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## ***Ganoderma curtisii*, Firstly Reported from Districts Lahore and Gujranwala of Punjab Province, Pakistan**

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**ABSTRACT:** *The current study presents a Ganoderma sp., as a new record from three different localities of districts Lahore and Gujranwala of Punjab province, Pakistan. Based on morpho-anatomical characteristics, collected specimens were identified as Ganoderma curtisii. The macroscopic and microscopic features of G. curtisii include; semicircular to kidney shaped laccate pileus that is beautifully lacquered, duplex context, resinous bands, no concentric growth zones, pore surface white to cream brown and sub circular to circular pores, stipe lateral making an angle with the pileus (45-90 degrees), basidiospores  $11.4 \times 5.7 \mu\text{m}$ , ellipsoidal with the hyaline vesicular appendix double walled, trimitic hyphal system and club shaped cutis. The detailed study of different Ganoderma species from the published literature and comparisons with those species confirmed the identity of our specimen as G. curtisii. In this article, taxonomic descriptions, micrographs and illustrations elaborate the morphological and anatomical characters of Ganoderma curtisii, a distribution map of this species across Pakistan and a comparison table of morphoanatomical characteristics of Ganoderma curtisii reported from neighbouring countries is given. Conduction of molecular studies, investigation of ecological roles, development of conservational strategies, and exploration of biotechnological exploration are future perspectives for this taxon.*

**Keywords:** Canal-bank Road Lahore, *Ganoderma curtisii*, Macrofungi, Polyporaceae

## INTRODUCTION

*Ganoderma* genus was founded by Karsten (1881) as a member of laccate pileated group of species, with *G. lucidum* as a type specimen. Genus *Ganoderma* has following subgenera, *Ganoderma* (which is classified in further sections *Ganoderma* and *Phaenema*), *Elfvingia*, and *Trachyderma* (Thawthong et al., 2017; Zhao and Zhang, 2000; Galappaththi et al., 2024). There are about 219 species assigned to genus *Ganoderma* (Moncalvo, 2000; Wachtel-Galor et al., 2012). This genus has distinguishing characters of laccate (shiny) surface with thick-walled pilocystidia that are present in an extracellular melanin matrix (Moncalvo, 2000), sessile to stipitate basidiomata and bilayered basidiospores with inter-wall pillars (Karsten, 1881; Moncalvo and Ryvardeen, 1997; Luangharn et al., 2021). Due to extreme phenotypic plasticity, the identification of *Ganoderma* species is difficult (Ryvardeen, 1994; Zhao and Zhang, 1994; Wachtel-Galor et al., 2012; Chen et al., 2024). More reliable criteria to identify and distinguish *Ganoderma* species includes context color and consistency, shape and size of spore and micro-anatomy of the pileal crust. *Ganoderma* is a cosmopolitan genus with global distribution in tropical and sub-tropical forests.

Specimens flourish in hot and humid conditions (Pilotti et al., 2004; Singh et al., 2014; Thawthong et al., 2017).

Basidiomes are commonly found in bracket form (Pilotti et al., 2004), with most *Ganoderma* species are pathogenic and grow as facultative parasites of trees and cause various diseases in plants such as stem rot, wood decay and white rot (Ryvardeen, 2004; Pilotti, 2005; Dai et al., 2007; Cao and Yuan, 2013; Coetzee et al., 2015). Some *Ganoderma* species are saprobes on rotting stumps and roots (Turner, 1981; Pilotti, 2005). This genus has a great pharmaceutical importance due to the presence of natural bioactive compounds, e.g., high and low molecular weight sterols, triterpenoids polysaccharides, and proteins (Ahmadi and Riazipour, 2007; Chan et al., 2007; Chen and Seleen, 2007; Luangharn et al., 2021). The bioactive constituents present in the genus *Ganoderma* are known to have broad therapeutic properties, such as antiviral, anticancer, anti-tumor, anti-inflammatory, anti-oxidant, anti-hypotensive, immunomodulatory, anti-diabetic, anti-viral, anti-bacterial, anti-fungal potentials (Liu et al., 2002; Paterson, 2006; Teng et al., 2011; De Silva et al., 2012a, b; Kao et al., 2013; Singh et al., 2014; Richter et al., 2015; Hyde et al., 2019; Luangharn et al., 2021).

Fruiting bodies of some *Ganoderma* species have been used as traditional medicine for more than 2,000 years in some countries for promoting longevity and health, boosting the immune system, lowering the risk of cancer, treatment of diabetes, insomnia, debility and weakness etc (Galor et al., 2004; Singh et al., 2014). The cultivated yield for *Ganoderma* has exceeded 10,000 tons per annum due to its unique pharmacological activity (El Sheikha, 2022; Khadbaatar et al., 2024), this is due to the presences of highly active compounds including polyphenols, polysaccharides, quinones, sterols, triterpenes (Seweryn et al., 2021; Fang et al., 2023; Khadbaatar et al., 2024).

In Pakistan, previously, about 18 species of this genus have been reported so far and these are *Ganoderma applanatum* (Pers.) Pat., *Ganoderma australe* (Fr.) Pat., *Ganoderma ahmadii* Steyaert, *Ganoderma boninense* Pat., *Ganoderma curtisii* (Berk.) Murrill, *Ganoderma colossus* (Fr.) C.F. Baker, *Ganoderma chalceum* (Cooke) Steyaert, *Ganoderma flexipes* Pat., *Ganoderma gibbosum* (Blume & T. Nees) Pat., *Ganoderma lucidum* (Curtis) P. Karst., *Ganoderma leucocontextum* T.H. Li, W.Q. Deng, Sheng H. Wu, Dong M. Wang & H.P. Hu, *Ganoderma multipileum* Ding Hou, *Ganoderma multistipitatum* S.

Ahmed, M. Awais, M.M. Sadiq, A. Umar, L. Dufosse, M.T. Khan, J. Alkahtani & R.M. Mahmoud, *Ganoderma multicornum* Ryvarden, *Ganoderma multiplicatum* (Mont.) Pat., *Ganoderma pakistanicum* Umar, A., Ahmed, S., & Gafforov, Y., *Ganoderma resinaceum* Boud., and *Ganoderma tornatum* (Pers.) Bres.

In this study, we are dealing with three specimens of *G. curtisii* species collected from three different localities of Lahore and Gujranwala districts. Previously this taxon has already been reported only from Murree Hills of Pakistan. The morphological and anatomical descriptions, macrographs of fruiting bodies, micrographs of anatomical features, comparison morpho-anatomical features of *Ganoderma curtisii* described from neighboring countries of Pakistan along with a map showing geographical locations of *G. curtisii* from Pakistan are given in this article.

## MATERIALS AND METHODS

Three of *G. curtisii* specimens were collected during the during rainy seasons of 2021 from Canal-bank Road (Lahore) Wazirabad tehsil and Uggo Chak, along Hafizabad road (Gujranwala). Each specimen was photographed, tagged with a specific code and documented with location, date

and host tree details. In laboratory, the specimens were dried using a fan heater and preserved in zip locked bags with their tags.

### Macroscopic Analysis

Macroscopic characteristics of fruiting body were analyzed including length and width, color and size of pileus, resinous bands, laccate surfaces, texture, margins of stipe and pileus, presence or absence of stipe, hymenial surface, discoloration, taste and odor.

### Slide Preparation and Micrometry

For the slide preparations, small pieces of the dried specimens were soaked in alcohol for 5 minutes then thin sections were placed on glass slides. Two drops of 5% KOH were used as mounting medium and 1% aqueous Congo red solution (w/v) was applied for hyaline structures. The fine sections of fruiting bodies were sharply cut and covered with cover slip for observation. Microscopic characteristics were measured and recorded under a light microscope at 40X magnification.

For anatomical analysis, at least 20 readings were taken, focusing on the length and width, shape, and quotient value and range of basidiospores, basidiole, cutis elements. The width range of generative hyphae, skeletal hyphae, binding hyphae and stipe hyphae were also measured. Additionally, extreme spore length

and width values were also noted. Total number of spores determined for each collection was  $n$  and  $Q_{avg}$  was the average of the  $Q$  coefficient (length/width ratio). Manual illustrations were created observing hymenial section. Identification of the species was conducted based on morpho-anatomical features.

## RESULTS

The morpho-anatomical explanation species *Ganoderma curtisii* (Berk.) Murrill, with voucher codes (KM-09; GM-95; GM-15) is given in the form of description, micrographs, and illustrations. This species was collected at young and mature stages and reported here as two new locality records from districts Lahore and Gujranwala of Punjab province, Pakistan.

### Taxonomy

*Ganoderma curtisii* (Berk.) Murrill, N. Amer. Fl. (New York) 9(2): 120 (1908)

(Fig. 1-4)

### Macroscopic Analysis

**Basidiocarp** 4-10 cm, annual, stalked or finger-like structure, aplanate, coriaceous to woody hard. **Pileus** 1.3-1.5 × 1-1.2 cm (when young), 3.5-12 × 9-10 cm, 2-6 cm deep (when mature) medium sized to large, upper surface smooth, flabellate to subflabellate, glabrous, semicircular to kidney shaped when mature, laccate, creamy

white to light brown when young and brownish red to reddish brown when mature with purple hues, reddish brown to orange-yellow and ultimately white from center to margins, uneven and bald surface, lacquered. Texture tough and hard but not woody. **Margins** slippery when wet, thin and sharp, unlike to the center softer and thinner, varies in color from orange red to brown from young to mature, have active developing hyphae, clearly laccate, thinner than center. **Hymenial surface** of the basidiocarp is fertile and porous, light brown and not even. Duplex context 0.35 cm thick, resinous bands (melanoid zone) present, no concentric growth zones as present in *G. sessile*, corky-woody, has club-shaped cuticle cells, thick at the base, clamped, and subsolid hyphae. **Pore surface** whitish to light brown color when young stage and medium dark brown when maturity, sub circular to circular in shape and 4-6 per mm, thin dissepiments. Tubes 0.5-2 cm deep, unstratified, have variations in thickness, scretched when dried, light brown, with a typical melanoid zone. **Stipe** (usually present) 6.8- 7.8 × 1.5- 2.2 cm, bald, reddish-brown color, glabrous lacquered, rough and solid texture, lateral making an angle with the pileus (45-90 degrees). **Taste and odor** not distinctive. **Habit and habitat** grow in solitary or group, present

at the base of *Eucalyptus camaldulensis* Dehnh in terrestrial area.

### Microscopic Analysis

**Basidiospores** 11.4 × 5.7 μm, with hyaline vesicular appendum; avgL 11.4 μm, avgW 5.7 μm, avgQ 2 μm, broadly ellipsoid with truncate apex, brown in KOH double walled, inamyloid, contains oil droplet, sharply spotted, utriculate, brown color overlaid by hyaline apex and the wall have a range of "pillars" that are intermediately thick (0.3-0.7 μm). **Hyphal system** trimitic; consist of generative hyphae that were difficult to observe, hyaline to yellowish, branched, clamped. Skeletal hyphae  $W_{\text{range}}$  2.85-5.7 μm, avgW 4.27 μm, from pileus width ranges from 2.85-8.55 μm, avgW 5.13 μm, grouped, thick walled, apically branched, aseptate, golden brown, broader in the context and thinner crustohymeniderm, Binding hyphae hyaline to yellow in color and narrower than skeletal hyphae. **Basidia** 28-40 × 7-8.5 μm, double walled, light yellowish in color, bisporic, clavate to cylindrical, clamped at base, thin, without oil droplets. Cutis element 22.8-28.5 × 5.7-8.55 μm, avgL 25.65 μm, avgW 7.125 μm, Orange 2.66-5 μm, avgQ 3.83 μm, clavate to sub-clavate shape, clustered, apedicellate, smooth, and golden brown-yellow, thin or thick laccate lines of melanoid

substance present that start off from the stipe and run to the upper surface correspondingly. **Stipe hyphae**; 3-7  $\mu\text{m}$ , branched, aseptate, thin walled, light golden yellowish color, narrow.

#### Collection Sites

**PAKISTAN:** Punjab, Lahore, Canal Bank Road, Green belt near H-3 block, 217 m above sea level, growing singly or groups, on the bottom of *Eucalyptus camaldulensis* Dehnh trunk, Khadija Malhi, KM-09, 14<sup>th</sup> October 2021; District Gujranwala, Wazirabad Tehsil, Neevan Ojla, Wazirabad, attached with wood, solitary, 226 meters (744 ft.) above sea level, S.

Rukhsar (GM-15) 30<sup>th</sup> August 2021; Gujranwala Tehsil, Uggo

with base of tree trunk, solitary, 226 meters (744 ft.) above sea level, S. Rukhsar (GM-95) 25<sup>th</sup> September 2021.

#### Literature Reviewed for Species Identification:

The description of morphological and anatomical characters of the reported species was confirmed from published literature of Steyaert (1980), Torres-Torres and Guzman-Davalos (2012), Torres-Torres et al. (2015), Loyd et al. (2018), Mardones et al. (2023), and Chouhan & Kaur (2023).

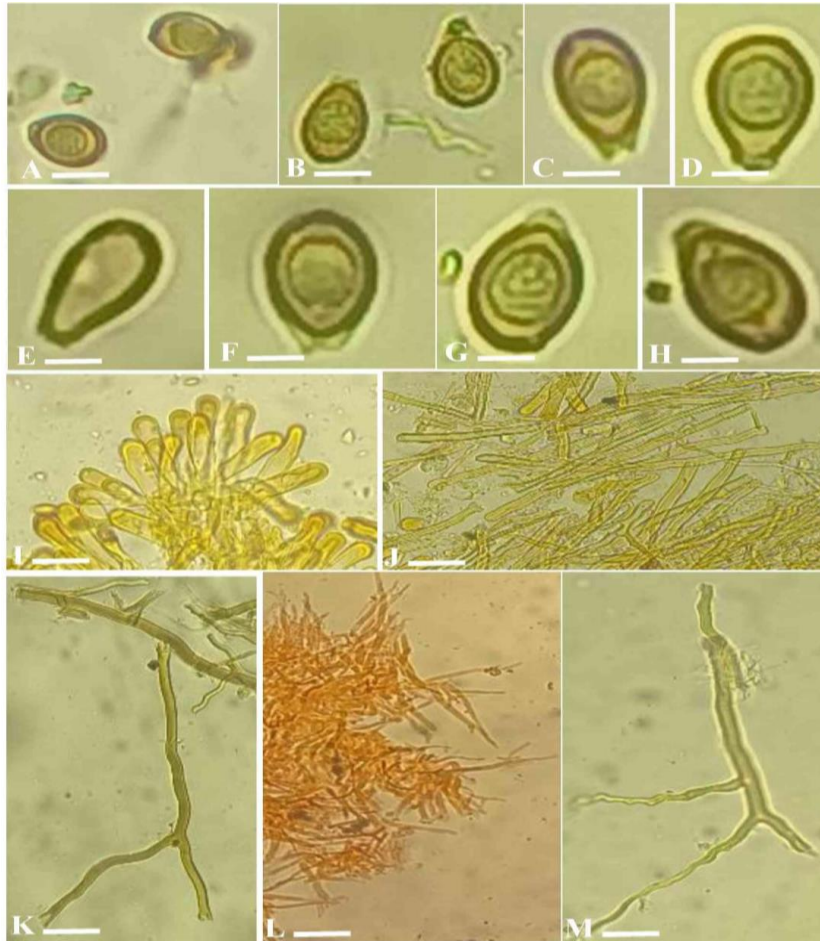


Chak, Hafizabad Road, attached

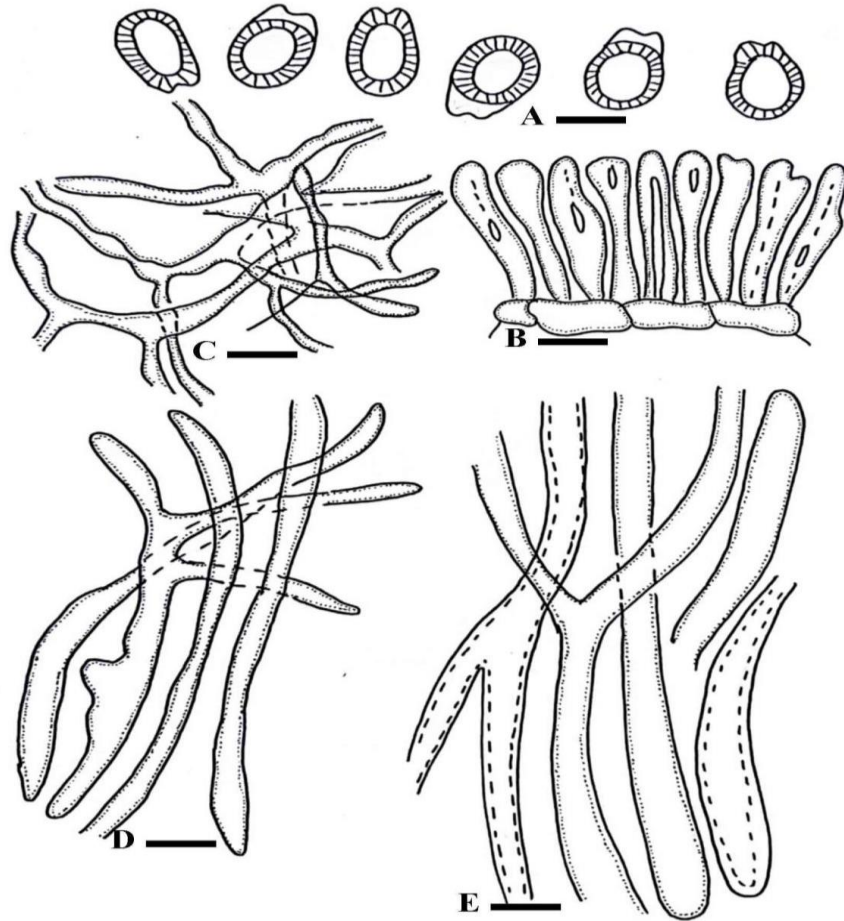
**Fig. 1:** Morphology of *Ganoderma curtisii* (KM-09). **A & B.** Basidiocarp in its natural habitat. **C & D.** Basidiocarp showing both white cap and stipe. **Scale bars:** **A.** 0.8 cm. **B.** 0.5 cm. **C.** 1.95 cm. **D.** 1.5 cm.



**Fig. 2:** Morphology of basidiomata of *Ganoderma curtisii* (GM-15; GM-95). **A–C.** Basidioma. **D–F.** Pore surface. **Scale bars:** **A, D & E.** 2 cm, **B & C.** 1.5 cm, **F.** 1 cm.



**Fig. 3:** Light micrographs of microscopic features of *Ganoderma curtisii* (GM-95). **A–H.** Basidiospores. **I.** Cutis elements. **J & K.** Generative hyphae. **L.** Skeletal hyphae. **M.** Stipe hyphae. **Scale bars:** **A.** 8  $\mu\text{m}$ , **B.** 7  $\mu\text{m}$ , **C.** 4.5  $\mu\text{m}$ , **D–F.** 4  $\mu\text{m}$ , **G & H.** 3.5  $\mu\text{m}$ , **I.** 17  $\mu\text{m}$ , **J & K.** 25  $\mu\text{m}$ , **L.** 30  $\mu\text{m}$ , **M.** 12.5  $\mu\text{m}$ .



**Fig. 4:** Illustrations of microscopic features of *Ganoderma curtisii* (GM-95). **A.** Basidiospores. **B.** Cutis elements. **C.** Skeletal hyphae. **D.** Stipe hyphae. **E.** Generative hyphae. **Scale bars:** **A.** 6.5  $\mu\text{m}$ , **B.** 14  $\mu\text{m}$ , **C.** 8  $\mu\text{m}$ , **D.** 8.5  $\mu\text{m}$ , **E.** 10  $\mu\text{m}$ .

**Table 1: Comparison of Characteristic Features of *Ganoderma curtisii* reported from Neighbouring Countries of Pakistan**

Species Name with country	Macroscopic characters						Microscopic Characterization							Source	
	Pileus						Stipe Length (cm)	Basidiospores		Type of Hyphal system	Hyphal system				Basidia size (µm)
	Size (cm)	Shape	Colour (at maturity)	Resinous Bands/ Non-Resinous Bands	Lactate/ Non Lactate	Concentric Zones (on the upper surface of the pileus)		Size (µm)	Shape		Generative Hyphae (Diameter in µm)	Skeletal Hyphae (Diameter in µm)	Binding Hyphae (Diameter in µm)		
<i>G. curtisii</i> (Pakistan)	1.3-1.5 × 1-1.2	semicircular to kidney shaped	brownish red to reddish brown with purple hues	resinous bands	lactate	concentric zonate	6.8-7.8	9.5-11.2 × 4.8-6.3	broadly Ellipsoidal	trimitic	2.4-4	2.85-5.7	2-3.1	12-27 × 5.6-11	Mukhtar, 2019
<i>G. curtisii</i> (India)	14 × 2.5	circular	creamish white, r	resinous bands	lactate	concentric zonate	8.0 × 3.0	14.0-16.5 × 9.0-10.5	ellipsoid	trimitic	5.9	4-5.5	3	21.8 × 3.12	Nagadesi & Arya, 2012 ; Chouhan & Kaur, 2023
<i>G. curtisii</i> (China)	10.5 × 4.4	reniform	yellowish-brown to reddish-brown with purple hues	resinous bands	lactate	concentric zonate	9.0 × 4.0	(9-)11-17 × (7-)8-10	ellipsoid to oblong	trimitic	3.5	1.5-6	3-5	not observed	Cao et al., 2012 ; Zhou et al., 2015 ; He et al., 2021

## DISCUSSION

*Ganoderma* was named due to its shiny or lustrous surfaces and *curtisii* represented the species name in the honor of Moses Ashley Curtis (1808-1872), an American Episcopal priest, botanist, mycologist and teacher. *Ganoderma curtisii* (Berk.) Murrill belongs to the laccate group of *Ganoderma* occurred in both temperate and sub-tropical forests. Initially, *Ganoderma curtisii*, was defined from North America (Moncalvo and Ryvarden, 1997; Thawthong et al., 2017). This species is saprobic and parasitic may be growing alone or in groups, found on the dead or decaying hardwood logs tree trunks or on the roots. From Pakistan this species was collected and reported from Murree Hills. In this study, *G. curtisii* was reported as new locality records from the base of *Eucalyptus camaldulensis*, and from base of an unknown tree trunk in terrestrial areas from Lahore and Gujranwala districts.

*G. curtisii* is a white root decay fungus, that causes the greater mass loss from the substrate where they are present, and this activity is comparable to the *F. ambrosius* as reported by (Kasson et al., 2016; Skelton et al., 2020).

*G. curtisii*, the mushroom of immortality or Ling-Chi, was characterized by semicircular to kidney shaped pileus beautifully

lacquered with brilliant yellow new growth and white tips, duplex context, resinous bands, no concentric growth zones (Cao et al., 2012) as present in *G. sessile*, corky- woody, club-shaped cuticle cells present (Haddow and Haddow, 1931; Steyaert, 1980; Ojeda-Lopez et al., 1986; Torres-Torres et al., 2015), Pore surface white to crème brown and sub circular to circular pores (Steyaert, 1980), stipe lateral at the angle of (45-90 degrees) with the pileus, basidiospores  $11.4 \times 5.7 \mu\text{m}$ , ellipsoidal (Steyaert, 1980; Torres-Torres and Guzman-Davalos, 2012) with the hyaline vesicular appendix double walled, trimitic hyphal system and club shaped cutis (Torres-Torres et al., 2015; Gurpreet et al., 2017).

*G. curtisii* by some adjustments has been used to determine antioxidant activity and in the food industries other nutritional manufacturing because of its effective natural antioxidants (Huang et al., 2002; Ivone et al., 2016; Rosales-Lopez et al., 2022).

*G. curtisii* has been used to make composites due to its ability to form resistant mycelial matrix (Cesar et al., 2023). By this species about 29 lanostane triterpenoids have been obtained, that are the lipopolysaccharides, inhabit the nitric oxide (NO) production in the activated BV-2 microglia cells (Jiao et al., 2016).

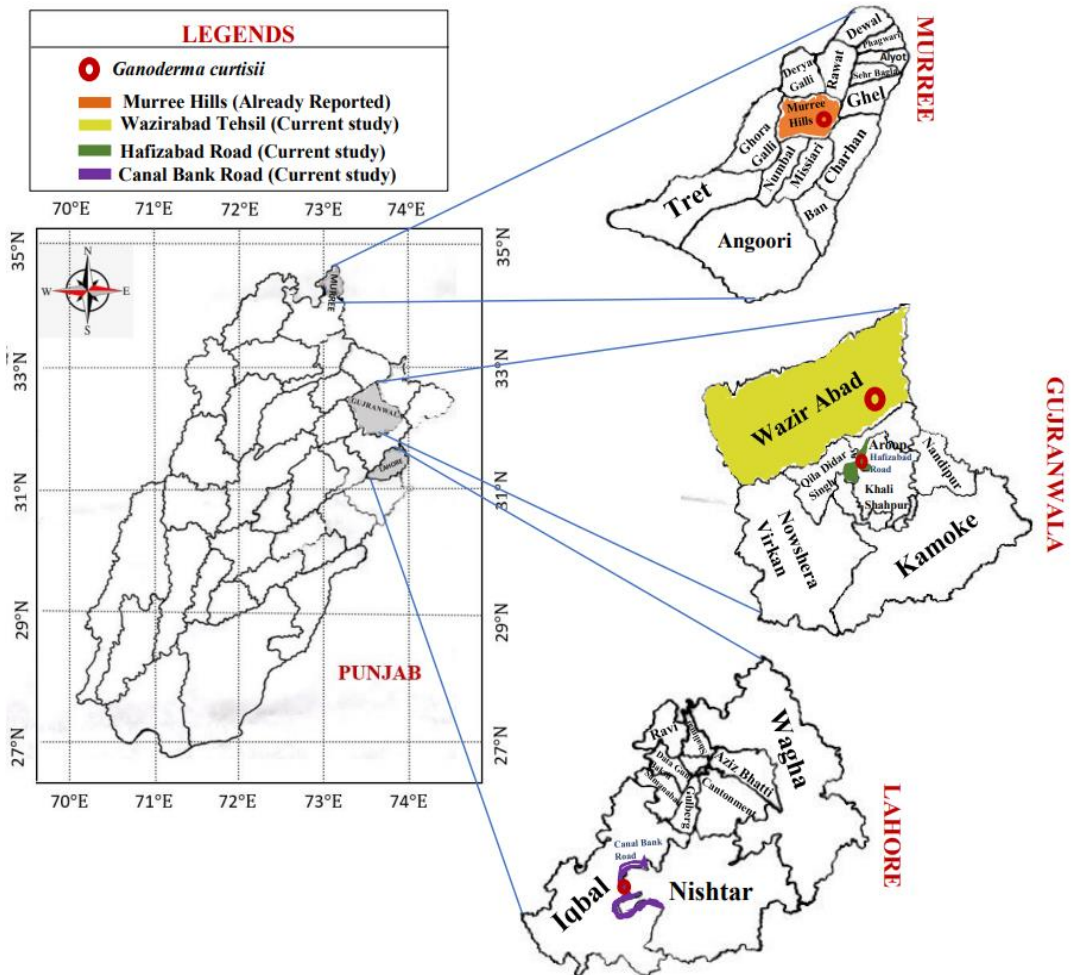
*G. curtisii* exhibits antibacterial activity, in addition to ganoderic acids, phenolic compounds are also involved in this activity (Li et al., 2012; Rempe et al., 2017). The compounds extracted from this species responsible for this antibacterial activity are phenols, terpenes, and fatty acids. (Desbois and Smith, 2010; Rempe et al., 2017). This inedible fungus can be used to lower the blood pressure and to reduce the blood clot. It can also be used in cancer treatments.

*G. curtisii* was morphologically related to the *G. sessile* as both were annual, perennial (*G. curtisii* is substipitate to stipitate or sessile while *G. sessile* is sessile), pileus surface laccate, flabelliform, dimidiate, lacquered (Steyaert, 1980; Torres-Torres et al., 2015; Nagadesi and Arya, 2016). The duplex context (Torres-Torres and Guzman-Davalos, 2012) have resinous bands (melanoid zone). There were no concentric growth zones in *G. curtisii* (Cao et al., 2012; Luangharn et al., 2021) but were present in *G. sessile*. Basidiospores ellipsoidal (Steyaert, 1980; Torres-Torres and Guzman-Davalos, 2012), the size of basidiospores in *G. sessile* were slightly larger as compared to *G. curtisii* (Luangharn et al., 2021), hyphal system is trimitic (Steyaert, 1980).

*G. curtisii* closely resembled *G. lingzhi* (*G. lucidum*) in phylogeny.

The basidiomata of both resembles each other in juvenile stage, pileal surface is yellow with white pore surface. When mature, *G. lucidum* has pileus reddish brown in color with a hard crust, however, *G. curtisii* still has yellowish brown to brick red pileus with a thin and soft crust. In *G. curtisii* the pore surface was white with thin dissepiments when mature, while in *G. lucidum* pore surface is yellow when mature with dissepiments thick. The cuticle cells in the *G. lucidum* are, closely packed, slender with narrower apical parts as compared to loosely arranged cutis cells inflated with wider apical parts in *G. curtisii* (Steyaert, 1980; Cao et al., 2012; Torres-Torres et al., 2015).

According to (Torres-Torres et al., 2015) *G. meredithiae* and *G. ravenelii* had identical morphology as that of *G. curtisii* in the basidiomata, pileus shape, color and texture, margins, context features, pore arrangements, stipe color and texture, and in microscopic characterizations. *G. meredithiae* (Adaskaveg and Gilbertson, 1988) had more diverticulated and differentiated cuticle cells. But in case of *G. ravenelii* there was a difference in terms of shape and size of basidiospores, as compared to *G. curtisii* they were more oblong to cylindrical (Steyaert, 1980) and in the context there were no resinous bands.



**Figure 5: Distribution of *Ganoderma curtisii* in Pakistan**

## CONCLUSION

In conclusion, new locality reports of *Ganoderma curtisii* from three different sites (one from District Lahore and two from District Gujranwala) signified the notable expansion of its geographical range. This finding suggested that Punjab supports the

growth of these macro-mycetes and there are many more that need to be explored. The morphological and anatomical features of *Ganoderma curtisii* was described by the illustrations, micro-plates and colored photographs of micro-plates, a distribution map of this species at Murree (already reported), Lahore and Gujranwala (current study) reported from

Punjab, and a comparison table of characteristic attributes of *Ganoderma curtisii* recorded from neighboring countries of Pakistan were presented in this article.

#### ACKNOWLEDGEMENT

N/A

#### CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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