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Effect of biotransformation process on yield and quality of patchouli oil

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Abstract
The patchouli (*Pogostemon cablin*) oil is well known for its warm, spicy, musky and sensuous scent. It is widely used in many industries like perfume, food, pharmaceutics, cosmetics, and cleaning products. In this study, the effect of microbial culture and incubation period on the recovery of the patchouli oil and its chemical composition were investigated. Three microbial cultures *Aspergillus foetidus*, *Penicillium citrinum and Trichosporon asteroids* were subjected to patchouli herbage for five different incubation period 0, 2, 4, 6, and 8 days. The oil was extracted by steam distillation of dried-treated patchouli leaves and analyzed by gas chromatography-mass spectrometry (GC-MS). The recoveries of patchouli oil increased with incubation period for all the treatments. The highest oil recovery and patchouli alcohol content were found to be 1.62% and 31.25%, respectively for the samples incubated with *Trichosporon asteroids* for 8 days. The quality of oil extracted from patchouli leaves treated with *Trichosporon asteroides* was better than the other treatments.

Keywords: patchouli oil, biotransformation, incubation period, oil recovery, patchouli alcohol

1. Introduction
Patchouli (*Pogostemon cablin*) essential oil is widely used in food and perfumery industry (Akhila and Tewari, 1984) [1]. Indeed, patchouli oil is used extensively in the flavouring industry, and serves as an ingredient in many foods and beverages, frozen dairy desserts, candy, baked foods, meat and meat products (Raghu, 2006) [8]. It is also used in soaps, scents, lotions, pre-shave and after-shave lotions and detergents. Patchouli oil has therapeutic properties, namely antidepressant, antifungal, antibacterial, anti-inflammatory, antiseptic, aphrodisiac, astringent, carminative, diuretic, febrifuge, sedative and tonic. The oil is used as a topical remedy for skin problems such as acne, eczema, cracked, chapped and irritated skin. It is known as a cell rejuvenator and helpful in healing wounds and scars. In Chinese medicine, decoction from the patchouli leaves is used with other drugs to treat nausea, vomiting, diarrhoea, cold and headache (Kader *et al.*, 2006) [6]. Moreover, the patchouli oil acts as a deterrent to insects. It exhibits excellent repellence effect towards mosquitoes (Jantan and Zaki, 1998) [5].

The commercial oil of patchouli is obtained by steam distillation of the shade dried leaves. The oil yield is in the range of 2-4% w/w, which depends on quality and maturity of leaves (Akhila and Tewari, 1984) [1]. Biotransformation is the chemical modification of a compound by microorganisms to produce high value products with low cost precursors (Wolfgang and Dirk, 2010) [11]. The ability of microorganisms to introduce functional groups into chemically inactive complex molecules has made microbial transformations an indispensable part of the manufacturing process of some molecules. Whole cell biocatalysts such as fungi, bacteria, and algae have been extensively applied in the flavour and fragrance industry over the last half a century (Gounaris, 2010) [3]. In recent years, the patchouli oil produced by local farmers has very low grade, making the selling price relatively cheaper. The cause of low-grade quality is due to presence of many unwanted chemical compounds which darkens the colour of the oil. Therefore, it is necessary to develop a suitable extraction technique for the removal of desirable soluble constituents, leaving out those not required. Recently, steam distillation method with biotransformation has gained increasing interest during the last decades. Oil yield is also comparable to conventional method and in some cases it is even higher (Khare *et al.*, 2018) [7].

The aim of this work was to evaluate the effect of biotransformation on patchouli oil recovery and its quality in respect of patchouli alcohol.

2. Materials and Methods

2.1 Materials

The patchouli herbage was collected from the Instructional cum Research Herbal Garden, Indira Gandhi Krishi Vishwavidyalaya, Raipur (Chhattisgarh). The leaves were dried up to 15% moisture content (db) for 8-10 days by laying it in the shade at room temperature. The three fungal strains *Aspergillus foetidus* (MTCC-10559), *Penicillium citrinum* (MTCC-6590), *and Trichosporon asteroids* (MTCC-7632) in lyophilized state were procured from M/s Microbial Type Culture Collection and Gene Bank, Chandigarh.

2.2 Inoculation of cultures

The lyophilized strains were revived separately on solidified media in Petri plates in the incubator at 28±1 °C. MGYP agar was used for the revival and maintenance of the strains. The pH was maintained at 7.0 using dilute HCl or NaOH. Single colony of each culture from the Petri plate was inoculated in the 50 ml MGYP broth in 100 ml conical flask separately and incubated for 48 hours at 28±1 °C. The 24 hr grown cultures was used as mother culture/pre-culture. Mother cultures were again inoculated in the broth to find out the log phase of the culture. Cultures were used as inoculums in all the experiment only from the specified time (log phase). After optimum growth the mycelia were separated from broth by filtration with Whattman No. 1 filter paper. The separated mycelia were blended with sterile distilled water to break the clumps; this was the inoculum (biocatalyst) for the experiment (Khare *et al*., 2018) [7].

2.3 Sample preparation

Samples were prepared by spraying each inoculum over 2 kg of dried patchouli herbage separately and kept for incubation for 8 days at room temperature. Herbage treated with distilled water only was kept as control for comparison. Extraction of oil and quality analysis were carried out after 0, 2, 4, 6 and 8 days of incubation.

2.4 Oil extraction

Oil was extracted from each 2 kg of treated patchouli leaves by steam distillation method. Distillation process was carried out for seven hours for maximum extraction of essential oil. The oil yield was calculated by using the following formula (Ranitha *et al*., 2014) [9]:

Fig 1: Steam distillation unit

$$\text{Yield of essential oil (\%)} = \frac{\text{amount of essential oil (g) obtained}}{\text{amount of raw materials (g) used}} \times 100 \quad \ldots (1)$$

2.5 Separation and dehydration

Oil separation and dehydration was done using chloroform and anhydrous sodium sulfate. Chloroform was added to the oil-water mixture in a separating funnel. After a few moments of shaking, the oil was partitioned into the chloroform layer. Then the very small quantity of anhydrous sodium sulfate was mixed with the separated oil and kept overnight for dehydration.

2.6 Gas chromatography-mass spectrometer (GC-MS) analysis

The chemical components of the extracted patchouli oil were analyzed by Gas Chromatography-Mass Spectrometer (Shimadzu Scientific Instruments, Inc. (SSI), Maryland, US). Compounds were separated on Chromatography Column (Rtx-5MS, Chromservis Company Ltd., Czech Republic) of dimension 30 m×0.25 mm×0.25 µm film thickness coated with 5% diphenyl dimethyl polysiloxane. Injector temperature was maintained at 280 ºC. Oven temperature program was set at 40 ºC for 0.5 min. The temperature was increased at the rate 5 ºC/min to 240 ºC and held for 8 min. The 5 µl oil sample was diluted to 200 µl with GC grade acetone. Diluted 1 µl sample was injected using Hamilton syringe with a split ratio of 100:1. Helium was used as a carrier gas at the constant flow rate 36 ml per min. The spectrophotometer was operated in EI mode and the mass range was 40-500 amu. The ion source temperature and interface temperature was set at 250 ºC and 220 ºC respectively. The different compounds were detected in GC-MS analysis at different retention time. The compositional analysis of the oil was carried out using the software provided along with the GC-MS (Khare *et al*., 2018) [7].

2.7 Statistical analysis

Factorial experiment was conducted using a complete randomized design with three replications. The effect of different fungal treatments on quantity and quality of extracted essential oil were studied by Analysis of variance (ANOVA) using SPSS version 21.0 Statistical Software Package (IBM Corp., 2012) [4]. The confidence level used to determine statistical significance was 95%.

3. Results and Discussion

3.1 Effect of Cultures on patchouli oil recovery different incubation period

The oil recovery obtained after completion of steam distillation process from the control patchouli herbage was 1.03% and remains nearly constant for all incubation period. The oil obtained from the samples incubated with *Aspergillus foetidus* for 0, 2, 4, 6, and 8 days were 1.06%, 1.17%, 1.33%, 1.42%, and 1.43%, respectively. Similarly, the oil recoveries obtained from samples incubated with *Penicillium citrinum* and *Trichosporon asteroides* for different incubation periods were 1.09%, 1.22%, 1.35%, 1.48%, 1.49%; and 1.21%, 1.35%, 1.47%, 1.60%, 1.62 %, respectively. Fig 4 shows the patchouli oil recoveries after treatment with microorganism *Aspergillus foetidus*, *Penicillium citrinum*, and *Trichosporon asteroids* for different incubation periods. Data indicates that the oil recovery is significantly affected by the type of culture as well as the incubation period. Oil recovery gradually increased with the incubation period; however the recovery at 6th day was at par with the 8th day. Similar outcomes were reported by Sowbhagya *et al*. (2010) [10] with celery seeds. The highest oil recovery of 1.62% was obtained from the samples incubated with *Trichosporon asteroids* for 8 days.

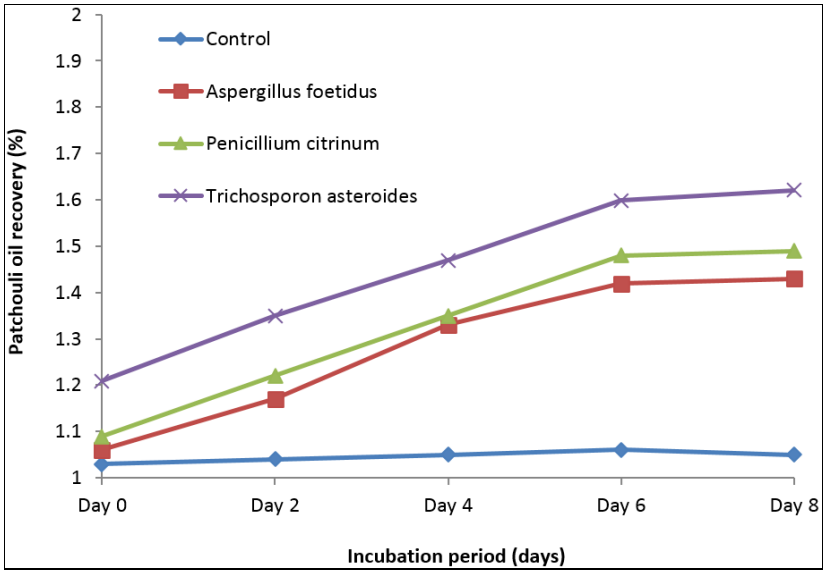

Fig 2: Changes in patchouli oil recovery with incubation period for different treatments

3.2 Gas chromatography mass spectrometer (GC-MS) analysis of patchouli oil

In this study, only 15 compounds were detected in all samples of patchouli oil during GC-MS analysis; they were (1) 2-Pentanone, (2) Copaene, (3) Caryophyllene, (4) Naphthalene, (5) Azulene, (6) Patchoulene, (7) Thujopsene, (8) Cyclohexane, (9) p-Menthane, (10) Longifolenadehyde, (11) Caryophyllene oxide, (12) Epiglobulol, (13) Patchouli alcohol, (14) α –Bisabolol, (15) β–humulene. Fitri *et al.* (2017) [2] reported 25 compounds to be found in patchouli essential oil extracted using the water-steam distillation technique. It indicates that, the good quality of patchouli oil free from undesirable compounds can be obtained using biotransformation technique. The results of GC MS analysis are shown in Fig 3 to Fig 7. The patchouli alcohol is the major compound in patchouli oil and could be used as a marker for quality control. On 0th day, the patchouli alcohol obtained from the samples treated with *Aspergillus foetidus*, *Penicillium citrinum*, and *Trichosporon asteroids* were 25.75%, 25.78%, and 25.82%, respectively. The highest amount of patchouli alcohol was 31.25% in oil extracted from patchouli leaves treated with *Trichosporon asteroides* after 8 days of incubation.

Table 1: Analysis of variance of essential oil recovery from treated patchouli leaves

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	509.776[a]	14	36.413	32.295	0.000
Intercept	33092.367	1	33092.367	29349.867	0.000
Culture	93.726	2	46.863	41.563	0.000
Incubation Period	414.689	4	103.672	91.948	0.000
Culture * Incubation Period	1.361	8	.170	.151	0.996
Error	33.825	30	1.128		
Total	33635.968	45			
Corrected Total	543.601	44			
a. R Squared = .938 (Adjusted R Squared = .909)					

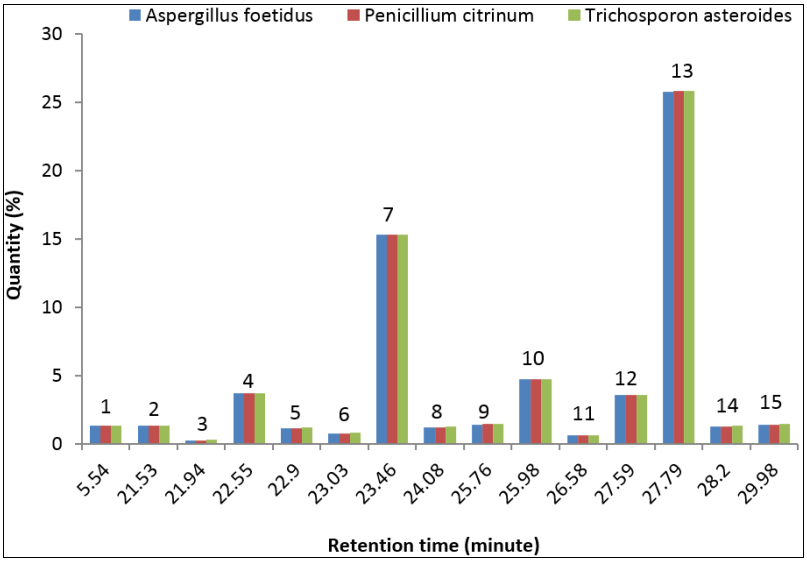

Fig 3: Chemical compounds detected in the oils obtained from different cultured patchouli leaves on 0th day; (13) Patchouli alcohol

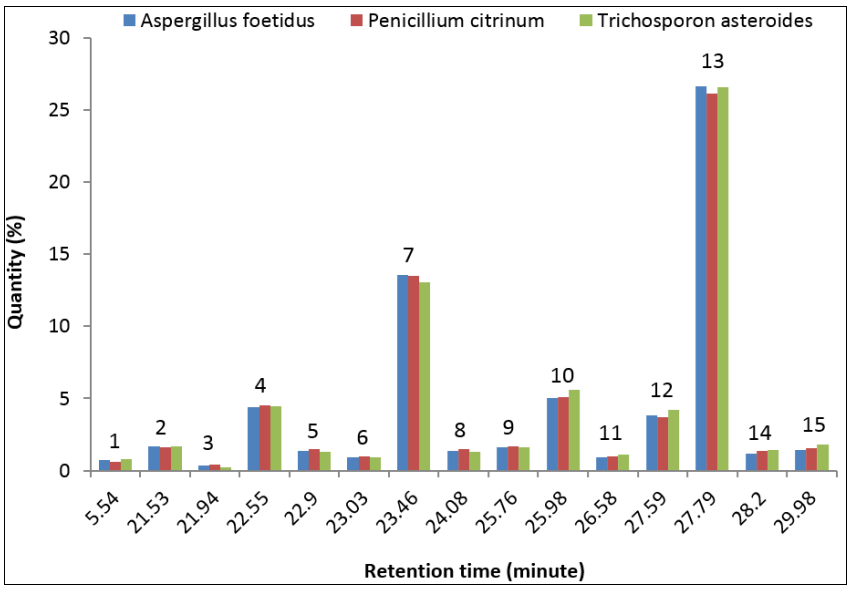

Fig 4: Chemical compounds detected in the oils obtained from different cultured patchouli leaves on 2nd day; (13) Patchouli alcohol

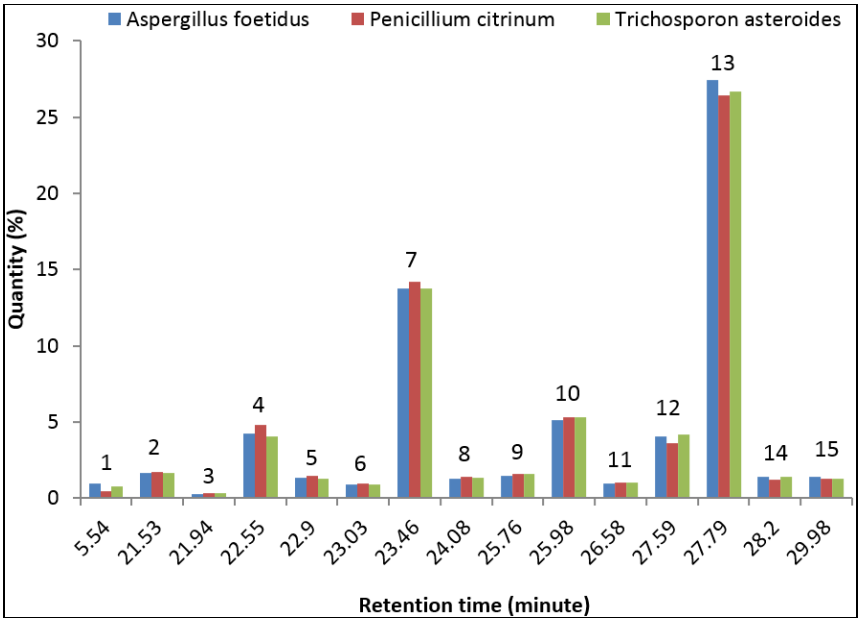

Fig 5: Chemical compounds detected in the oils obtained from different cultured patchouli leaves on 4th day; (13) Patchouli alcohol

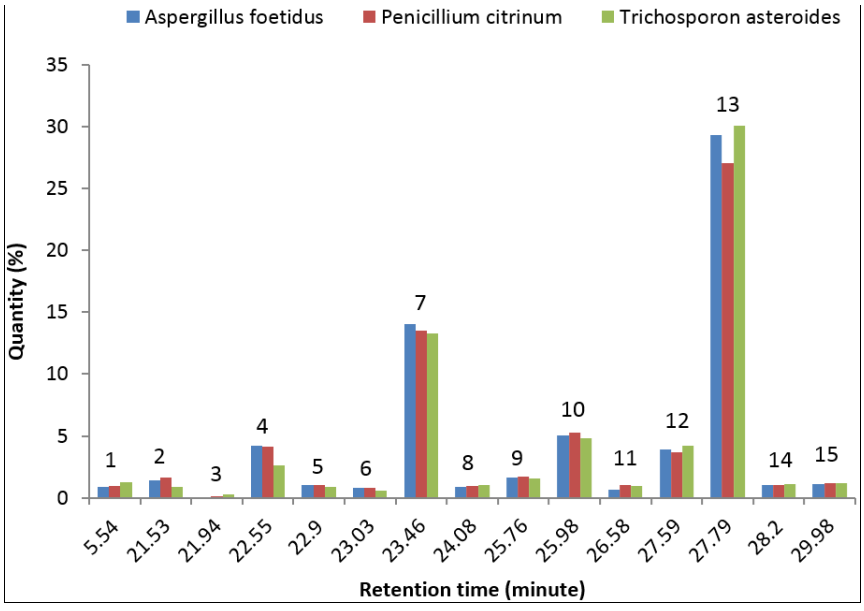

Fig 6: Chemical compounds detected in the oils obtained from different cultured patchouli leaves on 6th day; (13) Patchouli alcohol

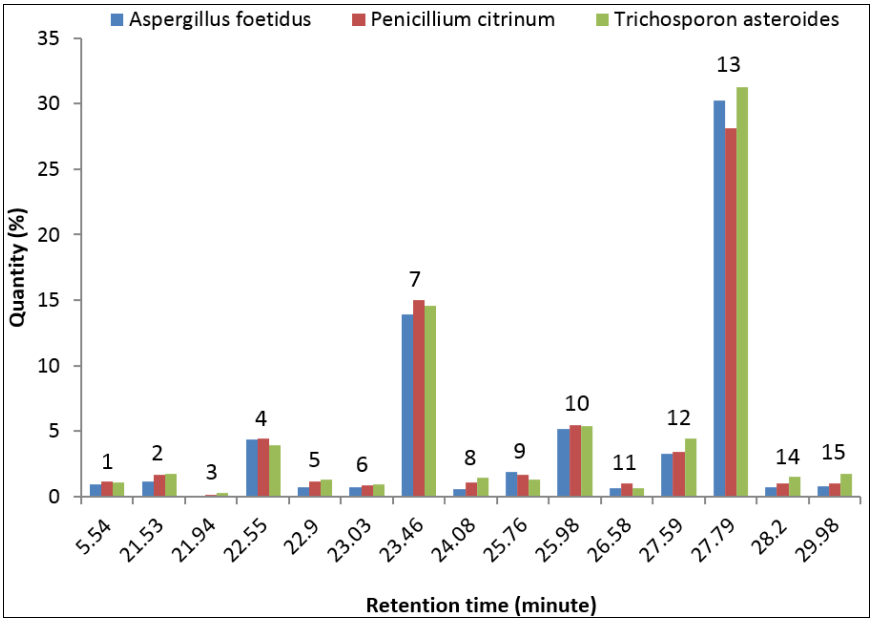

Fig 7: Chemical compounds detected in the oils obtained from different cultured patchouli leaves on 8th day; (13) Patchouli alcohol

4. Conclusions

The recoveries of patchouli oil by all treatments increased with incubation period. The highest oil recovery and patchouli alcohol content were observed for the samples incubated with *Trichosporon asteroids* for 8 days. It was found that the quality of oil extracted from patchouli leaves treated with *Trichosporon asteroides* was better than the other treatments. From the present study it may be concluded that the biotransformation method can be utilized to improve oil recovery along with the oil quality. The findings from this study will be useful for the commercial exploitation, also for the researchers, academics, and anyone seeking to better understand the patchouli oil extraction using biotransformation.

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