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Optimization of mycelial growth and mycochemical screening of *Lentinus sajor-caju* (fr.) from Banaue, Ifugao Province, Philippines

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Lentinus sajor-caju (fr.). locally known as "ulat" in the Ifugao province, is one of the wild mushrooms used as food by the indigenous communities here. In this study, the optimal conditions for the mycelial growth of *L. sajor-caju* was studied to develop a protocol for its mass cultivation. Initially, *L. sajor-caju* was collected from a forest site in Banaue, Ifugao, Northern Philippines. Mycelial growth on different indigenous culture media and culture conditions, i.e. pH, aeration, illumination, and temperature were evaluated. Results showed highest secondary mycelial growth on coconut water gelatin (CWG) medium after 5 days of incubation. Secondary mycelia also grew best at pH 5, at room temperature (28-33℃), and in lighted and sealed or unsealed incubation conditions. The study also identified the chemical components and antioxidant activity of *L. sajor-caju*. Mycochemicals were extracted from dried mycelial mat with hot water. Results of the study showed the presence of terpenoids, glycosides, alkaloids, and saponins. Aqueous extracts of *L. sajor-caju* also contained a high amount of phenolics (75.29 mg/1g dried mycelial) but showed low radical scavenging activity (31.03%) as compared to the standard Catechin (97.41%).

Keywords: antioxidants, edible mushroom, functional chemicals, indigenous media

Introduction

Approximately 30,000 mushrooms are described worldwide (Kirk *et al.*, 2008), but only about 2,000 species are edible with around 20 species being cultivated commercially (Labarere and Gemini, 2000). Mushroom cultivation is a lucrative business especially in developing countries where agricultural wastes are utilized by mushroom growers as low-cost substrates (Khan *et al.*, 2012). Mushrooms are also abundant sources of a wide range of useful compounds including alkaloids, terpenoids, phenols, and steroids with documented bioactivities against diabetes, hypertension, hypercholesterolemia, and even cancer (Jonathan and Fasidi, 2003, Nwachukwu *et al*., 2010).

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In the Philippines, *Lentinus* species are cultivated for its nutritional and medicinal properties. In fact, two species, i.e. *Lentinus squarrosulus* (De Leon *et al*., 2013) and *L. tigrinus* (Dulay *et al*., 2012), were successfully cultivated using rice straw-based substrate formulations. Interestingly, of the 40 species of *Lentinus* Fr. known around the world (Kirk *et al.*, 2008), most are edible and/or used by the local people. For example, *L. sajor-caju,* which naturally grows on fallen logs during the rainy season (Eguchi *et al.*, 2014), is considered as an edible mushroom in the Philippines. Locally known as "ulat" in the Ifugao province in northern Luzon Island, this mushroom is used directly as food or as ingredient for local delicacies by the indigenous communities, albeit collected only in the wild and no commercial cultivation has yet been established. The aim of this research is to provide critical experimental design for a successful cultivation of *L. sajor-caju,* thereby potentially generating livelihood, ensuring food security, and promote environmental protection among the indigenous people in the countryside. The study also reports the mycochemical components and its antioxidant properties, providing additional support to the nutritional and pharmaceutical values of this mushroom.

Materials and Methods

Mushroom used in this study

Wild strain of *L. sajor-caju* (Figure 1) was collected from Banaue, Ifugao Province in Northern Philippines. This macrofungus is a wood decaying fungi and also referred to as a white rot fungus. It has a central to eccentric stalk, a fibrous or scaly cap and there are lamellate gills on the lower surface. The margins of cap are rolled under when old and dry. The spores are smooth, elliptic to cylindric and inamyloid with a whitish, yellowish or orangish spore print. It is found growing on dead wood and is edible, however the fruiting body is extremely tough (Bessette *et al*., 1997).

Figure 1. *Lentinus sajor-caju*

Tissue culture of L. sajor-caju

Initially, fruiting bodies of *L. sajor-caju* were brushed to remove any adhering wood particles and soaked in 10% sodium hypochlorite for 1 minute following the protocol of De Leon *et al*., (2013). The surfaced-sterilized mushrooms were aseptically sliced longitudinally to expose the sterile inner tissue. With a sharp, sterile scalpel, the inner tissue was cut and placed on previously prepared potato dextrose agar (PDA). A total of 10 explants were placed in 10 PDA plates. All culture plates were incubated at room temperature until the mycelia of the mushroom were fully ramified in the medium.

Screening of indigenous culture media for the mycelial growth of L. sajor-caju

The mycelial growth of tissue-cultured *L. sajor-caju* was evaluated on different indigenous culture media: potato sucrose gelatin (PSG), crack corn decoction gelatin (CCDG), and coconut water gelatin (CWG). For the preparation of PSG and CCDG, one liter decoctions of potato or yellow corn grits were mixed with 20 g shredded, commercially available, white gelatin bars and 10 g sucrose. To prepare the CWG, one liter of coconut water was filtered with cheese cloth, boiled for 20 minutes and mixed with 20 g shredded gelatin. All prepared indigenous culture media were sterilized by autoclaving at 121 ℃ (15 psi) for 15 minutes and plated. To evaluate the mycelial growth of *L. sajor-caju*, 10 mm diameter mycelial agar disc from a 7 day old cultures was inoculated centrally on all prepared indigenous culture media. All inoculated plates were incubated at room temperature and the mycelial growth in diameter was measured and recorded daily. Mycelial density was also observed visually and rated as (+) very thin, (++) thin, (+++) thick and (++++) very thick. The best indigenous culture medium is defined as the medium that produced the most luxuriant mycelial growth in the shortest incubation period.

Assessment of mycelial growth of L. sajor-caju in different culture condition

pH. Coconut water gelatin (CWG) was prepared as previously described with the pH adjusted to 4, 5, 6, 7 and 8 using 0.1 M NaOH or 0.1 M HCl prior to autoclaving. Mycelial discs (10 mm in diameter) from a 5 day old culture was inoculated at the center of the plates. All cultures were incubated at room temperature until the plates were fully ramified by the mycelia. Daily mycelial growth was measured and recorded and mycelial density was also observed visually and rated based on the scale that was previously described in the assessment of indigenous culture media.

Aeration. To evaluate the aeration requirement, the CWG adjusted to optimal pH (5) was initially inoculated with mycelial discs as previously

described and incubated at room temperature for 4 days under two conditions: sealed with layers of parafilm and unsealed. Mycelial growth was measured and recorded daily, while the mycelial density was also observed visually.

Illumination. For the assessment of the effect of light in the mycelial growth, a 10 mm diameter size of the mycelial disc was inoculated in the optimum culture media (CWG) with the optimum pH level (5) and aeration (sealed/unsealed) condition. These were incubated in lighted, full dark conditions and in alternating light and dark condition at room temperature until the plates were fully covered on by the mycelia. To provide full light condition, inoculated plates were exposed to artificial light and for full dark condition, the inoculated plates were covered with clean black paper. On the other hand, for the alternation of light and dark condition, set-ups were incubated for 12 hours in light and 12 hours in dark condition. Similarly the daily mycelial growth was measured and noted and mycelial density was also assess visually.

Temperature. Finally to evaluate the temperature effect, the most appropriate medium (CWG), pH (5), aeration (sealed/unsealed) and illumination (lighted) were used to evaluate the optimum temperature for mycelial growth. Petri dish plates with best culture medium and optimum pH level were inoculated with mycelial discs (10 mm diameter) and incubated at optimum aeration and illumination with different temperature conditions; room temperature (28-33^{o}C), air-conditioned (23-25^{o}C) and refrigerated (7-10^{o}C). Daily mycelial growth in all the petri dish plates were measured using a vernier caliper until the medium was totally covered on by the mycelia of *L. sajor-caju*.

Statistical Analysis

All the treatments were laid out in complete randomized design under laboratory conditions. One-way analysis of variance (ANOVA) was used to determined significant differences between treatments means using least significant differences (LSD) at 5% level of significance. The SAS 9.1 program were used for the analysis of data.

Screening for mycochemicals on mycelial mat of L. sajor-caju

Culture of *L. sajor-caju*. Initially, 100 ml mature coconut water was dispensed in a sterile culture dish and autoclaved at 121^{o}C (15 psi) for 30 minutes. These were inoculated with mycelial discs of *L. sajor-caju* and incubated at room temperature for 10 days. The mycelial mats were then harvested and air-dried for 5 days.

Preparation of powdered mycelia and aqueous extraction. Air-dried mycelial mat of *L. sajor-caju* was cut into pieces and powdered with a blender.

To obtain the aqueous extracts, 20 g powdered mycelia was dissolved in 600 ml distilled water and boiled in double boiler water bath (80-90 ℃) for two hours as described in the protocol of Eguchi *et al.* (1999). The aqueous mycelial extract was filtered and stored in a refrigerator prior to the assays.

Qualitative tests for mycochemicals. The hot water extracts and powdered mycelia of *L. sajor-caju* were screened for the presence of steroids, terpenoids, flavonoids, glycosides, tannins, saponins and alkaloids. Test were done in duplicates. Results were compared with distilled water as control and interpreted as: (+) if chemicals are present in trace amount, (++) if the chemicals are present in substantial amount, and (-) if chemicals are absent (Guevarra and Recio, 1985).

Test for Steroids. Two ml each of acetic anhydride and sulfuric acid (H_2SO_4) was added to 5 ml aqueous extract. A color changed from violet to blue or green indicates the presence of steroids.

Test for Terpenoids. Five ml aqueous extract was mixed with 2 ml chloroform ($CHCl_3$) in a test tube. Three ml concentrated H_2SO_4 was carefully added to the mixture to form a layer. An interface with reddish brown coloration was formed if terpenoids were present.

Test for Flavonoids. A few drops of 1% ammonia (NH_3) solution were added to 5 ml aqueous extract in a test tube. A yellow coloration indicates the presence of flavonoids.

Test for Glycosides. Five ml aqueous extract were mixed with 2 ml glacial acetic acid (CH_3CO_2H) containing 1 drop of ferric chloride ($FeCl_3$). The above mixture was carefully added to 1 ml concentrated H_2SO_4. The presence of brown ring indicates glycoside constituents.

Test for Tannins. Powdered mycelia (0.5 g) was boiled in 20 ml distilled water and filtered. To which, 0.1% ferric chloride ($FeCl_3$) was added to observe for brownish green or blue black coloration indicating the presence of tannins.

Test for Saponins. Two gram powdered mycelia was boiled in 20 ml distilled water in a water bath and filtered. The filtrates (10 ml) was mixed in 5 ml of distilled water in a test tube and shaken vigorously to obtain a stable persistent froth. The frothing was mixed with 3 drops of olive oil and observed for the formation of emulsion, which indicates the presence of saponins.

Test for Alkaloids. Five gram powdered mycelia were mixed with 200 ml of 10% CH_3CO_2H in ethanol (C_2H_5OH). The mixture was allowed to stand for 4 hours, filtered and the extract was concentrated in a water bath until it reaches ¼ of the original volume. Concentrated ammonium hydroxide (NH_4OH) was added and the formation of white precipitate or turbidity indicates the presence of alkaloids.

Assay for Antioxidant Activities

DPPH radical scavenging assay. The radical scavenging activity of the aqueous extracts of *L. sajor-caju* was assayed following the procedure described by Kolak *et al.* (2006). The aqueous extract was dissolved in methanol to a final concentration of 500 ppm. A 0.1 mM DPPH in methanol was freshly prepared by diluting 1 mL DPPH stock solution (3.49 mg DPPH in 10 mL methanol) to 100 mL methanol. Then, 1 mL of each extract and 4 mL of DPPH solution was mixed and incubated in the dark at 37^{o}C for 30 minutes. Triplicate test were done in each extract. The absorbance reading was monitored at 517 nm using UV-Vis spectrophotometer (APEL-100) and the ability to scavenge the DPPH radical was calculated using the equation: % DPPH scavenging effect = $[(A_{control} - A_{sample})/ A_{control}] \times 100$ where $A_{control}$ was the absorbance of the control and the A_{sample} was the absorbance of the test sample containing the mixture of DPPH and the aqueous extract. The synthetic antioxidant cathechin was used as positive control.

Test for phenolic compounds. Phenolic compounds of the aqueous extracts was determined using the Folin – Ciocalteu method as described by Hodzic *et al.* (2009). A calibration curve was made at different concentrations, i.e. 0.25, 0.5, 1.0, 2.0 and 4.0 mg/ml of ascorbic acid, using APEL-100 UV-Vis spectrophotometer (PD-303UV). These ascorbic acid solutions (volume of 20 µL were placed in vials. To each vial, 200 µL of Folin – Ciocalteu reagent was added and incubated at room temperature for 5 minutes. Then, 1 ml of sodium carbonate (1 mg/ml concentration) was added to the mixture of ascorbic acid and FC reagent, and then transferred to cuvettes and read using UV-Vis spectrophotometer at 680 nm wavelength. A standard curve was then prepared from these data. To text for the TPC, 200 µL of the aqueous extract was mixed with 200 µL FC reagent and incubated at room temperature for 5 minutes. Then, 1 ml sodium carbonate was added to the mixture and read using the APEL-100 UV-Vis spectrophotometer (PD-303UV) at 680 nm wavelength. Absorbance values of the extracts were compared with the calibration curve using the ascorbic acid. The phenolic compound was calculated based on the standard curve of the ascorbic acid and its linear regression as shown in the equation: $y = mx+b$**.** Where y represents the OD, m represents the slope of ascorbic acid, and b represents the y-intercept. Ascorbic Acid Equivalent (AAE) is expressed as mg AAE/gram sample.

Results

Growth of L. sajor-caju on different indigenous culture media

The luxuriance and rapidity of growth of a certain mushroom partly depend on the appropriate culture medium used in its cultivation in the

laboratory (Reyes *et al*., 2009). The growth of mycelia is influenced by the nutritional content of the organic matter of the substrates. Nutritional evaluation is therefore necessary to determine the best medium which favors the efficient growth of mushroom species. To determine the most suitable media that provides nutrients for the growth of *L. sajor-caju*, this study evaluated three indigenous culture media such as potato sucrose gelatin (PSG), coconut water gelatin (CWG) and cracked corn decoction gelatin (CCDG). Table 1 shows the mean mycelial growth diameter and mycelial density of *L. sajor-caju* on different indigenous culture media. CWG recorded the widest mycelial diameter with mean of 94.01±0.31 mm while PSG recorded the smallest mycelial diameter with a mean of 88.75±2.88 mm after 5 days of incubation period. Analysis of variance revealed that the mycelial growth in CCDG is comparable to PSG and CWG. However, thin mycelial density was observed in CCDG while very thick mycelia ramification was recorded in CWG and PSG after 5 days of incubation period (Figure 2).

Table 1. Mycelial diameter and density of *L. sajor-caju* on various indigenous culture media for its mycelial growth

	Mycelial Diameter (mm)	**Incubation Days**	**Mycelial Density**
Indigenous Culture Media			
PSG	88.75^{b}±2.88	5.00^{a}	++++
CWG	94.01^{a}±0.31	5.00^{a}	++++
CCDG	91.15^{ab}±0.79	5.00^{a}	++

Means with the same letter are not significantly different from each other at 5% level of significance. Note: (+) very thin, (++) thin, (+++) thick, (++++) very thick, (-) no growth

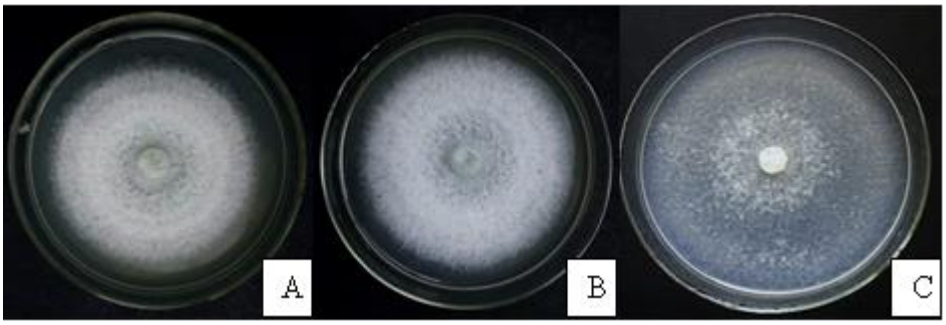

Figure 2. Mycelial growth performance of *L. sajor-caju* on different indigenous culture media: (A) PSG, (B) CWG and (CCDG) after 5 days of incubation period

Growth of L. sajor-caju in different culture conditions

pH, temperature, light and aeration are the key factors affecting the mycelial run of different mushroom species (Stott and Mohammed, 2004). Since mycelial growth of *L. sajor-caju* grew best in CWG, this medium was used in the evaluation of physical factors required for the optimum mycelial growth of *L. sajor-caju*.

pH. Among the different pH levels (4, 5, 6, 7, 8), *L. sajor-caju* cultured on coconut water gelatin at pH 5.0 registered the largest mycelial growth with a mean of 97.31±4.65 mm while the smallest growth was recorded in pH 8.0 with a mean of 78.110.92 mm (Table 2). The results showed that, luxuriant mycelial density were obtained in acidic, neutral and alkaline environment (Figure 3). However, pH 8.0 has the longest incubation period of 8 days while pH 4.0 -7.0 showed no significant difference in incubation period of 5 days. The study shows that rapid mycelial growth of *L. sajor-caju* was observed at pH 4.0 to pH 7.0. Although, analysis of variance revealed that pH 5.0 was significantly different to other pH level which means that 5.0 is the most suitable pH for mycelial growth of *L. sajor-caju.*

Table 2. Mycelial diameter and density of *L. sajor-caju* on various physical requirements for its mycelial growth

	Mycelial Diameter (mm)	Incubation Days	Mycelial Density
pH Levels			
4	91.38^{b}±1.66	6.00^{a}	+++++
5	97.31^{a}±4.65	5.33^{a}	+++++
6	91.00^{b}±1.84	6.00^{a}	+++++
7	91.16^{b}±2.23	6.00^{a}	+++++
8	78.11^{c}±0.92	8.00^{b}	+++++
Aeration			
Sealed	74.36^{a}±2.54	4.00^{a}	++++
Unsealed	81.25^{a}±4.78	4.00^{a}	++++
Illumination			
Light	100.00^{a}±0.00	5.00^{a}	++++
Dark	90.18^{a}±8.50	5.67^{a}	+++
Light and Dark	94.06^{a}±5.27	5.67^{a}	++++
Temperature			
Refrigerated	10.00^{c}±0.00	N/A	-
Room Temp.	100.00^{a}±0.00	5.00^{a}	++++
Air-condition	81.38^{b}±0.85	6.00^{a}	++++

Means with the same letter are not significantly different from each other at 5% level of significance. Note: (+) very thin, (++) thin, (+++) thick, (++++) very thick, (-) no growth

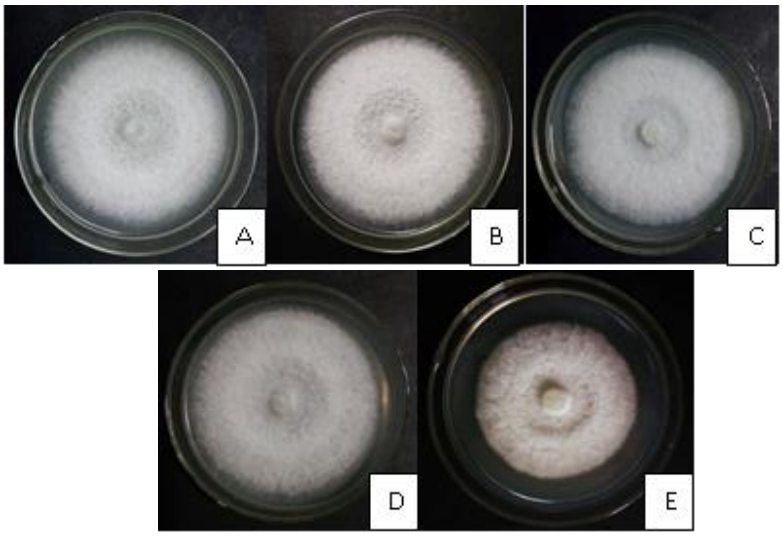

Figure 3. Mycelial growth performance of *L. sajor-caju* on coconut water gelatin with different pH levels: (A) 4.0, (B) 5.0, (C) 6.0, (D) 7.0 and (E) 8.0 after 5 days of incubation period

Aeration. The effect of aeration on the mycelial growth and mycelial density of *L. sajor-caju* was evaluated and is shown in Table 2 and Figure 4. The study revealed that parafilm sealing did not significantly influenced the rate of colonization. However, unsealed petri plates produced larger mycelial diameter with a mean of 81.25±4.78 mm while the sealed petri plates produced smaller mycelial diameter with a mean of 74.36±2.54 mm after 4 days of incubation. Although, statistical analysis showed that there is no significant difference between the two aeration conditions after incubation. In both conditions, very thick mycelial density was observed.

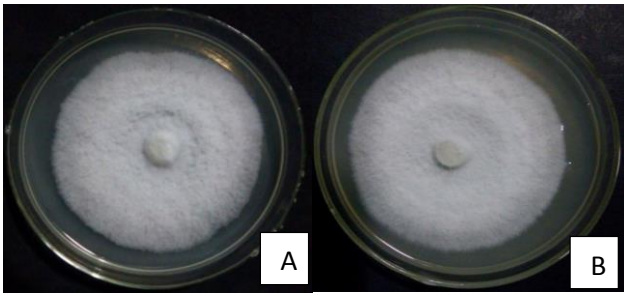

Figure 4. Mycelial growth performance of *L. sajor-caju* on different aeration conditions: (A) Sealed and (B) Unsealed after 4 days of incubation period

Illumination. The influence of illumination on the mycelial growth of *L. sajor-caju* was evaluated by incubating the cultured plates in different illumination condition. Plates incubated in lighted condition recorded the highest mean mycelial diameter (100.00±0.00) compared to incubated in dark (90.18±8.50) and alternating light and dark (94.06±5.27) conditions (Table 2).

However, statistical analysis revealed that there is no significant difference in mycelial diameter on the three illumination conditions indicating that *L. sajor-caju* can be incubated in either lighted or dark conditions. Furthermore, very thick mycelial density was observed in lighted condition and in the alternating light and dark condition while only thick mycelial density was produced in plates incubated in full dark condition (Figure 5).

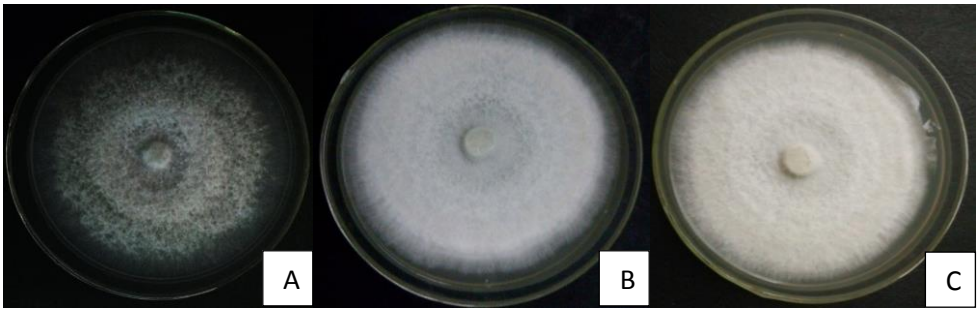

Figure 5. Mycelial growth performance of *L. sajor-caju* on different illumination conditions: (A) full dark, (B) lighted and (C) alternating light & dark condition after 5 days of incubation period

Temperature. Temperature is one of the most important and critical physical factors affecting mycelial growth in mushroom cultivation (Chang & Miles 2004). Varying temperature conditions could affect the mycelial growth of *L. sajor-caju*. Among the three temperature conditions such as room temperature (28-33 ℃), air-conditioned (23-25 ℃), and refrigerated condition (7-10 ℃), the largest mycelial growth was observed at room temperature. Analysis of variance revealed that those incubated at room temperature (30 ℃) produced significantly higher mycelial diameter with a mean of 100.00±0.00 mm compared to the other temperature conditions (Table 2). Moreover, *L. sajor-caju* incubated at room temperature and air conditioned produced very thick mycelial density after 5 days of incubation period. In contrast, no mycelial ramification was observed under refrigerated (9 ℃) condition after 5 days of incubation period (Figure 6).

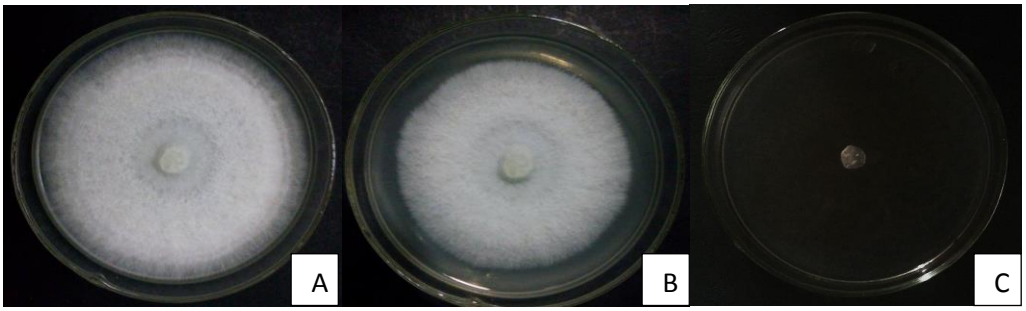

Figure 6. Mycelial growth performance of *L. sajor-caju* on different temperature conditions: (A) room temperature (30 ℃), (B) air-conditioned (24 ℃) and (C) refrigerated (9 ℃) after 5 days of incubation period

Mycochemicals found in L. sajor-caju

Mycochemicals are chemicals that fungi particularly mushrooms produce to perform metabolic functions and also for protection for themselves. These are naturally occurring constituents of fungi particularly mushroom. The mycochemical screening of hot water extract of *L. sajor-caju* was conducted in the present study. Table 3 and Figure 7 presents the mycochemical components of the hot water extract of *L. sajor-caju.* Apparently, among the seven mycochemicals tested, four were found present in *L. sajor-caju*hot water extract.

These mycochemicals exhibit significant human healthful benefits. In this study, mychochemicals such as terpenoids, cardiac glycosides, saponins and alkaloids were detected in the hot water extract of *L. sajor-caju*. Terpenoids, cardiac glycosides and alkaloids were found present in appreciable amount while saponins were present in trace amount. However, tannins, steroids and flavonoids were not detected in hot water extract.

Table 3. Chemical analysis of hot water extract of mycelial mats of *L. sajor-caju*

Mycochemicals	**Reactions**	**Presence**
Tannins	no reaction	-
Steroids	no reaction	-
Terpenoids	reddish brown coloration interface	++
Flavonoids	no reaction	-
Saponins	emulsion and frothing formed	+
Cardiac Glycosides	formation of brown ring	++
Alkaloids	turbidity formed	++

Note: (+) = present in trace amount, (++) = present in appreciable amount, (-) = absent

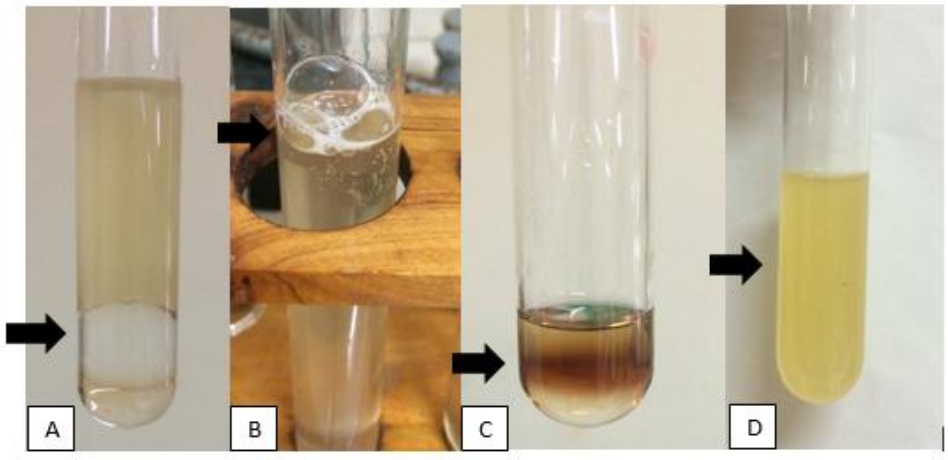

Figure 7. Positive results exhibited by hot water extract of mycelial mat of *Lentinus sajor-caju.* (A) Terpenoids- reddish brown coloration interface (B) Saponins- emulsion and frothing (C) Cardiac glycosides- formation of brown ring (D) Alkaloids- turbidity.

Discussion

Growth of L. sajor-caju on different indigenous culture media

CWG recorded the widest mycelial diameter in the shortest incubation period of 5 days. The superiority of coconut water could be due to its chemical components which can be attributed for the efficient mycelial growth. Based on previous reports, coconut water contains a variety of nutrients including protein (0.3 g), potassium (310 mg) and iron (1.1 mg) (Snowdon *et al*., 2003), nitrogen (0.05%), calcium oxide (0.69%) (Campbell et al. 2000), vitamins, minerals, amino acids, enzymes, growth factors, and trace elements such as zinc, selenium, iodine, sulfur, manganese, boron, molybdenum and others (Fife, 2008). Similarly, Cuevas *et al*. (2009) also reported that the higher mycelial diameter of *L. sajor-caju* was recorded in coconut water gelatin with a mean value of 54.7 mm. Furthermore, mycelial growth of both *L. squarrosulus* and *P. grammocephalus* was found significantly highest in CWG with mean mycelial growth of 80 mm in diameter on the 8th day of incubation (De Leon *et al*., 2013). In addition, Dulay *et al*. (2012) indicated that CWG was the most suitable and favorable medium for mycelial growth of *L. tigrinus* due to the significantly highest mean mycelial growth of 90 mm and very thick mycelial density on the 4th day of observation. Also, very thick mycelial density of *P. djamour* was also observed in CWG (Valdez, 2014) and thin mycelial density were recorded in CDG for *L. squarrosulus* (De Leon *et al*., 2013). This results suggests that coconut water is more suitable medium in order to enhance the biomass in the mycelia cultivation of *L. sajor-caju*.

Growth of L. sajor-caju in different culture conditions

pH. Although, pH is one factor that greatly influences the vegetative growth rate of the mushroom but it can also limits the further proliferation of mycelia in the medium (Krishna and Adholeya, 2003).The result of the study revealed that substrate pH did not affect the mycelial density of *L. sajor-caju.* This result was similar to the report of Lin (2004) who stated that although mycelia grow in the medium at a pH 4.0 – 7.5, the most suitable pH range for efficient mycelial growth is 5.5–6.0. The results obtained in this study is also congruent to the study of Gbolagade *et al.* (2006), who reported that pH range from 4.0-8.0 could support the mycelial growth of *Lentinus subnudus* and the acidic medium (pH 5.0-5.5) is the best for mycelial growth. In addition, this finding is also similar to the report of Cuevas *et al.* (2009) that the most suitable pH for *P. pulmonarius* ranged between pH 4.0 to 7.0.

Aeration. In this study, *L. sajor-caju* could grow either under aerobic or anaerobic conditions which is similar to the results reported on the mycelial growth response of *Schizophyllum commune* (Bulseco *et al.*, 2005) and *L. tigrinus* (Dulay *et al*., 2012). The result of the present study also conforms to the observation obtained by Cuevas *et al.* (2009), who reported that *L. sajor-caju* grow either under aerobic or anaerobic conditions. These results indicate that aeration was not a major physical factor affecting the growth of mycelia.

Illumination. The rapid mycelial growth of *L. sajor-caju* in lighted condition could be due to the combination of light, carbon and nitrogen sources in the medium. Magday *et al.* (2014) reported that the presence of light in combination with the carbon and nitrogen sources present in CWG serves as a signal for *G. lucidum* to be in flight, thus stimulated rapid mycelial growth in order to yield maximum biomass in the shortest period of 5 days. This result however is contrary to the results obtained by Cuevas *et al*. (2009) where larger mycelial diameter of *L. sajor-caju* was observed in dark condition while smaller mycelial diameter was observed in illuminated condition. Similarly, it did not conform to the previous report of Dulay *et al*. (2012), that very thick mycelial growth of *L. tigrinus* was recorded in dark condition while thick mycelial growth was observed in plates incubated under lighted conditions. This result suggests that *Lentinus* species had different characteristic in their responses to light. However, this finding conforms to the previous reports about the positive effect of light on the mycelial growth of other basidiomycetes such as *V. volvacea* (Reyes *et al.*, 1998). In addition, light controls the growth, metabolism and reproduction of fungi and therefore this is important for the survival and distribution of fungi. Therefore, the effects of light on fungal development has been intensively studied for decades, mainly in model fungi such as the Basidiomycetes genus Coprinus and the Zygomycetes genus Phycomyces (Cheng *et al.*, 2012). The present study also revealed that the mycelial growth of *L. sajor-caju* in terms of diameter is not sensitive to illumination condition.

Temperature. The result of the study wherein thick mycelial density was observed in room temperature condition after 5 days of incubation is similar to the result of Klomklung *et al.* (2014), who reported that the best temperature for mycelial growth of other *Lentinus* species such as *L. conatus* was observed at 30 ℃ with the growth rate of 10.89 ± 0.19 mm/day and the best mycelial growth and density of *L. roseus* occurred at 30 ℃ with the growth rate of 14.15 ± 0.28 mm/day. Moreover, several studies have also shown that *Pleurotus* and *Lentinus* species could be grown at 25 ℃ or higher temperatures (45 ℃) (Chang and Quimio 1982, Gbolagade *et al*. 2006, Vargas-Isla & Ishikawa 2008, Klomklung *et al*., 2014) which is similar to the mycelial response of *L. tigrinus* (Dulay *et al.*, 2012). The irresponsive mycelial growth of *L. sajor-caju* in refrigerated condition (9 ℃)

could be due to the inactivation of enzymes at lower temperature. Previous study reported that *G. lucidum* appeared to be drastically suppressed at 9 ℃ in proportion due to the drop of temperature caused by the denaturation and inactivation of essential enzymes which catalyze metabolic processes of *G. lucidum* (Lee *et al*., 2008). These findings revealed that temperature is a major factor to be considered for the mycelial growth of *L. sajor-caju.*

Mycochemicals found in L. sajor-caju

In the present study, terpenoids were present in the hot water extract of *L. sajor-caju*. Terpenoids (isoprenoids) are secondary metabolites with molecular structures containing carbon backbones made up of isoprene. The compounds have been reported to show a wide range of pharmacological benefits that include anti-malarial, anti-inflammatory and anti-cancer (Roslin and Annular, 2011).

Saponins were also detected in *L. sajor-caju* hot water extract. Saponins comprise a large family of structurally related compounds containing a steroid or triterpernoidaglycone. They are reported to have a wide range of pharmacological properties that exert various benefits, such as anti-inflammatory and anti-diabetic properties (Lee *et al*., 2012). It also prevents disease invasion by parasitic fungi (Okwulihie and Odunze 2004, Okwulihie *et al.*, 2010). Saponins are also potent antioxidants that neutralize free radicals to prevent disease and stimulate the production of antibodies, which help in fighting bacterial and fungal infections (Imaga *et al*., 2013). Saponins can also inhibit the growth of cancer cells, boost immune system and energy, lower cholesterol, act as natural antibiotic, and anti-oxidant, and can reduce the uptake of certain nutrients including glucose and cholesterol at the gut through intralumenal physicochemical interaction (Aberoumand, 2012, Ray-Sahelian, 2012, De Silva *et al*., 2013).

Cardiac glycosides which are responsible in cardio-activity, increase the function of myocardial circulation (Prasad and Sethi 2013) and is used to treat congestive heart failure and cardiac arrhythmia (Pongrakhananon, 2013). These compounds were present in appreciable amount in the hot water extract of *L. sajor-caju* but absent in some mushrooms such as *Agaricus bisporus, Bunapi shimeji,* and *Flammulina velutipes* (Prasad and Sethi, 2013). Similarly, these are present in *Cantharellus cibarius, Termitomyces robustus, Termitomyces manniformis, Pleurotus ostreatus, Pleurotus pulmonarius, Auricularia* sp., *Hericium erinaceus, L. deliciousus* and *Ganoderma* sp. (Unekwu *et al*., 2014, Ogbe *et al*., 2009).

In addition, hot water extracts of *L. sajor-caju* had appreciable amounts of alkaloids which has been reported to act as powerful pain reliever and topical anaesthetic in ophthalmology, and has stimulating effects and antipuretic action among other uses (Edeoga and Enata, 2001). The presence of alkaloids in the mushroom indicates antibacterial activity as

explained by Idowu *et al.* (2003). One of the most important biological properties of alkaloids is the toxicity against cells of foreign organisms. This bioactivity has been widely studied for their potential use in the elimination and reduction of human cancer cell lines (Nobori *et al.*, 1994). This suggests that *L. sajor-caju* have a potential anticancer agent.

Tannins, steroids and flavonoids were not detected in the hot water extract of *L. sajor-caju*. However, in the study conducted by Johnsy and Kaviyarasan (2014), steroids and flavonoids were found in methanol extracts and water extracts of *L. sajor-caju* then again tannins were not detected on both extracts. The absence of these three chemicals in the hot water extract suggests that chemical compositions of *L. sajor-caju* maybe substrate dependent. The substrate used can influence the chemical composition of mushroom (Silva *et al.*, 2002).

Antioxidant Activity of L. sajor-caju

Phenols are important plant constituents because of their scavenging ability due to their hydroxyl groups (Hatano *et al*., 1989). The phenolic compounds may contribute directly to the antioxidative action (Duh *et al*., 1999). In addition, it was reported that phenolic compounds were associated with antioxidant activity and play an important role in stabilizing lipid peroxidation (Yen *et al.*, 1993). In the recent study *L. sajor-caju* contains 75 mg/g phenolic content. This phenolic is higher compared to *Lentinus tigrinus* which has 26.59 mg AAE/g sample (Dulay *et al.*, 2015a) and *Panaeolus antillarium* which has 25.07 $\pm$ 0.02 mg AAE/g sample (Dulay e*t al.*, 2015b). The high phenolic content of *L. sajor-caju* extracts might account for the better results found for their antioxidant activity. It had been reported that the antioxidant activity of plant materials was well correlated with the content of their phenolic compounds. Polyphenols, such as BHT (butylated hydroxytoluene) and gallate, are known to be effective antioxidants (Halliwell and Gutteridge, 2003) so, it is important to consider the effect of the total phenolic content on the antioxidant activity of mushroom extracts. But in this study, the results for the radical scavenging activity of *L. sajor-caju* were quite low compared to the standard antioxidant, Cathechin. According to Gan *et al.* (2013), this could be due to the fact that, mushroom absorbs nutrients from the substrates where they grow, and so the substrate where the mushroom grow could be a big factor for its radical scavenging activity. In addition, maturation stages of fruiting bodies could affect antioxidant contents which results to low scavenging activities.

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