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Bioprospecting optimal phenology for bioactive molecules in native golden yellow *Pleurotus citrinopileatus* Singer

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ABSTRACT

Objective: To bioprospect optimal phenological phases as source of novel molecules from native golden yellow *Pleurotus citrinopileatus* across four phenologies in both aqueous and ethanol extracts, and identify novel molecules responsible for these activities.

Methods: Standard qualitative assay, Folin–Ciocalteu assay; aluminium chloride spectrophotometric, 2, 2-diphenyl-1-picrylhydrazyl, 2, 2′-azinobis (3-ethylbenzothiazoline-6-suslfonic acid, ferricyanide reducing antioxidant power were used to determine total flavonoid, polyphenols, radical scavenging, and reducing power. Spectrophotometric methods were used for lycopene, β-carotene, and total carotenoids, while liquid chromatography quadrupole time of flight mass spectrometry was used for identification and comparative quantitation of polyphenols and flavonoids across the four phenological states. ChemSpider™ database was used for the identification of compounds based on their empirical formula, accurate mass and literature review of previously reported compounds in mushroom.

Results: Primordial phases exhibited higher contents of secondary metabolites than mature basidiocarps. Polyphenols content differed across physiological phases with primordials exhibiting significant high contents ($P < 0.05$) [(13.803 ± 0.797) mg gallic acid equivalent/g dry weight]. Distribution of total flavonoids was significantly different ($P < 0.05$) across physiological states and ranged from (3.311 ± 0.730) to (14.824 ± 0.890) mg quercetin equivalent g dry weight. Ten polyphenol acids and seven flavonoids compounds identified varied across these phases with primordials exhibiting relatively high peak areas. Total antioxidant activities showed a positive correlation with total polyphenols ($r = 0.969$; $P < 0.05$) and total flavonoids ($r = 0.960$; $P < 0.05$) across these phenologies.

Conclusions: These findings provide evidence that primordials of golden yellow mushroom as opposed to their fruiting bodies are potent sources of bioactive health molecules.

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1. Introduction

Edible fungi are delicacies that are rich sources of health promoting molecules like polyphenols, flavonoids with radical scavenging properties [1]. The value added health benefits of these basidiomycetes are being ascertained because they also possess protein, minerals, vitamins, unique taste, and flavour [2,3]. Studies have shown that edible fungi in the genera *Pleurotus*, *Agaricus* possess antioxidant properties [4,5]. These have elicited interests to bioprospect for natural pharmacological and nutraceutical antioxidants that can quench free radicals. Previously, Athanasakis *et al.* [6] reported that natural molecules with potent antioxidants would help in quenching such radicals. These radicals are manifested when the enzymatic systems protecting the body from oxidative stress are overwhelmed due to excessive generation of reactive oxygen species resulting in imbalances [7]. Studies by Barros *et al*. [8,9] demonstrated that different parts of mushroom have varied health properties. Seemingly, studies have shown that mature fruiting bodies have reduced amounts of bioactive molecules associated with radical scavenging properties [10]. As the fruiting body ages, bioactive molecules declines due to their involvement in defence mechanisms [8,9]. Radical scavenging properties are largely attributed to phytochemicals such as flavonoids and polyphenols, that contribute largely to plant ecophysiology and survival against biotic and abiotic stressors [11]. However, little effort has been done to identify appropriate phenological phases for obtaining the fruiting bodies with optimal quantities of these biomolecules. This elicited interest to elucidate distribution of health molecules across four phenological phases from the unique native golden yellow *Pleurotus citrinopileatus* (*P. citrinopileatus*), which was recently collected from the forest and tissue culture developed for domestication [12]. To our knowledge, this is the first study ascertaining both phytochemical, total antioxidative properties, and the phenological distribution of health promoting molecules of the Kenyan native golden yellow *P. citrinopileatus* Singer using biochemical assays and liquid chromatography-quadrupole time of flight mass spectrometry (LC-QToF-MS).

2. Materials and methods

2.1. Source of Pleurotus specie mushroom cultures

P. citrinopileatus was provided by Dr. Fredrick Musieba of Kenya Industrial Research Development Institute. Starting cultures were obtained through tissue culture techniques and cultures maintained on potato dextrose agar medium at 5 °C [13].

2.2. Reagents

Potassium ferricyanide, 1,1-diphenyl-2-picrylhydrazyl (DPPH), hexane, 2,2′-azinobis (3-ethylbenzothiazoline-6-suslfonic acid) (ABTS), gallic acid, quacertin, L-ascorbic acid (L-AA), α-tocopherol (TOC), butylated hydroxytoluene (BHT), Folin–Ciocalteu reagent, sodium carbonate, aluminium chloride, silica gel, petroleum ether, acetone, ethanol, ferric chloride, acetic acid, Mayer's reagent were of analytical grade supplied by Sigma Aldrich (Germany).

2.3. Developing spawn and substrate inoculation

Development of spawn was conducted according to previous method with slight modification [14]. In this case, media bottles and bird millet were utilized instead of spawn bags and wheat grains. Ten-day-old pure spawn with fully colonized mycelium was used in the inoculation of substrate. Sugarcane bagasse (*Saccharum officinarum*) and wheat straw (*Triticum aestivum*) substrates were treated according to previous studies [12,15] and spawned at 10% w/w; with each bag carrying 500 g w/w substrate. Sugarcane bagasse (*Saccharum officinarum*) and wheat straw (*Triticum aestivum*) substrates, spawn run for efficient substrate colonization took 14 days, and primordials emerged on the second pinning. Fruiting bodies were picked at predetermined phenological stages (Figure 1). These phases were ten-day-old fully-grown spawn mycelium (SPM), first primordials two days after pinning, second primordials three days after pinning, and mature fruiting bodies collected a week after pinning.

2.4. Preparation of samples

Intact SPM, two phases of primordials, and a fruiting body phase of *P. citrinopileatus* were picked separately (Figure 1) and dried in oven at 42 °C for 3 days and milled to powder using electric blender (Kenwood: BL370 400W 1.6L, USA). A total of 10 g of powder was placed in high performance liquid chromatography (HPLC) grade bottles and mixed separately with 100 mL of distilled water and 100 mL of analytical grade ethanol (Sigma Aldrich, Germany) and left in a 24-h shaker set at 150 revolutions per minute at room temperature in darkness. The liquid was decanted and the residue resuspended in 100 mL of the solvent for re-extraction for 24 h. The filtrate was pooled and filtered through Whatman paper No. 1 (12.5 cm) and stored at 4 °C awaiting further analysis [16]. The filtrate was concentrated using a vacuum rotary evaporator (40 °C). Pooled samples were screened for secondary metabolites whiles samples across phenological states were analysed for total polyphenols, total flavonoids, and total radical scavenging properties using various *in vitro* assays and LC-QToF-MS.

2.5. Screening of phytochemicals of phenological samples

Screening of phytochemicals was carried out according to previously described standardized methods [17]. Briefly, extracted samples were prepared and screened for bioactive secondary metabolites like flavonoids, phenols, terpenoids, saponins, alkaloids, tannins, resins, phytosterols, cardiac glycoside, saponins, and anthraquinones constituents.

2.6. Total carotenoids content

Carotenoids were determined as previously described with a few modifications [18]. Ten gram of sample and 5 g of celite 454