Volume 3, Issue 5, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

PHYTOCHEMICAL PROFILING AND ANTIBACTERIAL POTENTIAL OF *MORUS ALBA* L. LEAF EXTRACTS AGAINST SALMONELLA TYPHI

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DOI: https://doi.org/10.5281/zenodo.16633194

Keywords
Morus alba, leaf extracts, agar well diffusion, *Salmonella typhi*

Article History
Received: 31 April, 2025
Accepted: 16 July, 2025
Published: 31 July, 2025

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Abstract

Typhoid fever, commonly referred to as typhoid, is a bacterial infection caused by Salmonella typhi. Its treatment relies on antibiotics; however, this approach poses challenges such as drug resistance, high costs, and limited accessibility, particularly for rural populations. This research aimed to identify the bioactive metabolites in the leaves of Morus alba and evaluate their antimicrobial activity against Salmonella typhi. The antibacterial potential of Morus alba leaf extracts prepared through aqueous maceration, decoction, infusion, and ethanol maceration was assessed using the agar well diffusion technique to explore its viability as an antibacterial agent. Additionally, the phytochemical constituents, Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC) of the extracts were analyzed. Phytochemical screening revealed the presence of saponins, flavonoids, tannins, phenolics, and coumarins, while phlobatannins and oxalates were absent. Among the extraction methods, the decoction exhibited the highest antibacterial activity against S. typhi, with a zone of inhibition of 15 ± 2.7 mm at 1000 mg/ml. MIC values for aqueous maceration, infusion, and decoction were recorded at 62.5 mg/ml, whereas ethanol maceration demonstrated an MIC of 15.625 mg/ml. Despite these findings, the study concluded that the antibacterial activity of Morus alba leaf extracts against S. typhi was limited, given the exceptionally high concentrations required to achieve effectiveness.

INTRODUCTION

Natural products have been a crucial source of new medicines throughout history (Manan et al., 2025). While natural compounds may not always serve as the final active ingredients in drugs, many modern medicines originate from natural sources (David & Craig, 2020). It is estimated that about 25% of today's medicines are derived, directly or indirectly, from medicinal plants (Irshad et al., 2025). Examples include artemisinin, paclitaxel, vincristine, and salicylic acid (Robinson & Zhang, 2011). According to the World Health Organization (WHO), approximately 80% of people in developing countries rely on traditional medicine for healthcare (WHO, 2013). The process of drug discovery

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ISSN: (e) 3007-1607 (p) 3007-1593

involves identifying new chemical entities (NCEs) with therapeutic potential (Ullah et al., 2025a). These NCEs can be obtained through isolation from natural sources, chemical synthesis, or a combination of both (Krause & Tobi, 2013). Medicinal plants, in particular, provide an alternative treatment option for infectious diseases, ranging from mild to severe cases (Fabricant & Farnsworth, 2001). This is increasingly important as more pathogens develop resistance to standard antibiotics (Ullah et al., 2025b).

Morus alba, known as white mulberry, common mulberry and silkworm mulberry, is a fast-growing, small to medium-sized mulberry tree which grows to 10–20 m (33–66 ft) tall (Eseyin *et al.*, 2007). It is generally a short-lived tree with a lifespan comparable to that of humans, although there are some specimens known to be more than 250 years old (Akindele et al., 2018). The species is native to China and India and is widely cultivated and naturalized elsewhere (including United States, Mexico, Australia, Kyrgyzstan, Argentina, Turkey, Iran, Iraq, and many others) Africa (Badifu & Ogunsua, 1991; Ullah et al., 2025c). The flowers are single-sex catkins; male catkins are 2–3.5 cm (0.8–1.4 in) long, and female catkins 1–2 cm (0.4–0.8 in) long (Asif et al., 2025). Male and female flowers are usually found on separate trees although they may occur on the same tree (Akoroda, 1990). The fruit is 1–1.5 cm (0.4–0.6 in) long. In the wild it is deep purple, but in many cultivated plants it varies from white to pink (Akoroda, 1990; Eseyin *et al.*, 2014). It is sweet but bland, unlike the more intense flavor of the red mulberry and black mulberry (Stevels, 1990; Ullah et al., 2024). The seeds are widely dispersed in the droppings of birds that eat the fruit. White mulberry leaves are the preferred feedstock for silkworms, and are also cut for food for livestock (cattle, goats, etc.) in areas where dry seasons restrict the availability of ground vegetation (Alada, 2000; Olaniyan & Adeleke, 2005; Okokon *et al.*, 2009). The leaves are prepared as tea in Korea. The fruit are also eaten, often dried or made into wine (Nwozo *et al.*, 2005). Previous studies had reported the anti-anemic (Alada, 2000; Khan et al., 2024), hepatoprotective (Nwozo *et al.*, 2005), hypoglycemic (Eseyin *et al.*, 2007), antinociceptive and anti-inflammatory (Abidemi *et al.*, 2015) activities of extracts of *Morus alba* (Ullah et al 2023). The aims and objectives of this research was to identify the phytochemical components of the leaf of Morus alba, to determine the antimicrobial activities of the leaf extract of Morus alba, to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Morus alba* on *Salmonella typhi*.

MATERIAL AND METHOD

Plant collection

The fresh leaves of *Morus alba* were carefully harvested from Ghahi Village, located in the District Bajaur Mountains, during the months of June and July 2023 (Ullah et al., 2024). The selection focused on mature, healthy leaves to ensure the quality and consistency of the samples. Alongside the leaves, other plant materials such as fruits, buds, and stems were also collected. These additional materials were included to aid in comprehensive identification and taxonomic verification by the National Herbarium. The identification process was conducted under the supervision of botanist Shakir Ullah, an expert associated with the Government Postgraduate College Timergara Herbarium (Ullah et al., 2023). To confirm the identity of the plant samples, a comparative analysis was performed using a voucher specimen from the Botanical Collection, identified as No. GPG12347. This voucher specimen had been previously registered at the Government Postgraduate College Timergara Herbarium, where it was cataloged under the reference number GPGC_12347. This meticulous identification process ensured the accurate classification of the plant as *Morus alba*, providing a reliable foundation for the subsequent research and analysis of its phytochemical properties and antimicrobial potential (Shakir et al., 2023a).

Preparation of plant extracts

The leaves of the plant were air-dried for 4 weeks in a shade, and ground to a fine powder before storing in an air-tight container to avoid the absorption of moisture. A quantity of 50 g was weighed and placed into 4 conical flasks. In flask 1, 500ml of distilled water was added to it and shaken thoroughly to homogenize then allowed to macerate (Shakir et al., 2023b). In flask 2, 500 ml of ethanol 95° was added and shaken and left to macerate. In flask 3, 500ml of

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ISSN: (e) 3007-1607 (p) 3007-1593

distilled water was added, adequately shaken to homogenized and placed in a hot water bath to boil. In flask 4, distilled water heated to 100°C is poured on the powder, shaken to homogenize and allowed to infuse. Each flask is set aside after proper labelling for 72 hours while shaking or stirring from time to time. Later they were individually sieved using a clean mousseline cloth and then filtered using N° 1 Whatman filter paper. The filtrates were oven dried at 55°C. Each extract was separately preserved in sterile well sealed bottles until use (Ullah et al., 2018h).

Phytochemical screening of the extracts

The phytochemical screening of the extracts was conducted following the protocols outlined by Odebiyi and Sofowora (1978) and Trease and Evans (2009). These methods are well-established for the qualitative and semi-quantitative analysis of bioactive compounds in plant materials. In our study, the phytochemical tests targeted a wide range of secondary metabolites known for their pharmacological significance (Ullah et al., 2018). The screening included tests for the presence of alkaloids, steroids, terpenoids, cardiac glycosides, saponins, tannins, total phenols, flavonoids, mucilage's, oxalates, quinones, resins, phlobatannins, and betacyans. Each of these compounds plays a crucial role in medicinal efficacy, contributing to activities such as antimicrobial, anti-inflammatory, antioxidant, and anticancer properties (Ullah et al., 2019c).

The comprehensive screening approach ensured the detection of key phytochemicals, providing valuable insights into the bioactive potential of the *Morus alba* extracts. This analysis forms the basis for further studies to explore the therapeutic applications of the plant in combating Salmonella typhi and other pathogens (Ullah et al., 2018).

Microorganism and growth condition

The microorganisms used in this study consisted of clinical isolates of *Salmonella typhi* strains. These strains were obtained from the Microbiology/Bacteriology Laboratory of the Timergara Dir Lower Teaching Hospital Centre. To confirm their identity and purity, the isolates were subjected to standard biochemical tests as described by Aneja (2003).

After verification, the isolates were sub-cultured on Nutrient Agar (NA) to ensure viability. They were then preserved in sterile cryotubes and stored at 4°C for use in subsequent experiments. This process ensured the reliability and consistency of the bacterial cultures throughout the study (Ullah et al., 2018b).

Preparation of microbial suspensions

Microorganisms were sub-cultured prior to each testing in Muller Hinton agar at 37°C for 24 hours. Stock bacterial inoculums suspensions were prepared in sterile normal saline. Three-four well isolated colonies were picked using the inoculating loop and suspended in sterile saline under aseptic conditions. Bacterial suspensions were adjusted visually to 0.5 McFarland turbidity standards $1x10^8$ CFU/ml (Teke et al., 2013; Teke & Fokunang, 2020).

Anti-microbial Susceptibility Test

The agar well diffusion method was employed to evaluate the anti-salmonella activity of various concentrations of *Morus alba* leaf extracts. This technique aimed to determine which extract exhibited the highest inhibitory effect against Salmonella typhi. Varying concentrations of the extracts were prepared, specifically at 5, 50, 100, 250, and 500 mg/ml (Khan et al., 2018).

Mueller-Hinton agar was prepared according to the manufacturer's instructions, sterilized at 121°C, and cooled to a workable temperature. Ciprofloxacin, a standard antimicrobial agent, was used as a positive control at a concentration of 5 mg/ml. To conduct the assay, an inoculating loop was used to transfer a specific volume of the microbial suspension onto the prepared agar plates, which were uniformly seeded by streaking. A sterile cork borer or pipette tip was then used to aseptically create wells in the agar. Each well was carefully filled with one of the prepared extract concentrations (Ullah et al., 2018g).

The susceptibility plates were incubated at 37°C for 24 hours. After incubation, the plates were observed for zones of microbial inhibition, and the diameters of these inhibition zones were measured. The extract concentration that produced the largest diameter of

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ISSN: (e) 3007-1607 (p) 3007-1593

inhibition was identified as having the highest anti-salmonella activity.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the plant extract was determined by serially diluting extract from 10^1 to 10^{10}. One millilitre (1ml) of each of the dilutions representing a known concentration of the extract was introduced into 1ml of sterile nutrient broth (Mueller Hinton broth) in a test tube. The mixture was then inoculated with 0.1ml of the test organisms previously standardized at 10^8. It was then incubated at 37 °C for 24 hours. The least concentration of the plant extract in the test tube with no turbidity was taken as the MIC (Andrews, 2001). These tests were carried out in triplicate (Khan et al., 2018a).

Determination of Minimum Bactericidal Concentration (MBC)

The plant extract was serially diluted from 10^1 to 10^{10}. One milliliter (1ml) of each of the dilutions representing a known concentration of the extract was introduced into 1ml of sterile nutrient broth in test tubes. The mixture was then inoculated with 0.1ml culture of the test organisms previously standardized to 10^8. It was then incubated at 37 °C for 24hours. The least concentration of plant extract in the test tube with no turbidity was taken as the MIC. Subsequently, tubes that indicated no turbidity were plated out on nutrient agar plates and absence of growth after incubation for 24hours confirms the MBC as described by (Andrews, 2001). These were done in triplicate (Ullah et al., 2018g).

RESULTS

Yield of the extraction

After the extraction the yield of each extract was calculated and the results were presented in the table 1 below. The highest yield was obtained for the decoction, followed by maceration with water. The lowest yield corresponded to the ethanol maceration.

Table 1 Yield of the extraction

Type of extraction	Mass of powder (g)	Volume of solvent (ml)	Mass of extract obtained (g)	Yield (%)
Aqueous infusion	50	500	6.8	13.6
Aqueous maceration	50	500	8.24	16.48
Decoction	50	500	8.41	16.82
Ethanol maceration	50	500	4.09	8.18

Phytochemical Screening of extract

Table 2 shows the phytochemical components of extracts of *Telfairia occidentalis*. The result indicated the presence of saponins, alkaloids, tannins, phenolics, and absence of oxalates, phlobatannins, and anthraquinones.

Table 2 Phytochemical components of *Telfairia occidentalis*

Secondary Metabolites	Ethanol maceration	Aqueous maceration	decoction	Infusion
Polyphenols	+	+	++	+++
Alkaloids	++	+++	+++	-
Saponins	-	-	+	+
Mucilage	_	+	++	+++
Oxalates	-	-	-	-
Total tannin	+++	++	++	++
Gallic tannin	+	+	+	-

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Catechic tannin	++	++	++	++
Flavonoids	++	++	+++	+++
Coumarins	+++	+++	+++	+++
Anthraquinone	-	-	-	-
Phlobatannins	_	_	_	_
Anthocyanin	++	++	++	++
Chalcones	++	++	++	++
Betacyanin	+	+	+	+
Terpenoids	_	++	++	++
Triterpenoid	+	+	+	+
Cardiac glycosides	+	-	-	-
Vitamin A	+	-	-	-
resins	-	-	-	-

Anti-microbial Susceptibility Test

The leaf extracts of *Morus alba* showed varying antimicrobial activities against strains of *Salmonella typhi*. The decoction of the leaves demonstrated the highest activity at 1000mg/ml against the microorganism as shown in Table 2 below.

Table 3 Diameters of Inhibition

Extracts	Maceration	Infusion	Decoction	Ethanol
Diameters at 5 mg/ml (mm)	-	-	-	-
Diameters at 50 mg/ml(mm)	-	-	-	-
Diameters at 250 mg/ml(mm)	-	-	-	-
Diameters at 500 mg/ml(mm)	-	-	-	-
Diameters at 1000 mg/ml(mm)	7±1.8	8±2.06	15±2.7	6±1.00

Minimum inhibitory concentration and minimum bactericidal concentration of *Morus alba* on *Salmonella typhi*

Table 4 shows the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of extracts of *Morus alba*. For 3 the extracts no growth was recorded above 62.5mg/ml whereas for the ethanolic extract minimum inhibitory concentration were recorded at 15.625mg/ml.

Table 4 MIC and MBC of the different extracts of *Morus alba*

Isolates	Maceration MIC	Maceration MBC	Infusion MIC	Infusion MBC	Decoction MIC	Decoction MBC	Ethanol MIC	Ethanol MBC
St1	62.5	62.5	125	125	125	250	62.5	62.5
St2	62.5	62.5	62.5	125	250	250	15.625	62.5
St3	62.5	125	250	250	250	250	62.5	62.5
St4	125	125	125	250	250	250	125	250
St5	62.5	125	62.5	250	250	250	62.5	125
St6	250	250	250	250	250	250	15.625	125
St7	125	125	250	250	250	250	62.5	125
St8	125	125	125	125	62.5	125	125	125
St9	125	62.5	125	125	125	250	62.5	125

St = *Salmonella typhi*

No growth was recorded in the control tubes containing ciprofloxacin at 5mg/ml

DISCUSSION

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Globally, a large number of medicinal plants and botanical drugs are being employed as major therapeutic agents or supplements for treatment of various human diseases (Leonard B *et al.*, 2001). Subsequently with this comes an increase in the acceptance of herbal remedy which is gaining renounce worldwide (Inoue *et al.*, 2011). As a result of abundant usage and propensity for prolonged use of the fresh leaves of *Morus alba* in traditional medicine especially in Pakistan and especially in Khyber Pakhtoonkhwa as a whole, a phytochemical screening and the evaluation of the anti-salmonella effects of different leaf extracts of the plant were carried out in this study (Ullah et al., 2018c).

The yield of the extractions showed the decoction having the highest yield at 16.82% and the ethanol maceration having the lowest at 8.18%. This contradicted the works done by Akindele and associates in 2018 who have a yield of 15% for their ethanolic extract after carrying a double maceration to render the extraction exhaustive (Akindele *et al.*, 2018; Ullah et al., 2019b).

The phytochemical screening of the decoction, infusion, aqueous and ethanolic maceration of the leaves of *Morus alba* showed the presence of tannins, phenolics, coumarins, alkaloids and flavonoids. Saponins were present but to lesser degree and a total absence of phlobatanins, oxalates and anthraquinones. This corroborates the findings done by Egharevba in 2014 who found similar components in an aqueous maceration (Egharevba, 2014; Ullah et al., 2021). Tannin a component found in excess in extracts of *Morus alba*, is a water-soluble polyphenol with anti-inflammatory and anti-microbial effect (Ullah et al., 2018d). It has both bactericidal as well as bacteriostatic effect against *Staph. aureus* and other micro-organisms (Akindele *et al.*, 2018). Prior studies have shown that the presence of tannins in the extract provides the effect for its use as purgative (Adeniyi *et al.*, 2010; Ullah et al., 2018e), anti-asthmatic, antitussives and anti-hay fever (Kuete et al., 2010). A study on the identification of bioactive chemical constituents by Oladele *et al* (2020) showed the presence of aromatic CH bend, C-O stretch, phenol, aromatic ring stretch, alkenyl stretch, hydroxyl group (alcohol) in the extract by the Fourier Transform Infrared, FTIR Techniques. (Ullah et al., 2019a). This result further corroborates the presence of phytochemicals in aqueous leaf extracts of *Morus alba* (Oladele *et al.*, 2020)

Based on the antimicrobial susceptibility test, the highest activity was registered for the decoction of the leaf extract. This is contrary to other studies carried out by Oyewole and Abalaka where the zones of inhibition of the plant were higher than the reference standard at 500mg/ml. Owing to the fact that they worked with just two extracts; maceration with distilled water and ethanol maceration, their results showed a marked difference (Oyewole & Abalaka, 2012).

Our research led us to the conclusion that the anti-salmonella activity of *Morus alba* is very low with activity only possible at 1000mg/ml. This therefore implies that the plant extract has insignificant action on *Salmonella typhi* (Ullah et al., 2018f). This is similar to the finding of Oboh *et al.* (2006) who reported inhibitory effects of ethanolic extract of *Morus alba* on *Escherichia coli, Pseudomonas aeruginosa* and *Proteus sp.* but no inhibitory effect on *Salmonella typhi.*(Oboh et al., 2006). On the other hand, the study carried out by Abalaka and Oyewole who determined that the ethanolic extract of *Morus alba* was tolerant to *S. typhi* at 5.0mg/ml. The minimum Inhibitory Concentration for *S.typhi* was found to be 5.0mg/ml (Oyewole & Abalaka, 2012; Tsafack *et al.*, 2017; Khan et al., 2028b).

CONCLUSION

The phytochemical analysis of the extracts showed the presence of saponins, flavonoids, tannins, phenolics and coumarin with the absence of phlobatannins and oxalates. The study also showed that the decoction had the greatest activity against *Salmonella typhi.* But the anti-salmonella activity was insignificant given that it was only observed as from concentrations of 1000 mg/ml which was relatively high. The use of *Morus alba* in the treatment of typhoid could possibly be attributed to the anti-anemic potential of the plant.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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ISSN: (e) 3007-1607 (p) 3007-1593

AUTHORS' CONTRIBUTIONS

Lubna Shakir and Mohammad Sohail conceived the study and designed the methods. Falak Naz, Azmat Noreen and Sajid Ali did the experimental and laboratory work, collected data and other materials. Hasnain Khan, Ghani Subhan and Naveen Dilawar drafted the manuscript. Shakir Ullah edited and finalized the manuscript for publication. All authors read and approved the final manuscript.

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ISSN: (e) 3007-1607 (p) 3007-1593

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