

Short Communications

Antioxidant activity of phenolic compound of *Astraeus hygrometricus*: A Case of Ranchi, India

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ABSTRACT

The forest region of Ranchi district of Jharkhand, India, is very rich with different types of wild mushrooms. This study collected two hundred samples. There are nine different species were identified and one of them was *Astraeus hygrometricus* that chosen for isolation, purification, and characterization of its compounds. This mushroom was chosen because of its nutritive value as for human consumption and also fewer studies done on it. It has got many compounds unrevealed. Various techniques such as solvent extraction including phase separation, TLC, FT-IR, and MS were employed in this study. Also, the total phenol content and antioxidant assay (DPPH) of the purified transparent compound of methanolic extract was carried out. The study showed that unknown transparent phenolic compound, established as astrakurkurool with molecular weight 485 was obtained.

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The nutritional and medicinal aspects of different mushrooms of Ranchi were analyzed and communicated for publication somewhere else (article accepted now). Of the several wild mushrooms of Ranchi, *Astraeus hygrometricus* is highly popular and consumable among all the other ones. It contains a very good amount of protein and vitamins, also less amount of carbohydrate and fats. Several medicinal properties such as antimicrobial, antidiabetic antioxidant and anti-AChE activity has also been shown by *A. hygrometricus* (Khan & Chandra 2019).

Considering the importance of above medical aspects and potential of *Astraeus hygrometricus*, the present work was undertaken to investigate the important compound which was present in this wild mushroom. This is the first report on the purification aspect of compounds of wild mushroom, *Astraeus hygrometricus* of Ranchi district.

Mushrooms were collected from various niches of Ranchi district. Macroscopic and microscopic studies were carried out for their identification purposes. Voucher specimens were deposited in the herbarium of the microbiology lab in the Department of Bioengineering, BIT Mesra, Ranchi. On the basis of these studies, the mushroom was identified and it was found to be *Astraeus hygrometricus*.

Extraction was carried out by the method followed by [Smedsgaard \(1977\)](#) with some modifications. 18g of dried mushrooms sample was taken and crushed. It was then mixed in 36 ml of solvent I (consisting of methanol: dichloromethane: ethyl acetate in 1:2:3 proportion). It was then left overnight at 5 °C. Then it was evaporated and suspended in solvent II (50 ml) [consisting of methanol (43.76 ml), hydrochloric acid (0.04 ml), formic acid (1.2 ml), and water (5 ml)]. This supernatant was stored in refrigerator at 4 °C for further use.

In this method, the equal volume of n-hexane (10ml) and methanolic extract (10ml) of *Astraeus hygrometricus* were taken in a separatory funnel and blended well. At that point, an equivalent volume of distilled water and sodium chloride were added to a separatory funnel to improve the separation phase. Steadily, the extract got exchanged to the hypo-phase (n-hexane phase). The epi-phase containing methanol and water-dissolvable contaminations were expelled. At long last, n-hexane phase was washed 4 to 5 times with distilled water to expel leftover methanol. The compound gathered was treated with 1N HCl (9:1; v/v) and it was concentrated by evaporation at 40° C.

In this method, the compound was analyzed by thin layer chromatography using silica gel (silica gel G, Himedia). The optimized solvent system for running phase consists of hexane: ethyl acetate (90:10; v/v). The extract was spotted on the silica gel plates and air-dried. After the TLC plates have run, retention factor (Rf) was calculated ([Gogoi et al. 2016](#)).

Purified compound was characterized by Fourier transforms infrared spectroscopy (FT-IR) and Mass spectrometry. This technique works in the fact that bonds and groups of bonds vibrate at selected frequencies. The compound was further characterized using Fourier transform infrared (FTIR) spectrophotometer (Model IR-Prestige 21, Shimadzu Corporation, Japan). Dried compound was mixed with KBr powder and pressed into pellets for FTIR spectroscopy with frequency range of 4,000–400 cm⁻¹.

Mass Spectrometry (MS) was adopted for the identification and determination of the molecular weights of the purified bioactive compounds. The methanolic fractions of purified compounds of *Astraeus hygrometricus* were subjected to MS analysis. It was performed on Thermo Fisher LTQ excel.

The quantity of phenol in transparent compound obtained in TLC was determined using a spectrophotometric method ([Orhan et al. 2009](#)) with some modifications. Folin-Ciocalteu assay method was applied for the determination of total phenolic content. In the procedure, purified compound (concentration of 0.190 mg/ml) was dissolved in 0.4 ml Folin-Ciocalteu reagent (diluted 1:10 v/v). Sodium carbonate solution (4 ml) was added after 5 minutes. Distilled water was added to make the final volume of the tubes to 10 ml and allowed to stand for 30 minutes at room temperature. Gallic acid was used as the standard. The absorbance of test sample and standard was taken at 550 nm with spectrophotometer.

It was conducted by the method of Orhan et al. (2009) with some modifications. 2.9 ml of DPPH solution (0.15 mM) was mixed with 0.5 ml of purified compound. Then, the mixture was shaken vigorously and was allowed to stand for 30 minutes in the dark. Finally, absorbance was taken at 517 nm.

Standard curve of Gallic acid was used as a reference.

$$\text{Scavenging effect \%} = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \times 100$$

The methanolic extract was purified by extracting with n-hexane. In the process of separation phase, the n-hexane hypo-phase extract was taken out and 0.35g of extract was found in 10 ml of n-hexane. Thus, it can be concluded that approximately 9.72g of extract was found in 100g of mushroom sample. Then, thin layer chromatography (TLC) was performed and two compounds were obtained. Out of these two, one was more prominent and intense (compound 2 as shown in Figure 1a) so it was chosen for further study and its R_f value was found to be 0.6. The TLC plate was viewed in the UV chamber at both ranges (254 nm and 366 nm). Then, the desired com-

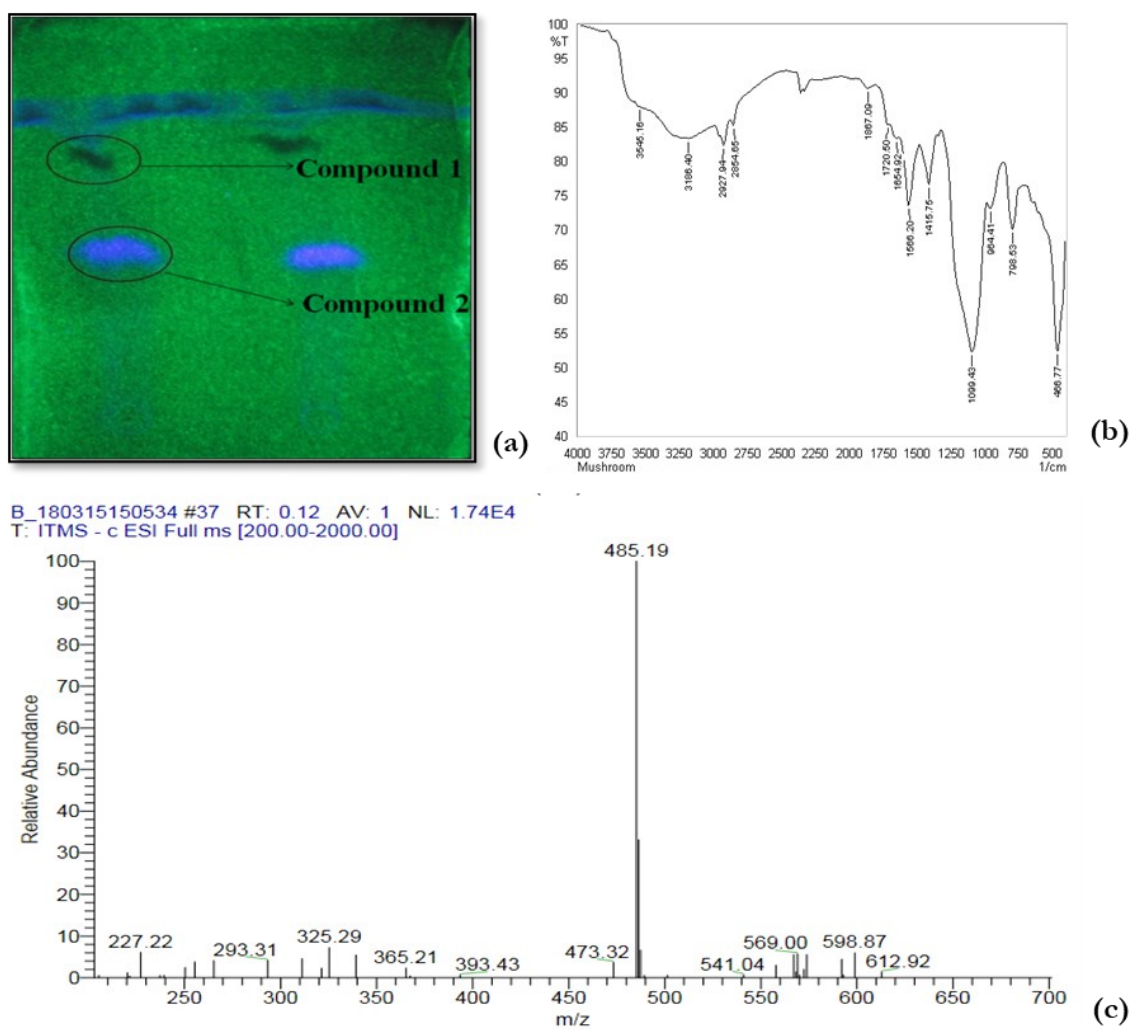


Figure 1. (a) Thin layer chromatogram of the compound single band of purified compound at visible range, plate viewed at 256 nm (short wavelength) UV range, (b) Fourier transform infrared spectrum (FTIR) of TLC purified transparent compound, (c) Mass spectrum of TLC purified transparent compound.

pound was scrapped out and dissolved in methanol; after that, it was then centrifuged to separate silica from the desired compound.

Finally, Fourier transform infrared (FTIR) was performed on the TLC purified sample (Figure 1b). The spectra of the compound showed strong bands at 1099 cm^{-1} (C-O stretch), 1566 cm^{-1} (C=O stretch), and 2927 cm^{-1} (O-H, C-H stretch) which is almost similar to the spectra of astrakurkurol. It was also supported by the studies of [Stanikunaite et al. \(2008\)](#) and [Sheldrick \(1997\)](#). Two sharp bands at 1566 and 1415 cm^{-1} were ascribed to CH_2 (bending) and Me(bending) vibrations, respectively.

The purified transparent compound was analyzed and characterized by mass spectrometry (MS). One major peak occurred on the mass chromatogram (Figure 1c). In positive ion mode, most of the m/z data were found in the form of $[\text{M}+\text{Na}]^+$ and provided molecular mass of 508.9 $[\text{M}+\text{Na}]^+$ for purified compound. The molecular formulae of transparent compound was established by ESI-MS as $\text{C}_{32}\text{H}_{54}\text{O}_3$ (m/z 508.9 $[\text{M}+\text{Na}]^+$). [Biswas et al. \(2017\)](#) also purified a compound with the same molecular formulae i.e. $\text{C}_{32}\text{H}_{54}\text{O}_3$. During MS analysis DBE (Double Bond Equivalent) was calculated and on the basis of the number of DBE, it can be concluded that the obtained compound has got five rings and one pi bond in its structure. This analysis concludes a structure for the compound that resembles the structure obtained by [Lai et al. \(2012\)](#), [Hill & Connolly \(2015\)](#), [Liziane et al. \(2016\)](#), and [Biswas et al. \(2017\)](#).

Thus, through the molecular weight, MS and FT-IR analysis of this transparent compound can be named as astrakurkurol as it is found and named by [Lai et al. \(2012\)](#), [Hill & Connolly 2015](#), [Liziane et al. \(2016\)](#), and [Biswas et al. \(2017\)](#).

The Phytochemical analysis of the transparent compound showed the presence of phenols. The phenolic compounds were reported for their direct contribution to anti-oxidative action ([Velioglu et al.1998](#)). Content of phenolic compounds correlates with the antioxidant activity as it has already been reported ([Blanche et al. 2017](#)). Therefore, for a mushroom to have antioxidant activity presence of phenolic compound is important. Phenolic is one of the major groups of nonessential dietary components that is related to the inhibition of cancer and atherosclerosis ([Williams et al. 1997](#)). Total phenol content was found to be 27.85 mg/100g. DPPH activity in this compound was found to be 56.7%. Total phenol content and DPPH activity in crude mushroom extract was 38.91mg/100g dw and 61.1%. Phenolic rich methanolic extract of this mushroom have rich total anti-oxidant activity as observed by [Ullah et al. \(2015\)](#). The phenolic fraction of plants is usually inter-linked to their antioxidant and antimicrobial activities. [Singh \(2010\)](#) quantified several phenolic compounds and found this mushroom to be rich in Protocatechuic acid, Ferulic acid, Salicylic acid, Anthralinicacid, and Syringic acid. Total phenolic content was also determined spectrometrically as 1.4% in inner and outer part of the mushroom respectively ([Singh 2010](#)) and it all correlates with our study.

As per literature review and studies done it may be concluded that *Astraeus hygrometricus* has several nutritional and medicinal properties. The methanolic extract of this mushroom contains two biochemical compounds as it is clear from our TLC result. Out of both compounds, one was more prominent and intense (compound 2) and this transparent compound was found to be astrakurkurool with a molecular weight of 485.19. This compound also showed good amount of phenol content and DPPH activity.

AUTHORS CONTRIBUTION

F.K. has done the research under the supervision of R.C.

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CONFLICT OF INTEREST

There is no conflict of interest.

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