol was added followed by 150 µl of chloroform. The tube was centrifuged at 13,000 rpm at 4°C for 1 hour and the upper aqueous phase was transferred to a new 1.5 ml tube.

An equal volume of chloroform was added and mixed gently. The tube was centrifuged at 13,000 rpm for 10 minutes and the upper aqueous phase was transferred to a new 1.5ml tube. DNA was precipitated with 0.54 volume of cold iso-propanol and was left overnight at -20°C. The tube was then centrifuged at 13,000 rpm for 30 minutes and the DNA pellet was washed with 70% alcohol. The pellet was then dried in a centrifugal evaporator and resuspended in 100 µl of sterile distilled water. RNAse and phenol treatment was carried out as described by Ranghoo *et al.* (1999, 2001).

RAPD protocol: Genetic diversity among the *Pleurotus* species was assessed using the RAPD reaction and 50 Operon primers were screened. Each 25 µl of reaction consisted of 0.016 U of *Taq* polymerase, 0.2 Mm dNTPs, 1.5 Mm $MgCl_2$, 0.5 µM primer, 1x reaction buffer and 50 ng of DNA template. 45 amplification cycles were performed in a Thermocycler (Biorad Thermal Cycler). One denaturation cycle of 90 seconds at 95° C, each 45 cycles consisted of denaturation at 92° C for 30 seconds, annealing at 35°C for 1minute and primer elongation at 72° C for 3 minutes. The final primer elongation segment of the run was extended at 72° C for 10 minutes and 15° C for 5 minutes. Reaction products were resolved in a 1.5% agarose gel at 90V for 3 hours. PCR products were visualised by UV-fluorescent staining with ethidium bromide. After gel electrophoresis the RAPD markers were converted into a matrix of binary data where the presence of band corresponded to value 1 and the absence to value 0.

DNA bands showing quantitative variation in brightness were scored as present, regardless of their intensities, and absent if they were undetectable.

Chemical and antibacterial screening

Chemical screening: Chemical screening for tannins, coumarins, terpenes, phenols, alkaloids, anthraquinones, saponins, leucoanthocyanins and flavonols was undertaken using qualitative methods described by Harborne *et al.* (1975) and Govinden-Soulange *et al.* (2008).

Antimicrobial assay: The serial dilution technique by Eloff (1998) was used to determine the Minimum Inhibitory Concentration (MIC) for antibacterial activity of crude extracts. 2ml cultures of three bacterial strains [one gram negative, *Escherichia coli* (ATCC 25922), and two gram positive, *Staphylococcus aureus* (ATCC 2928) and *Bacillus subtilis* (ATCC 6633) were prepared and incubated overnight at 37°C. The overnight cultures were diluted with sterile Mueller-Hinton broth to an absorbance of 0.4-0.6 at 600 nm. For each bacterium used, 100 µl of the each extract solution tested was two-fold serially diluted with 100 µl sterile distilled water in a sterile 96-well microplate. A similar two-fold serial dilution of chloramphenicol (0.01mg/ml) was used as positive control against each bacterium. Methanol was used as negative control and 100 µl of bacterial culture was added to each well. The plates were covered, sealed and incubated overnight at 37° C. Bacterial growth was tested with the addition of 40 µl of 0.2 mg/ml *p*-iodonitrotetrazolium violet (INT) to each well after incubation at 37° C for 30 minutes. Bacterial growth in the wells was indicated by a red colour, and colourless wells indicated inhibition by the tested extracts.

3. RESULTS

DNA extraction after RNAse treatment: Genomic DNA extracted from the different varieties of mushrooms was clear indicating that RNA and other contaminants were successfully eliminated. The designed protocol proved to have been successful in removing polyphenols, proteins, and polysaccharides to

yield DNA of acceptable quality for PCR amplification. The A_{260}/A_{280} ranged from 1.726-1.885.

RAPD analysis: Out of the fifty primers used maximum amplification were obtained using primers OPA 18, OPB 11, OPA 10, OPW 04, OPC 16, OPD 13, OPL 05 and OP 003

RAPD markers and polymorphism: As per Table 2 the primer OPA 10 gave the greatest number of markers. The primer OPL 05 gave the highest percentage of polymorphism. The eight primers gave 73 amplification products with all the *Pleurotus* species with 28.8% polymorphism.

Table 2. RAPD markers used and percentage of polymorphism observed.

Primers	Number of markers	Number of polymorphic bands	% polymorphism
OPA 18	9	3	33.3
OPB 11	13	5	38.5
OPA 10	14	4	28.6
OPW 04	10	2	20
OPC 16	9	2	22.2
OPD 13	8	1	12.5
OPL 05	3	2	66.7
OP 003	7	2	28.6
Total	**73**	**21**	**28.8**

The method used for DNA extraction from the *Pleurotus* species was the Phenol-Chloroform based DNA extraction described by Ranghoo *et al.* (1999, 2001). In fact, not every protocol is suitable for fungal DNA extraction since fungi have

high amounts of polysaccharides and secondary metabolites content which can be removed only by using specific reagents. Spectrophotometric analysis confirmed the overall efficacy of the DNA protocol in conjunction with the RNAse treatment in removing polyphenols, RNA, proteins and most polysaccharides, to yield DNA of acceptable quality for PCR amplification. The A_{260}/A_{280} ratio ranged from 1.726- 1.885. The quality and concentration of DNA were sufficient to obtain PCR products and distinguishable DNA patterns for RAPD analysis.

The genetic diversity and relationship among the *Pleurotus* strains was determined using the RAPD method. Due to a lack of useful markers, the study of variation within filamentous fungi has frequently been limited (Crowhurst *et al.* 1991). According to Achenbach *et al*. (1996), RAPDs are better to indicate all genetic relatedness than sequence analysis of a single region of the genome. The assay is fast, independent of gene expression and proved to be beneficial for the isolates of any fungus. During PCR amplification, considerable differences in the overall product pattern and yield are a result of the variations in the amplification efficiencies. Therefore, it is important to optimize the PCR to obtain interpretable results. Normally, the final PCR is derived from the exponential amplification of the DNA template. Maximum amplification were obtained using primers OPA 18, OPB 11, OPA 10, OPW 04, OPC 16, OPD 13, OPL 05 and OP 003. Amplification with primer OPC 16, OPD 13, OPL 05 and OP 003 produced monomorphic bands which are the 700bp, 300bp, 500bp and 1000bp respectively with all three varieties. Those bands could be associated to fruit shape, stalk colour, stalk shape, stalk size and gills colour as they are all in the *Pleurotus* genus. These morphological traits which were present in all three species are characteristic of typical *Pleurotus* species. The monomorphic bands observed in the species, represents the highly conserved region of primer binding sites in the genome (Abrol & Bhatnagar, 2006). Out of the 8 primers run on all the *Pleurotus* species during the RAPD reaction, all of them produced polymorphic bands which differentiated the species from each other. Primer OPL 05 had yielded the highest percentage of polymorphism (66.7%) while OPD 13 produced the lowest percentage (12.5%). The presence of polymorphic bands might be due to nucleotide changes in DNA sequence at primer binding sites (Williams *et al*. 1990). Analysis of these DNA bands clustered *Pleurotus sajor-caju* (CC114)

with *Pleurotus sajor-caju* (CC201) separately from *Pleurotus sajor-caju* (CC200).

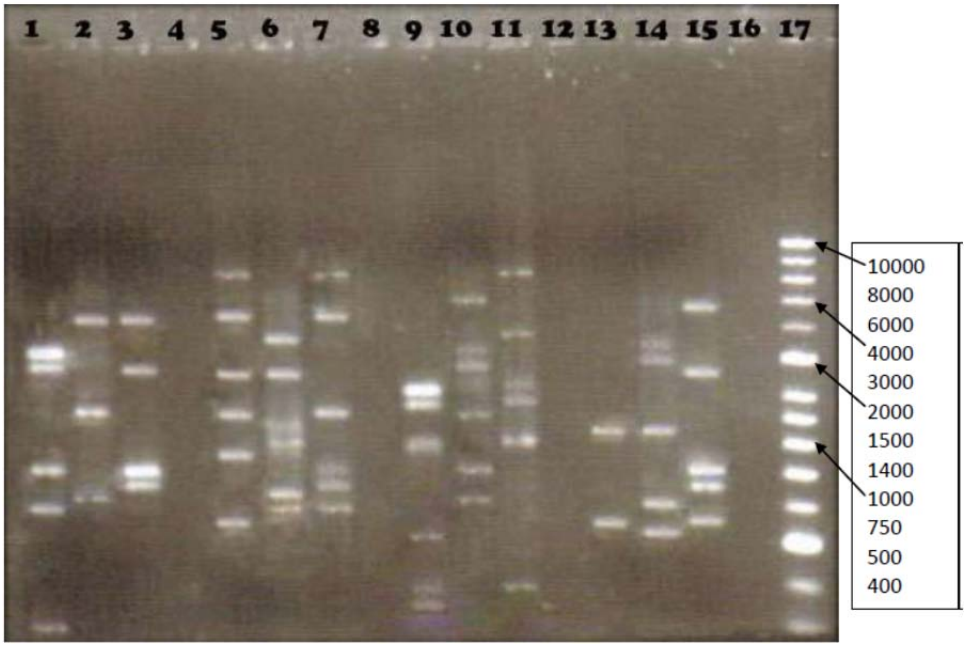

Fig. 1. RAPD Amplification of *Pleurotus* strains

Lanes 1, 5, 9, 13: *Pleurotus sajor caju* strain CC114; Lanes 2, 6, 10, 14: *Pleurotus sajor caju* strain CC200; Lanes 3, 7, 11, 15: *Pleurotus sajor caju* strain CC201; Lanes 4, 8, 12, 16: Negative control; Lane 17: Hyperladder II.

Lanes 1, 2, 3: Amplification with OPA 18; Lanes 5, 6, 7: Amplification with OPB 11; Lanes 9, 10, 11: Amplification with OPA 10; Lanes 13, 14, 15: Amplification with OPW 14.

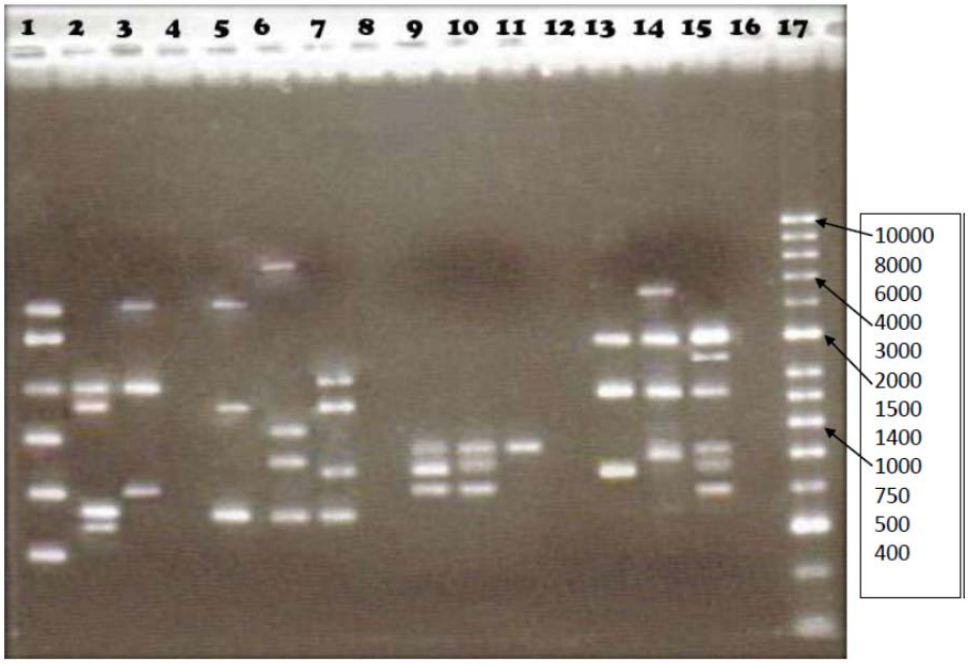

Fig. 2. **RAPD Amplification of *Pleurotus* strains**

Lanes 1, 5, 9, 13: *Pleurotus sajor caju* strain CC114; Lanes 2, 6, 10, 14: *Pleurotus sajor caju* strain CC200; Lanes 3, 7, 11, 15: *Pleurotus sajor caju* strain CC201; Lanes 4, 8, 12, 16: Negative control; Lane 17: Hyperladder II.

Lanes 1, 2, 3: Amplification with OPC 16; Lanes 5, 6, 7: Amplification with OPD 13; Lanes 9, 10, 11: Amplification with OPL 05; Lanes 13, 14, 15: Amplification with OPO 03.

Chemical screening: Terpenes, tannins, alkaloids, phenols and saponins were detected in all the strains whether in methanolic or dichloromethanolic extracts. Coumarins and anthraquinones were not detected in the extracts. Leucoanthocyanins were present only in *Pleurotus sajor-caju* strain CC114 and flavonols only in *Pleurotus sajor-caju* strain CC200.

Table 3- Compounds detected in *Pleurotus* crude extracts in different solvent systems+:

Tests	Methanol only(MeOH)			Methanol:Dichloromethane(1:1) MeOH:DCM		
	CC 114	**CC 200**	**CC 201**	**CC 114**	**CC 200**	**CC 201**
Coumarins	-	-	-	-	-	-
Terpenes	+	+	+	+	+	+
Tannins	+ (H)	+ (H)	++ (H)	++ (H)	+ (H)	++ (H)
Phenols	+	+	+	++	+	++
Alkaloids	+	+	++	++	+	++
Saponins	++	+	++	++	+	++
Anthraquinones	-	-	-	-	-	-
Leucoanthocyanins	+	-	-	++	-	-
Flavonols	-	+	-	-	+	-

+: Trace ++: Metabolite present -: Metabolite absent H: Hydrolysable tannins

Antimicrobial activity: The non- volatile extracts were tested against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* using the broth micro dilution assay. Table 4 represents the Minimum inhibitory concentrations (MIC) values of *Pleurotus sajor caju* strain CC114, *Pleurotus sajor-caju* strain CC200 and *Pleurotus sajor-caju* strain CC201 extracts against the tested bacteria**.** Lower MIC values were obtained for *Pleurotus sajor-caju* strain CC114.

Table 4. MIC crude extracts of *Pleurotus sajor-caju* (CC 114) *Pleurotus sajor-caju* (CC 200) and *Pleurotus sajor-caju* (CC201) against *E. coli, B. subtilis* and *S. aureus*.

	MIC (mg/ml)					
	E. coli		***B. subtilis***		***S. aureus***	
Extracts	**MeOH**	**MeOH:DCM**	**MeOH**	**MeOH:DCM**	**MeOH**	**MeOH:DCM**
CC 114	17.23	13.60	8.61	6.80	8.61	6.80
CC 200	NG	NG	NG	NG	NG	NG
CC 201	17.23	13.85	9.20	6.93	8.61	6.93
Chloramphenicol	6.25		1.56		6.25	

4. DISCUSSION

Pleurotus sajor-caju strain CC114 is different morphologically from the other two strains in that its cap colour at maturity is dissimilar from the other two. *Pleurotus sajor-caju* strain CC114 is white to cream in colour as compared to *Pleurotus sajor-caju* strain CC200 and *Pleurotus sajor-caju* strain CC201, which are grey and grey to cream respectively. The two *Pleurotus sajor-caju* strains (CC200 and CC201) are different from the typical *Pleurotus sajor-caju* strain CC114 as their cap colours at pinhead are greyish in colour. It could be set apart by the presence of RAPD markers unique to *Pleurotus sajor-caju* strain CC200 and *Pleurotus sajor-caju* strain CC201. For instance the primer OPA 18 produced a 1300bp band that was similar in *Pleurotus sajor-caju* strain CC200 and *Pleurotus sajor-caju* strain CC201 (Fig. 1). The band could be hypothesized to be associated with the cap colour at pinhead stage.

Analysis of RAPD data has shown that *Pleurotus sajor-caju* strain CC200 is relatively distant from *Pleurotus sajor-caju* strain CC114 and *Pleurotus sajor-caju* strain CC201. *Pleurotus sajor-caju* strain CC200 has a lower average yield of 710-745g compared to *Pleurotus sajor-caju* strain CC114 and *Pleurotus sajor-caju* strain CC201, which have a higher average yield of 750g and 760-815g respectively. The primer OPA 10 yielded unique polymorphic bands of 1400bp, 1100bp, 1000bp, 700bp, 500bp and 400bp in the variety *Pleurotus sajor-caju* strain CC200. However this same primer showed no amplification with the two

other varieties. These polymorphic bands among the others could be hypothesized to be associated with the average low yield. However, the use of morphological traits is not always the best way to evaluate genetic distance, since the degree of divergence between genotypes at the phenotypic level is not necessarily correlated with a similar degree of genetic difference.

Preliminary screening of the crude extracts of the mushroom species revealed the presence of terpenes, phenols, alkaloids, saponins and hydrolysable tannins in all the three *Pleurotus sajor-caju* strains (CC114, CC200, CC 201) irrespective of the solvent extraction system employed (Table 3). However flavonols were identified only in *Pleurotus sajor-caju* strain CC200 extracts only and leucoanthocyanins were detected only in both the MeOH and MeOH:DCM extracts of *Pleurotus sajor-caju* CC114 strain. The results obtained for the mushroom extract in mixture were in accordance to Iwalokun *et al.* (2007), who detected the same compounds except alkaloids, which could be due to the different growth stage and growing conditions of the mushrooms. The effect of conditions including biotic and abiotic stresses on secondary metabolism has been extensively reported. Besides, Ikewuchi (2009) and Ijeh *et al*. (2009) had previously reported the presence of alkaloids, flavonoids, tannins and saponins in crude methanolic extracts of *Pleurotus* spp. Iwalokun *et al.* (2007) had indicated the presence of terpenes in high amounts in petroleum ether extract of *Pleurotus ostreatus*.

The two *Pleurotus sajor-caju* strains CC201 and CC200 revealed a similar chemical profile with terpenes, tannins, phenols, alkaloid and saponins in crude extracts. We deduce from the above that the presence of flavonols in *Pleurotus sajor-caju* strain CC200 and leucoanthocyanins in *Pleurotus sajor-caju* strain CC114 extracts and the absence of these compounds in *Pleurotus sajor-caju* strain CC201 could possibly preliminarily discriminate the three strains of *Pleurotus sajor-caju* at the chemical level. In fact, anthocyanins represent one type of flavonoid compounds, which are widely distributed plant polyphenols. Flavonols, flavan-3-ols, flavones, flavanones, and flavanonols are additional classes of flavonoids that differ in their oxidation state from the anthocyanins hence the similarities detected between *Pleurotus* CC200 and CC114 strains.

It can be easily presumed (from Table 4) that the extracts with the most potent antimicrobial activities were those of *Pleurotus sajor-caju* strain CC200 as none of the test bacteria grew in either the MeOH or MeOH: DCM fractions of this strain.

Pleurotus sajor-caju strain CC114 and *Pleurotus sajor-caju* strain CC201 had the less significant antimicrobial activity with similar MIC values against *B. subtilis* and *S. aureus* for MEOH and MeOH:DCM extracts of the strain. The antimicrobial effect of the same extracts on *E. coli* was also insignificant as compared to *Pleurotus sajor-caju* strain CC200 extracts. The effective antimicrobial activity of *Pleurotus sajor-caju* strain CC200 extracts can probably be explained by the differences in chemical composition among the species. Flavonols were detected only in the extracts of *Pleurotus sajor-caju* strain CC200 and not in the other strains of *Pleurotus sajor-caju*. Antimicrobial activity of flavonols has been explained by their ability to react with cell wall proteins and to complex with bacterial cell walls (Cowan, 1999). Antimicrobial activity of *P. sajor-caju* extracts against *S. aureus* has been previously reported by (Ngai & Ng 2007). Other authors have described the antimicrobial effect of *Pleurotus* mushrooms against Escherichia *coli, Staphylococcus epidermidis*, *S. aureus* (Akyuz, 2010) and species of *Candida* (Wolf *et al.* 2008) ,*Streptococcus*, *Enterococcus* (Kotra & Mobashery 1998; Morschhauser *et al.* 2000; Sandven 2000; Thomson & Moland 2000). Akyuz *et al*. (2010) reported that bacteria are susceptible to chemotherapeutic compounds even against different strains. The antimicrobial activities of *Pleurotus* species are variable as reported by Akyuz *et al.* (2010) which may arise from the genetic composition of mushroom species, physical, chemical constituents, solvents, chemical differences between mushroom extracts and test micro-organisms.

Various secondary metabolites including flavonols could be responsible for the antibacterial effects of *Pleurotus* extracts including those detected in Table 4. According to Birk & Petri (1980), saponins combine with sterol in cells to produce changes in cell morphology leading to lysis. In the present study, tannins could also have contributed to the antimicrobial activity of the extracts as tannins decrease bacterial cell proliferation by blocking key enzymes of microbial

metabolism (Geidam, 2007). Funatogawa *et al.* (2004) demonstrated promising antibacterial of hydrolysable tannins activity against *Helicobacter pylori*. Terpenes have been implicated as the constituents responsible for the antibacterial activity of mushrooms including *Cuminum cyminum* and *Carum carvi* (Iacobellis *et al.* 2005). Alkaloids which also have antimicrobial effects (Goshal *et al.* 1996) and antidiarrhoeal effect (Cowan, 1999) were also present in the mushroom extracts and since *E. coli* are diarrhoea causing microorganisms, alkaloids could have contributed to the inhibitory effect of the mushrooms extracts.

5. CONCLUSION

The present study revealed the presence of common bioactive molecules such as terpenes, tannins, phenols, alkaloids, saponins, leucoanthocyanins and flavonols with the exception of coumarins and anthraquinones in the *Pleurotus* strains. However, flavonols were present only in the *Pleurotus sajor-caju* strain CC200 while leucoanthocyanins were present only in the *Pleurotus sajor-caju* strain CC114. RAPD analysis confirmed genetic similarities especially at the chemotaxonomic level, since the three varieties of *Pleurotus* are morphologically similar except for differences in colour of the fruiting bodies. On the basis of the chemical screening, *Pleurotus sajor-caju* strain CC200 is slightly different from *Pleurotus sajor-caju* strain CC114 *and Pleurotus sajor-caju* strain CC201 and could be differentiated by the presence of flavonols. *Pleurotus sajor-caju* strain CC114 *and Pleurotus sajor-caju* strain CC201 have been shown to share similar compounds and antimicrobial properties. The antimicrobial assays revealed that *Pleurotus sajor-caju* strain CC114 and *Pleurotus sajor-caju* strain CC201 demonstrated nearly similar antimicrobial activity as compared to *Pleurotus sajor-caju* strain CC200 which had more significant antimicrobial effect against the bacteria assayed. Flavonols which were detected only in *Pleurotus sajor-caju* strain CC200 and leucoanthocyanins which were present in only *Pleurotus sajor-caju* strain CC114 could be used to as chemotaxonomic markers to differentiate between the three *Pleurotus* strains. These individual compounds could also be responsible for the difference in colour with respect to their concentration and chemical structure. The RAPD analysis has generated sufficient polymorphism

that has successfully differentiated the three strains of *Pleurotus* sajor-caju at the molecular level. Some of the RAPD markers were typical of the *Pleurotus* genus and some specific to each strain. Therefore, the RAPD technique can be said to be reliable and promising for the characterization of *Pleurotus* species as confirmed by Hyeon-Su *et al.* (2007) and Stajic *et al*. (2005). The molecular results obtained in the study corroborate with the chemotaxonomy of the strains of *Pleurotus* which moreover are reflected in their antimicrobial attributes. This work contributes to include the three *Pleurotus* strains consumed in Mauritius: CC114, CC201 and CC 200 in the group of functional foods, by highlighting their antimicrobial attributes and differentiating their molecular and chemical profile.

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