

Quantitative Estimation of Gallic Acid and Rutin of Non-Gilled mushrooms Collected from North-Western Himalayas by HPTLC

Shaveta Singh, Isha Sharma, Astha Tripathi



Abstract: To carry out the quantitative estimation of Gallic acid and Rutin from the methanolic extract of four non-gilled mushrooms by high-performance thin layer chromatography (HPTLC) method. Material and method: Four non-gilled mushrooms (*Auricularia auricula-judae*, *Auriuclaria polytricha*, *Ganoderma lucidum* and *Trametes elegans*) were collected from North Western Himalayas during the rainy season. Stationary phase for this study was TLC aluminium sheets precoated with silica gel 60 F₂₅₄ and mobile phase was Toluene: Ethyl acetate: Formic acid (3:6:1v/v) and Chloroform: Ethyl acetate: Methanol: Formic acid (3.5:5:0.5:1 v/v) for Gallic acid and Rutin respectively. The detection and quantification of Gallic acid and Rutin were performed at 280 nm and 254 nm respectively. Results: R_f value of Gallic acid and Rutin were recorded to be (0.43) and (0.70) respectively. Both HPTLC peak areas for standards Gallic acid (6467.8) and Rutin (16592.4) were compared with corresponding peak areas of four non-gilled mushroom extract and the amount of Gallic acid was calculated. All the four non-gilled mushrooms extracts showed the good concentration of Gallic acid and Rutin. But the amount of Gallic acid was higher in comparison to Rutin. Conclusion: This study results showed that the four non-gilled mushrooms extracts are potential sources of antioxidant activity.

Keywords: Gallic acid, HPTLC, Non-gilled mushrooms, Rutin.

I. INTRODUCTION

Macrofungi without gills are normally abbreviated as non-gilled mushrooms. The non-gilled mushrooms are generally developed as saprophytes on wood, logs, branch and, twigs. The existence of such non-gilled mushrooms produces severe degrees of white rotting to forest trees [1]. Most common and widely distributed non-gilled mushrooms include *Auricularia*, *Morchella*, *Ganoderma*, *Cordyceps*, *Hericium erinaceus*, and *Lycoperdons*. There are several non-gilled mushrooms exist which can be conveniently placed in ten distinct groups namely Gill like, with the pore, with teeth like projection, underwater coral, vase-shaped, club-shaped, cup or saucer-shaped, ball-shaped, amorphous jelly like and mold on other mushrooms [2].

High-performance thin layer chromatography (HPTLC) has developed as a useful analytical method for qualitative and quantitative estimation of chemical constituents present in plant materials [3]. This technique also introduces fingerprint profiles of TLC and detection of chemical markers and biomarkers [4]. Gallic acid (3, 4, 5-trihydroxybenzoic acid) is naturally obtained polyphenolic compounds which exhibit astringent, antioxidative, antimicrobial activity [5, 6, 7] and integrated as unavoidable component of the diet. The Gallic acid had been studied for its preventive activity on the enzyme lysosomes when infected in the myocardial rats treated with isoproterenol. In this study a significant increase was observed in the peroxidation of lipid products and also significant decrease in the level of glutathione. In the presence of gallic acid the activity of β -galactosidase, β -galactosidase and β -glucuronidase showed significant increase in the plasma and heart of cardiotoxic induced rats. The increase in the level of these enzymes shows that gallic acid can act as a cardio-protective agent [8]. Rutin are phenolic compounds which possess antiulcer, anti-inflammatory, antioxidant, antimicrobial, antiallergic activity [9, 10,11,12]. Rutin presence has been reported from many medicinal mushrooms. A study conducted on *Ganoderma lucidum* and *Cordyceps sinensis* mushroom showed the presence of high rutin [13]. Rutin has been reported to suppress the production of TNF- α in the microglia of brain. This activity can be used in the treatment of Alzheimer's disease [14]. The research has also shown the anti-diabetic property of rutin by showing decrease in the level of glucose and increase in insulin level in the streptozotocin induced diabetic rats [15]. Antiasthmatic activity of rutin has also been investigated in guinea pigs sensitized with ovalbumin. Rutin showed significant inhibition of airway resistance in these guinea pigs thus used as an antiasthmatic agent [16]. Rutin has also reported to have inhibition activity against to inhibit serine proteases trypsin and thrombin [17]. The present study was done to estimate two bioactive compounds, Gallic acid and Rutin (Fig.1) presence in the four non-gilled mushrooms extracts by using HPTLC.

II. MATERIALS AND METHODS

Four non-gilled mushrooms (*Auricularia auricula-judae*, *Auriuclaria polytricha*, *Ganoderma lucidum* and *Trametes elegans*) were selected for HPTLC analysis. from North-Western Himalayas in July-Oct (monsoon) and identified on the basis of molecular taxonomy. The aligned nucleotide sequence of samples were submitted to NCBI and accession numbers were provided by gene bank.

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The fruiting body and mycelial cultures were submitted in the Herbarium of Punjab University, Chandigarh and Culture bank of Directorate of Mushroom Research Centre, Solan, Respectively (Table-1). **A. High-performance thin layer chromatography (HPTLC):** High-performance thin-layer chromatographic (HPTLC) method has been used for quality control estimation of Gallic acid and Rutin.

- **Preparation of extracts:** The mycelia of the mushrooms were cultured in Potato Dextrose Broth (PDB) and incubated at 25°C in a rotator shaker at 150-200 rpm for 14 to 30 days. The liquid cultures were centrifuged along with mycelium at 4000 rpm for 15 minutes. The supernatant was lyophilized and then dried powder was used for further study. All the lyophilized non-gilled mushroom samples were extracted with methanol. By using Whatmann filter paper No.1 methanolic extract was filtered and then concentrated at low temperature. The stock solutions were further diluted to produce a uniform concentration of 10 mgml⁻¹ for all the samples.
- **Preparation of standard Gallic acid and Rutin solution:** The stock solution of Gallic acid and Rutin was prepared by taking 10 mg accurately weighed compounds into 10 ml volumetric flask, and then the solution was made up to 10ml with methanol (1mgml⁻¹) [18]. The final concentration of 100 µg/ml was prepared by taking 1ml of stock solution of Gallic and Rutin and diluting it further upto 10 ml.

B. Chromatographic conditions: Samples of methanolic extracts of all the four non-gilled samples and standard Gallic acid and Rutin were compared in the HPTLC technique. 8mm wide band of methanolic extract and standard was spotted by using automatic TLC applicator Linomat V on 0.2mm thick TLC aluminium sheets precoated with silica gel 60 F254 (10x10cm). The Mobile phase used was Toluene: Ethyl acetate: Formic acid (3:6:1v/v) and Chloroform: Ethyl acetate: Methanol: Formic acid (3.5:5:0.5:1 v/v) for Gallic acid and Rutin. The TLC plates were kept in twin trough chamber for fifteen minutes so that solvent can move without disturbance. Later the TLC plates were dried in air. The analysis of results were done by using CAMAG Scanner III at 280 nm and 254 nm for Gallic acid and Rutin respectively [19].

III. RESULTS

A. TLC analysis: Thin layer chromatography screening using solvent Ethyl acetate: Formic acid (3:6:1v/v) for Gallic acid and Chloroform: Ethyl acetate: Methanol: Formic acid (3.5:5:0.5:1 v/v) for Rutin separate many compounds from the mushroom extracts as shown in Fig.2 for gallic acid and Fig.3 for Rutin. The bands of compounds were compared with bands of both standards. These two compounds were presented in the *Auricularia auricula-judae*, *Auriuclaria polytricha*, *Ganoderma lucidum* and *Trametes elegans* extracts.

B. High performance thin layered chromatography: HPTLC profile analysed for the estimation of Gallic acid and Rutin (Fig.4). All the four non-gilled mushrooms extracts showed good concentration of Gallic acid and Rutin which proves its antioxidant nature.

C. Quantification of Gallic acid and Rutin in four non-gilled mushrooms extract:

- **Gallic Acid:** The peak area and Rf value of standard Gallic acid was observed to be 6467.8 and 0.43

respectively shown in Table-3 and Fig.5. Single peak values having Rf 0.41, 0.39, 0.40, 0.48 and peak area 979.2, 3065.5, 3329 and 1085.4 (Fig.6) were showed by the methanolic extract of four non-gilled mushrooms *Auricularia auricula-judae*, *Auricularia polytricha*, *Ganoderma lucidum* and *Trametes elegans* respectively. These single peak values were coinciding with the Rf value of standard (0.43). The amount of Gallic acid were found to be 30.3 µg/ml, 94.79 µg/ml, 102.94 µg/ml and 33.56 µg/ml respectively in *Auricularia auricula-judae*, *Auricularia polytricha*, *Ganoderma lucidum* and *Trametes elegans* shown in Table-2.

- **Rutin:** The peak value and Rf value of standard Rutin was observed to be 16592.4 and 0.70 respectively as shown in Table-3 and Fig.5. Single peak value having Rf 0.74 and peak area was 357.4 (Fig.7-A) was showed by methanolic extract *Auricularia auricula-judae*. The Rf value of this single peak was coinciding with standard Rf value (0.74) and the amount of Rutin was found to be 8.62 µg/ml. Two peaks were observed in case of methanolic extract of *Auricularia polytricha* and second peak value having Rf 0.71 was coinciding with standard Rf value. The peak area of this second peak was 694.6 (Fig.7-B) and the amount of Rutin was calculated to be 16.74 µg/ml. Methanolic extract of *Ganoderma lucidum* showed single peaks value having Rf 0.67 was coinciding with standard Rf value. The peak area of this single area was 698.1 shown in Fig. 7-C and the amount of Rutin was calculated to be 16.83 µg/ml. Three peaks were showed by methanolic extract of *Trametes elegans*. The second peaks value having Rf 0.69 was coinciding with standard Rf value. The peak area of second peak was 306.3 as shown in Fig.7- D and the amount of Rutin were calculated to be 7.38 µg/ml (Table-2).

$$\text{Amount of sample} = \frac{\text{Amount of standard}}{\text{Area of sample}} \times \text{Area of sample}$$

IV. DISCUSSION

High-performance thin layer chromatography (HPTLC) technique is used for the detection of chemical components in various samples. The main advantage of HPTLC is that many samples can be evaluated unitedly by using a small quantity of mobile phase. Some important active constituents like Gallic acid, Rutin and Quercetin are estimated by HPTLC method [3, 20]. Gallic acid, Rutin and Caffeic acid were detected from *Russula delica* Fr. Rutin in a good quantity of around 2.821% [21]. According to Hu,[22], the highest phenolic content in *Ganoderma lucidum* extract was around 91.5 ug /mg. The results from our study are in agreement with this report that *Ganoderma lucidum* extract showed the highest phenolic content (Gallic acid 102.94µg/ml). Many studies reported that a variety of phenolic compounds were present in mushroom samples such as chlorogenic acid, vanillin, ascorbic acid, and Gallic acid [23, 24]. According to the study conducted by Barros [25,26] phenolic acid quantity in *L. deliciosus* fruiting bodies was 2.26 mg kg⁻¹ and 35.67 mg kg⁻¹ in *Ramaria botrytis*.

Other edible mushroom species like *Agaricus bisporus* and *Lentinus edodes* were demonstrated to contain relatively low amounts of p-hydroxybenzoic acid, even lower quantities of trans-cinnamic acid and protocatechuic acid, and trace amounts of cinnamic acid. Kim studies showed a high level of phenolic compounds in ten edible mushroom species and also identified the presence of Gallic, homogentistic, chlorogenic and protocatechuic acids in *P. ostreatus* [27]. Božena investigations revealed the presence of p-hydroxybenzoic, syringic and Gallic acid in *Agaricus bisporus* [28]. In this study, we found that methanol was the most effective solvent for the extraction of all the four non-gilled mushrooms samples since it produced the highest Gallic acid levels while Rutin was found in low amount.

V. CONCLUSION

The standard Gallic acid and Rutin were quantitatively determined in methanolic extracts of four non-gilled mushrooms. This study is important as it quantitatively determined the presence of Gallic acid and Rutin in four non-gilled mushrooms extracts which is a potential source of antioxidant activity. Research on phenolic compounds is of current interest, they have important biological and pharmacological properties.

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CONFLICTS OF INTEREST

Both authors declare that they have no conflicts of interests

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AUTHOR PROFILE



Dr. Shaveta Singh. I have completed my Post Graduation in microbiology and started my research career in microbiology by joining PhD in 2014 from Shoolini University, Solan. I was started my PhD on the topic, "Antimicrobial properties and phytochemical analysis of non gilled mushrooms collected from North Western Himalayas" under the guidance of Dr. Astha Tripathi. During my PhD I have collected different types of wild non-gilled mushrooms and identified them on the basis of traditional and molecular taxonomy. I have published four research papers during my PhD.



Isha Sharma, I have started my research career in microbiology by joining MPhil in 2011 from Shoolini University. I was worked on ligninolytic enzymes of white-rot fungi in MPhil under the guidance of Dr. Astha Tripathi. I have four years teaching experience in microbiology. I continued my research in the same field by joining PhD in 2016 on the topic, "Isolation and purification of bioactive compounds from wild *Irpex lacteus* fungi. I have published three research papers on different functional properties

of mushrooms. Recently I am working on the purification of bioactive compounds from wild *Irpex lacteus* fungi to check their anti-cancerous properties.



***Dr. Astha Tripathi**, I have completed my Under graduate and Post graduate (Microbiology) from CSJM University, Kanpur. I started my research career in September, 2004 when I was joined council of scientific and industrial

research, Govt. of India (CSIR) sponsored project as a project assistant. In September, 2006, I was selected as Senior Research fellow in ICAR sponsored project and this is how my journey started with wild mushrooms. In 2006, I was started my Ph.D. on the topic "Mineralization of phenol, chlorophenols and nitrophenols by wild edible and non edible fleshy fungi" under the guidance of Dr. R.C. Upadhyay, Principal Scientist, DMR, Solan. My Ph.D. was awarded in August, 2011 in Microbiology. I have published five research papers during my Ph.D. In August, 2011, I was joined Shoolini University as Assistant Professor Microbiology in Faculty of Applied Sciences and Biotechnology. I was awarded as DBT-BioCARE fellow on April, 2012. This project was based on bioactive compounds of wild mushrooms of North-western Himalaya. One start-up grant has been approved by Himalayan Startup Trek, 2019 held at IIT Mandi in October, 2019 on chewable tablets of medicinal mushrooms. I have published thirty one research papers on different aspects of mushrooms. I am life member of Mushroom Society of India. 6 PhD, 13 MPhil and 82 UG/PG students have completed their research thesis under my supervision.

Table-1: Information of Collected non-gilled mushrooms

S.No.	Name	Accession Number		
		Gene Bank	Herbarium	Culture Bank
1	<i>Auricularia auricula judae</i>	MF770159	27219	DMRO-1096
2	<i>Auricularia polytricha</i>	MF774107	27218	DMRO-1097
3	<i>Ganoderma lucidum</i>	MF770158	27220	DMRO-1098
4	<i>Trametes elegans</i>	MF770160	27217	DMRO-1099

Table-2: HPTLC analysis indicating the presence of Gallic acid and Rutin

Samples	Gallic acid		
	Amount of Standard (µl)	Area (AU)	Amount in sample (µlml ⁻¹)
<i>Auricularia auricula judae</i>	20	979.2	30.3
<i>Auricularia polytricha</i>	20	3065.5	94.79
<i>Ganoderma lucidum</i>	20	3329	102.94
<i>Trametes elegans</i>	20	1085.4	33.56
	Rutin		
<i>Auricularia auricula judae</i>	10	357.4	8.62
<i>Auricularia polytricha</i>	10	694.6	16.74
<i>Ganoderma lucidum</i>	10	698.1	16.83
<i>Trametes elegans</i>	10	306.3	7.38

Table 3: Rf value of standard and four non- gilled mushrooms

Rf Value	<i>Auricularia auricula judae</i>	<i>Auricularia polytricha</i>	<i>Ganoderma lucidum</i>	<i>Trametes elegans</i>	Standard
Gallic acid	0.41	0.39	0.40	0.48	0.43
Rutin	0.74	0.71	0.67	0.69	0.70

Figures:

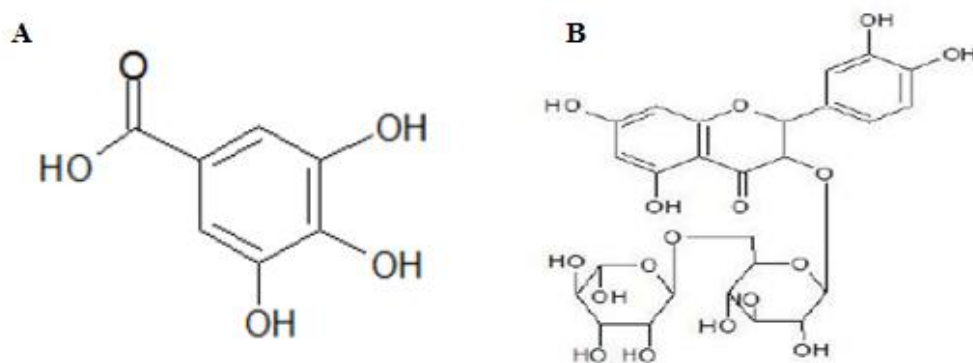


Fig.1: Chemical structure of Gallic acid (A) and Rutin (B)

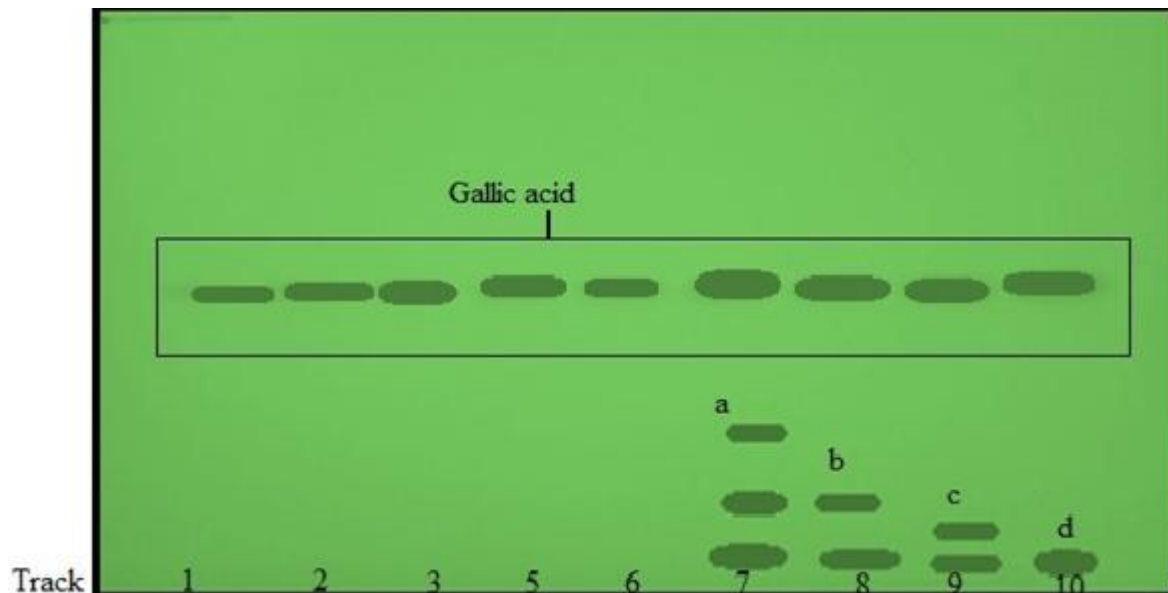


Fig.2: TLC profiles: a- Gallic acid in *Auricularia auricula-judae* extracts (Track -7); b-Gallic acid in *Auricularia polytricha* extracts (track-8); c- Gallic acid in *Ganoderma lucidum* extract (Track -9) (d) Gallic acid in *Trametes elegans* extract (Track-10); Gallic acid (Track 1-6) at 280 nm

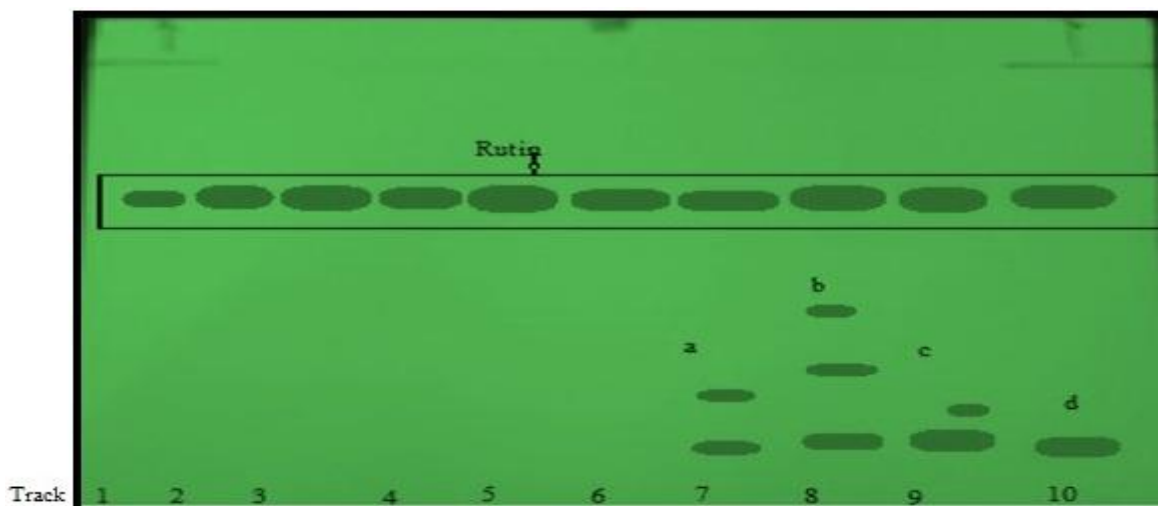


Fig.3: TLC profiles: a- Rutin in *Auricularia auricula-judae* extracts (Track -7); b-Rutin in *Auricularia polytricha* extracts (track-8); c- Rutin in *Ganoderma lucidum* extract (Track -9) (d) Rutin in *Trametes elegans* extract (Track-10); Rutin (Track 1-6) at 254nm

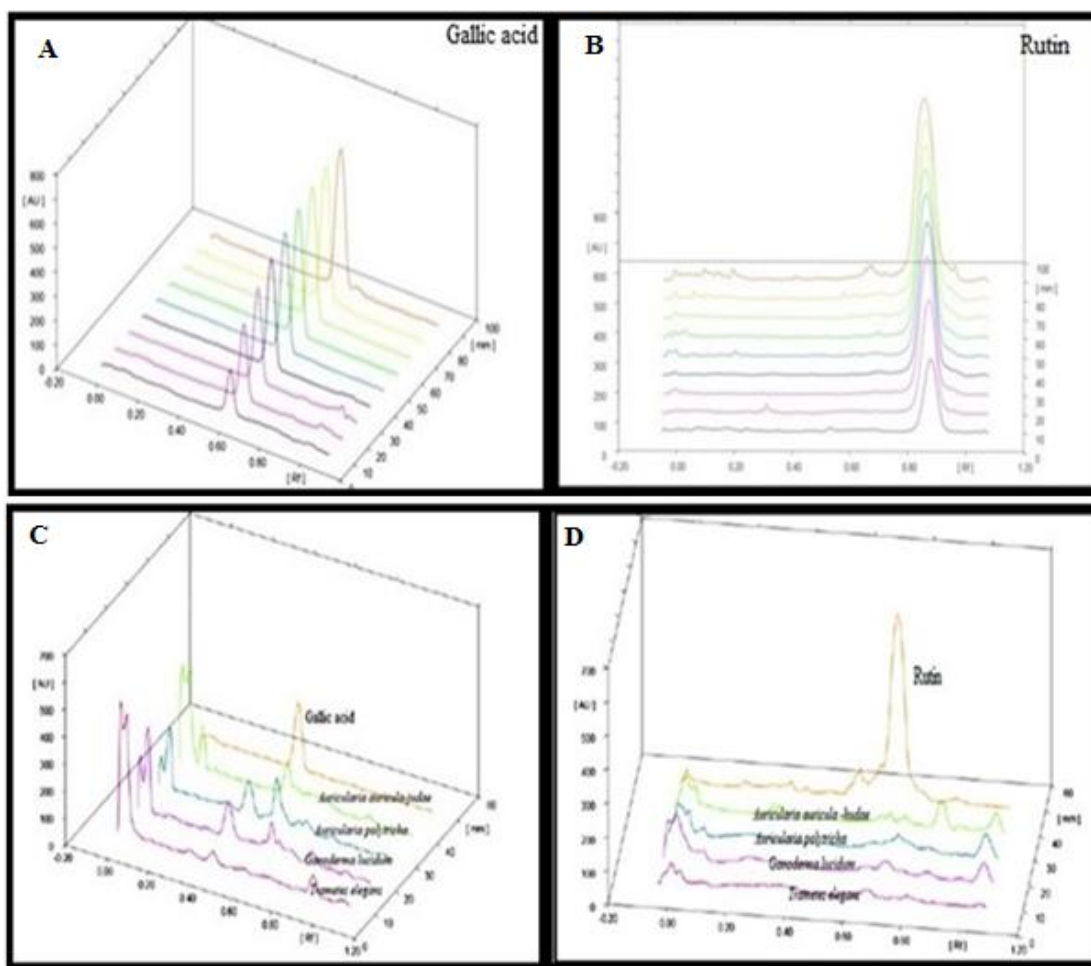


Fig.4: 3D chromatogram of Standard of Gallic acid and Rutin (A, B) and non-gilled mushroom samples (C, D)

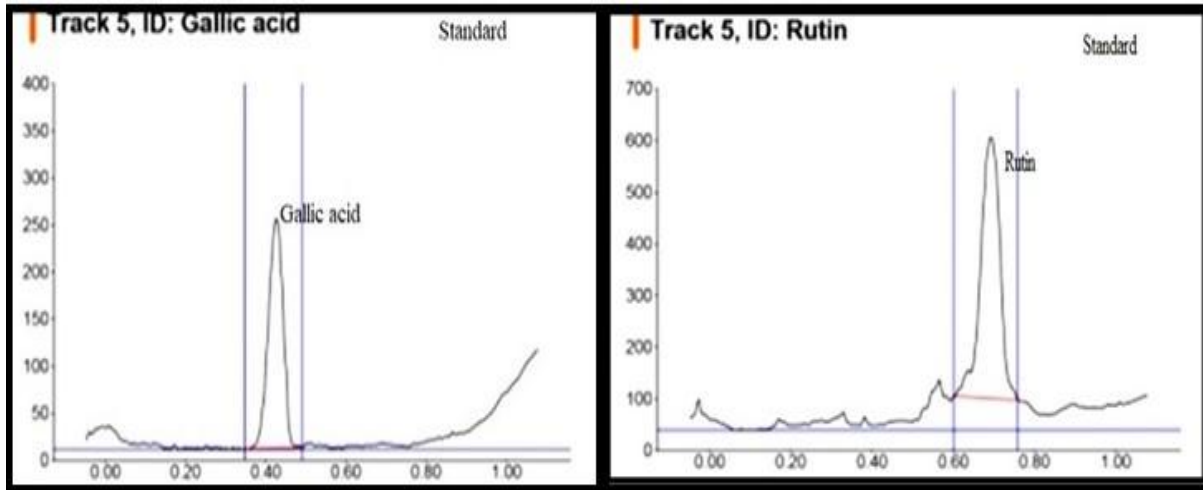


Fig.5: HPTLC Chromatogram of standard Gallic acid and Rutin

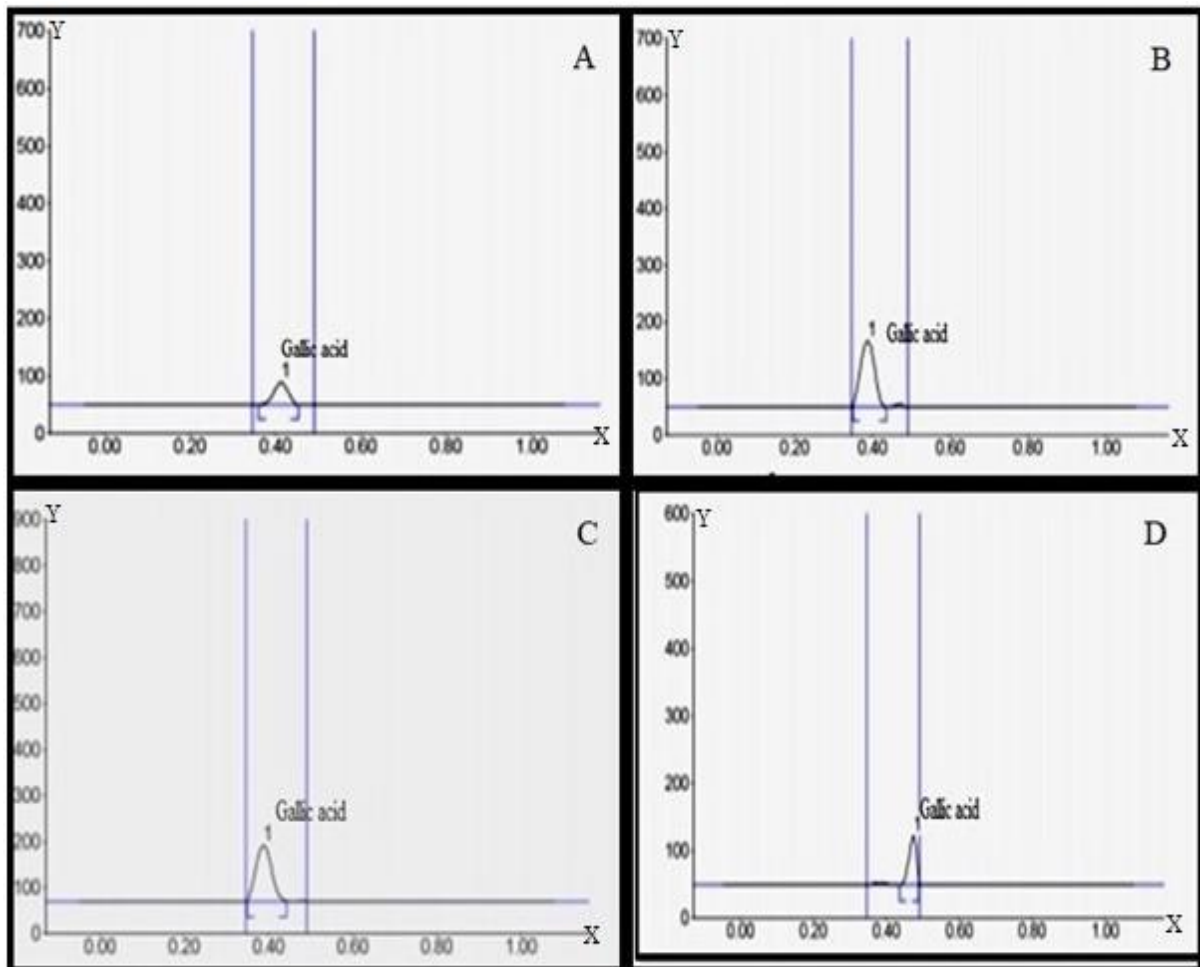


Fig.6: HPTLC Chromatograms of A-*Auricularia auricula-judae*, B-*Auricularia polytricha* C- *Ganoderma lucidum*, D-*Trametes elegans* of methanolic extract (Gallic acid); X axis corresponds to R_f and Y axis corresponds to absorbance

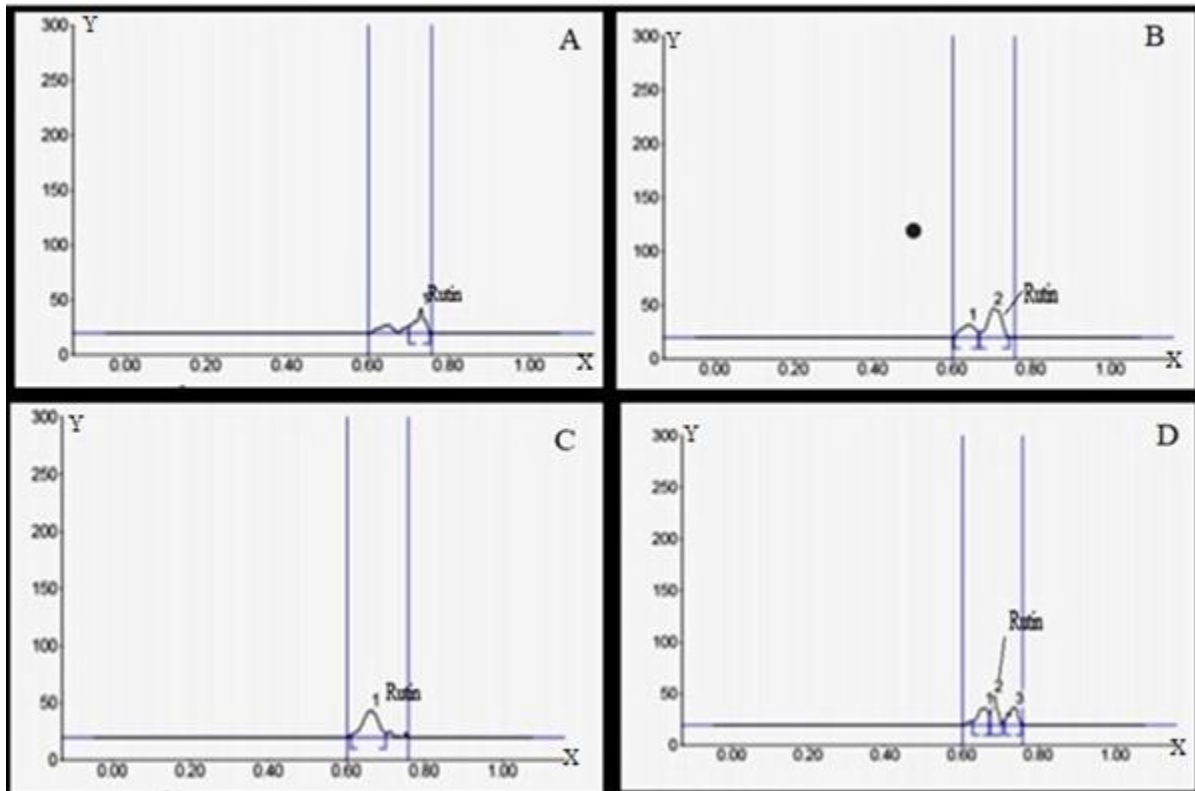


Fig. 7: HPTLC Chromatograms of A-*Auricularia auricula-judae*, B-*Auricularia polytricha* C- *Ganoderma lucidum*, D- *Trametes elegans* of methanolic extract (Rutin); X axis corresponds to R_f and Y axis corresponds to absorbance