

ISSN: 2395-4108



Abrahamia

An International Journal of Plant Sciences



Paphiopedilum druryi (Bedd.) Stein

VOLUME 6 • NUMBER 2 • 2020



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Phytochemical and pharmacological evaluation of *Merremia tridentata* (L.) Hallier F.- An appraisal

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Abstract

Merremia tridentata (L.) Hallier f. belongs to the family Convolvulaceae, is a perennial prostrate herb with an elongated stem and pale-yellow flowers. It is globally distributed in tropical Africa, Asia, Australia, and also the Indian Ocean Islands. In South India, the plant is one of the sources of the Ayurvedic medicine 'Prasarani', which is attributed with astringent, aphrodisiac, laxative, and bitter properties. The plant is found in all districts of Kerala. The present study was undertaken to investigate the phytochemical, antioxidant, hepatoprotective and antibacterial properties of the plant. The phytochemical analysis was performed on both the hot (Soxhlet) and cold methanolic extract of the plant parts (shoot and root). The qualitative phytochemical screening of methanolic extract of *Merremia tridentata* showed the presence of secondary metabolites such as flavonoids, phenolics, tannins, terpenoids, alkaloids, saponins, glycosides, and phytosterols. The quantitative study confirmed that the plant is a rich source of phytochemicals such as phenolics, flavonoids, tannins and saponins. Higher amounts of phytochemicals were observed in the cold extract of the plant parts than the hot extract. Therefore, the antioxidant, hepatoprotective and antibacterial activity of the plant was evaluated in the cold extract of the plant parts. In the hepatotoxicity assay, the root extract showed a potent effect against Chang liver cell line. The

root extract exhibited good antioxidant activity in a concentration-dependent manner. But the antibacterial effect was greater in the shoot extract. Thus, the plant has considerable pharmacological value in the modern medicinal system.

Keywords: Antibacterial, antioxidant, vold extract, Hot extract, Hepatoprotective, phytochemicals.

Introduction

Plant-derived products have recently become of great interest due to their wide range of applications in healthcare. Thus, the consumption of this indigenous herbal medicine around us promotes human health and helps to treat various disorders. Generally, medicinal plants are considered as cost-effective and devoid of side effects (Arunkumar et al., 2009). Sometimes these products can cause health problems if not used in the appropriate dosage. In recent years, many researchers have focused on natural products derived from medicinal plants due to their wide range of pharmacological significance (Shukla et al., 2010). Nowadays, we are constantly exposed to free radicals in this highly polluted environment. They harm our immune system leading to many degenerative diseases. An overload of free radicals accumulation in our body, generates a phenomenon called oxidative stress, which leads to damage of proteins, lipids, DNA, and is associated with chronic degenerative

diseases such as hypertension, diabetes and cancer. Antioxidants are considered as nature's way of protecting the body and cells from damage caused by free radicals (Pham-Huy et al., 2008). Many plant-derived medicines are rich in phenolics, flavonoids, alkaloids, and tannins, and exhibit strong antioxidant activity, thus protecting our body from various degenerative diseases. Liver disease is very common among our community and is mainly due to changing lifestyle and imbalance in the antioxidant level. Hepatoprotection or anti-hepatotoxicity is the ability of a chemical substance to prevent damage to the liver. Today a variety of plants has been reported to show hepatoprotective activity and so may be useful in the treatment of these diseases (Karan et al., 1999). Medicinal plants are considered as a rich source of antimicrobial agents against various infectious diseases. The antimicrobial compounds from plants have the ability to reduce microbial growth through different mechanisms (Britto et al., 2012). Antibacterial activity is the most common type of antimicrobial activity reported for the acridone alkaloids. The compounds extracted from plants are thus used as an alternative to chemically synthesized drugs (Katiyar et al., 2012).

Medicinal plants have been proved to be of great importance to the health of the individual and communities. *Merremia tridentata* was chosen for the current study due to their valuable medicinal properties as well as being a native plant. *Merremia tridentata* (L.) Hallier f. is a member of the Family Convolvulaceae. This family consists of 60 genera and more than 1,650 species. In India, 15 genera and 150 species were recorded, 20 genera and 129 species reported in China, and 80 species were recorded in tropical regions of Africa, Asia, Australia, North and south America (Neyanila et al., 2013). *Merremia tridentata* is considered a medicinal creeper (Austin, 2014). In Ayurveda, *Merremia tridentata* has been used in indigenous systems of medicine for treating various disorders like rheumatic arthritis, toothache (Ambika et al., 2019), hemiplegia, pile swelling, and urinary disorders (Bidkar, 2009), ulcer (Sowndhararajana et al., 2014), skin infections, inflammations, fever, diabetes, diarrhea, and also promote hair growth (Neyanila et al., 2013). The main aim of the present study is to evaluate phytochemical, antioxidant, hepatoprotective and antibacterial properties of *Merremia tridentata*.

Materials and Methods

The fresh and healthy plant parts of *Merremia tridentata* were collected separately in the

sterile polythene bags from the area around Kariavattom Campus, the University of Kerala, during the month of January 2020. The systematic identification of plant material was confirmed and submitted to KUBH (Kerala University Botany Herbarium) for further reference with Voucher/ Accession No. KUBH 10277. The collected plant materials were washed thoroughly with water and rinsed with distilled water. The cleaned, healthy plant materials were air-dried and subsequently powdered with an electric grinder. The shoot and root of the plant were taken separately for the investigation.

Preparation of Extract

The fine powder of the plant parts was then subjected to extraction by two different methods, namely hot extraction (Soxhlet) and cold maceration with methanol. Then the extract was filtered and stored at 4°C. It is then used for preliminary phytochemical screening and pharmacological studies.

Preliminary phytochemical screening

In preliminary phytochemical evaluation, crude extracts of *Merremia tridentata* were subjected to various qualitative chemical methods (Tiwari et al., 2011). The test helps to determine the presence of various phytoconstituents by characteristic colour reactions. Qualitative test for carbohydrates, proteins, fats and oil, gums and mucilage, flavonoids, phenolics, tannins, phlobatannin, terpenoids, alkaloids, saponins, glycosides, phytosterols and quinones were conducted for the preliminary screening. Extraction yield was calculated using the standard formula.

Quantification of phytochemicals

The quantitative estimation of total phenolic, flavonoid, tannin and saponin content was done according to standard procedures of Singleton et al. (1965), Chang et al. (2002), Padma et al. (2013) and Hiai et al. (1976)

DPPH free radical scavenging assay

One ml of 0.1 mM DPPH solution (0.004gm in 100 mL methanol) was mixed with 1 ml of plant extract solution of varying concentrations (0.2, 0.4, 0.6, 0.8 and 1 mg/ml). A corresponding blank sample was prepared and ascorbic acid (20 – 100 µg/ml) was used as a reference standard. The control sample contained all the reagents except the extract (Brand-Williams et al., 1995). The reaction was carried out in triplicate, and after 30 minutes of incubation in the dark, the decrease

in absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The percentage of inhibition of DPPH free radical was calculated.

In vitro hepatoprotective activity

The hepatoprotective activity of the extracts was evaluated using Chang liver cells. IC₅₀ concentration of H₂O₂ (73 µM) was used as a hepatotoxicant. Confluent Chang liver cells were cultured in growth media (DMEM + 10% FBS) at a density of 5 × 10⁴ cells/well in a 96-well tissue culture plate and incubated overnight. Post-incubation, cells were treated with varying concentrations of extracts (25, 50, 100 µg/ml) and incubated for 24 h, thereafter; IC₅₀ concentration of H₂O₂ (73 µM) was added and allowed for further 24 h incubation. Post-incubation, the treated cells were washed with PBS and incubated with MTT containing growth media. Finally, the medium was removed, and the formazan crystals were dissolved using DMSO. The optical density was measured at 570 nm. Untreated cells were kept as control, and the percentage of cell viability in treated cells was calculated (Chandrasekaran, 2010; Siddiqui 2018).

Antibacterial assay

Agar- disc diffusion assay (Bauer *et al.*, 1966) was used to study the antibacterial potential of the methanolic shoot and root extracts of *Merremia tridentata* against target microorganisms. The antibacterial activity was reported as the diameter of the inhibition zones.

Test microorganisms

Bacterial strains used in the study were pathogens procured from the MTCC, Chandigarh. The test microorganisms used in this investigation included both Gram-negative and Gram-positive Bacteria such as *Bacillus subtilis* MTCC 441, *Escherichia coli* MTCC 443, *Salmonella typhi* MTCC 98, *Staphylococcus aureus* MTCC 3160, *Acinetobacter baumannii* MTCC 9829.

Reagents for the assay

LB (Luria Bertani) agar medium: The culture medium was prepared by dissolving 4 g of LB agar in 100 mL of distilled water. The prepared medium was at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured into 100 mm sterile petri-plates.

Nutrient broth: The nutrient broth was prepared by adding 1.5 g of peptone powder in 100 ml distilled water. The medium was poured into sterile culture tubes with 5 ml in each tube. This medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes.

Preparation of inoculum

The selected bacterial strains were inoculated into 5 mL of the nutrient broth in sterile conditions. This was incubated at 37°C for 12 hours.

Preparation of extracts

The increasing concentrations of the methanolic shoot and root extracts, like 250 µg/ml, 500 µg/ml, 1000 µg/ml were prepared using DMSO. The antibiotic drug Amoxicillin trihydrate was used as standard. The paper discs of 4 mm in diameter were cut out from Whatman No.1 filter paper and impregnated with the different concentrations of the methanolic shoot and root extracts. 20 µg/ml concentration of Amoxicillin trihydrate was used as positive control, and DMSO was used as a negative control.

Agar-Disc Diffusion Assay

The selected bacterial suspensions were aseptically swabbed onto the surface of Agar plates with the help of a sterile inoculation loop. The paper discs prepared were placed on the surface using sterile forceps. The petri-plates were incubated at 37°C for 24 hours. After incubation, the zone of inhibition was measured.

Result

The percentage yield of hot extract of *Merremia tridentata* shoot (MTSA) was 30.13% whereas, hot extraction of *M. tridentata* root (MTSR) exhibited 16.49% of yield. The percentage yield of cold extraction of *M. tridentata* shoot (MTCA) was 41% whereas, cold extraction of *M. tridentata* root (MTCR) showed 70% of yield.

Qualitative phytochemical analysis

The preliminary phytochemical screening in methanolic shoot and root extract of *Merremia tridentata* was carried out and revealed the presence of carbohydrates, proteins, fats and oil, gums and mucilage, flavonoids, phenolics, tannins, terpenoids, alkaloids, saponins, glycosides, and phytosterols. The presence and absence of phytochemicals were noted in Table 1.

Table 1
Preliminary qualitative phytochemical screening in methanolic shoot and root extracts of *Merremia tridentata*

Sl No:	Phytoconstituents	Methanolic Extract of Shoot		Methanolic Extract of Root	
		Hot Extract (MTSA)	Cold Extract (MTCA)	Hot Extract (MTRSR)	Cold xtract (MTCR)
1.	CARBOHYDRATES	+	++	+++	++
2.	PROTEINS	+	++	++	+++
3.	FATS AND OIL	++	++	+++	+
4.	GUMS AND MUCILAGE	++	+	+++	+
5.	TERPENOIDS	+	+++	++	+
6.	PHENOLS	++	+	+++	++
7.	FLAVONOIDS	++	+++	+++	++
8.	TANNINS	+	++	+++	+
9.	PHLOBATANNIN	-	-	-	-
10.	ALKALOIDS	++	+	+++	++
11.	GLYCOSIDES	++	+++	++	+
12.	SAPONINS	++	+++	+	+++
13.	PHYTOSTEROLS	++	+++	++	+
14.	QUINONES	-	-	-	-

(-) Not detected, (+++) Present in high concentration, (++) Present in moderate amount, (+) Present in trace.

Quantitative phytochemical analysis

Total phenolic content (TPC)

The total phenolic content of root extract is found to be higher than the shoot extract of *Merremia tridentata* (Figure 1). The total phenolic content in *M. tridentata* is higher in the cold shoot extract (340.7 mg/g) when compared to hot shoot extract (314.4 mg/g) whereas methanolic cold root extract (838.5mg/g) showed a slightly higher amount than the hot root extract (835.9 mg/g).

Total flavonoid content (TFC)

The total flavonoid content in *Merremia tridentata* is higher in the cold shoot extract (177.0 mg/g) than the hot shoot extract (136.0 mg/g). In

contrast, hot root extract (145.6 mg/g) showed a higher amount of flavonoid (140.0 mg/g) as compared to cold extract of the root.

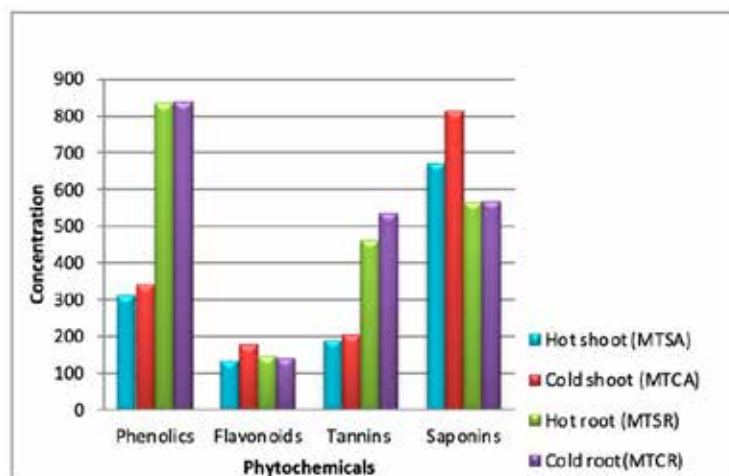
Total tannin content

The total tannin content was spotted maximum in the cold shoot extract (203.3 mg/g) and cold root extract (536.2 mg/g) than the methanolic hot shoot (192.7 mg/g) and root extract (461.1 mg/g) of *Merremia tridentata*.

Total saponin content

The total saponin content showed a maximum in the cold shoot extract (814.4 mg/g). The hot and cold roots showed almost the same amount (565.3 mg/g) of saponins in *Merremia tridentata*.

Fig. 1
Quantitative evaluation of phytochemicals in hot and cold methanolic extract of *Merremia tridentata*

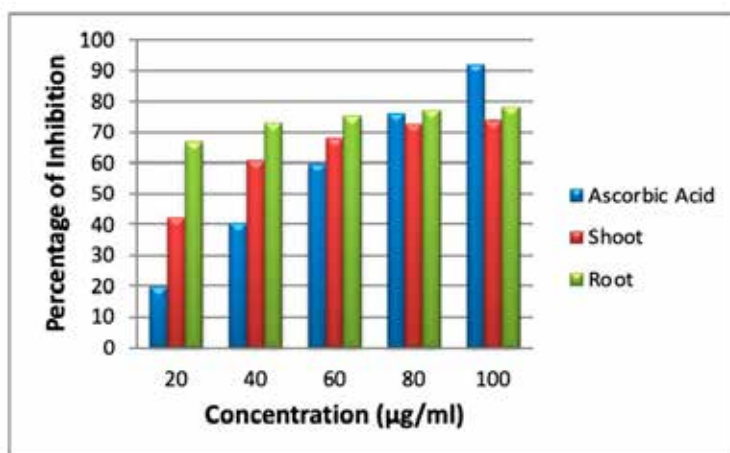


DPPH free radical scavenging activity

DPPH free radical scavenging activity of the methanolic shoot and root of *Merremia tridentata* is given in Fig. 2.

The IC₅₀ value of methanolic shoot extract of *M. tridentata* was found to be 24.4 µg/ml, which indicates IC₅₀ value is lower than standard (51.6µg/ml). This result showed that the shoot extract possesses good antioxidant capacity against the standard, ascorbic acid. The IC₅₀ value of the methanolic root extract of *M. tridentata* was found to be 12.4µg/ml. This result revealed that the root extract exhibited good antioxidant activity against the standard ascorbic acid.

Fig. 2
DPPH Free Radical Scavenging Activity of the Cold Methanolic Extracts of *Merremia tridentata*



In vitro Hepatoprotective Activity

The hepatoprotective activity of the plant was evaluated by hydrogen peroxide-induced cytotoxicity in Chang liver cells. Hydrogen peroxide was used as the hepatotoxicant on Chang liver cell line. The IC₅₀ concentration of H₂O₂ (73 µM) was used to induce hepatotoxicity. The hepatoprotective action of both shoot and root methanolic extracts is represented in Table 2 and 3 (Fig. 3 & 4).

Table 2
Hepatoprotective activity of the methanolic shoot extract of *Merremia tridentata*

Sample MTCA	Percentage cell viability
Untreated	100
H ₂ O ₂ (73 µM) + 25 µg/ml	47.7
H ₂ O ₂ (73 µM) + 50 µg/ml	63.3
H ₂ O ₂ (73 µM) + 100 µg/ml	71.3

The IC₅₀ value of methanolic shoot extract of *Merremia tridentata* was found to be 21.37 µg/ml. Untreated cells showed 100% viability. On increasing the concentration of extract along with H₂O₂ (73 µM), the cell viability also increased (Table 2 and Fig. 3).

Fig. 3
Cell viability of untreated and shoot extract on chang liver cells

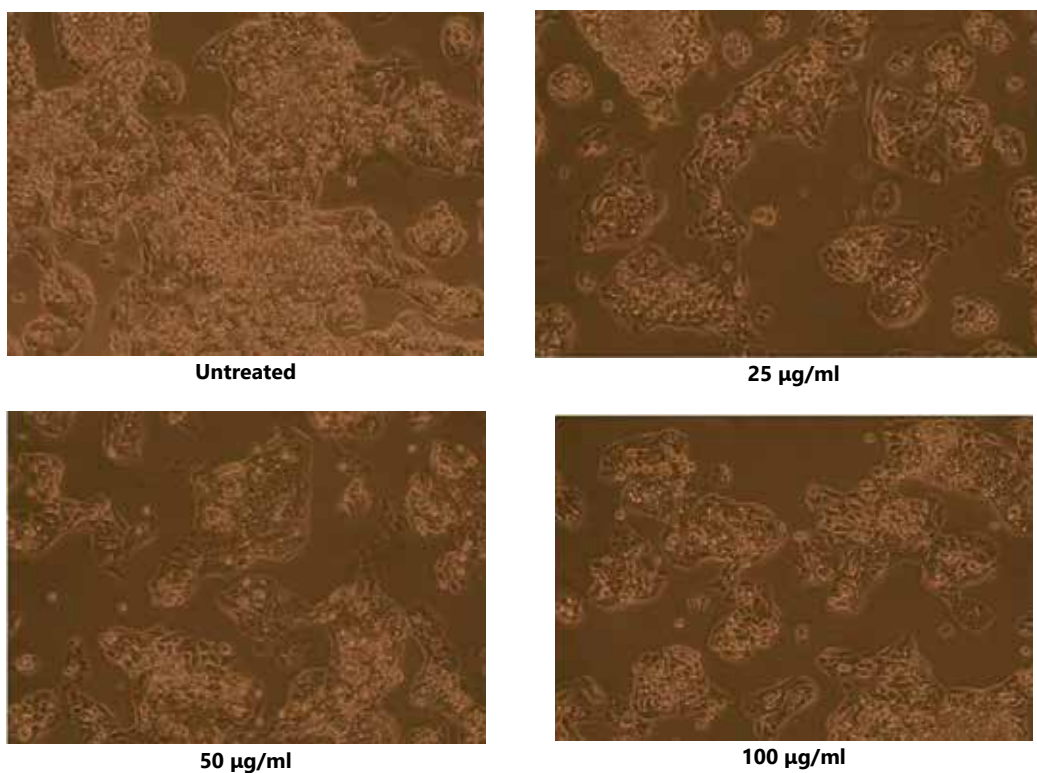
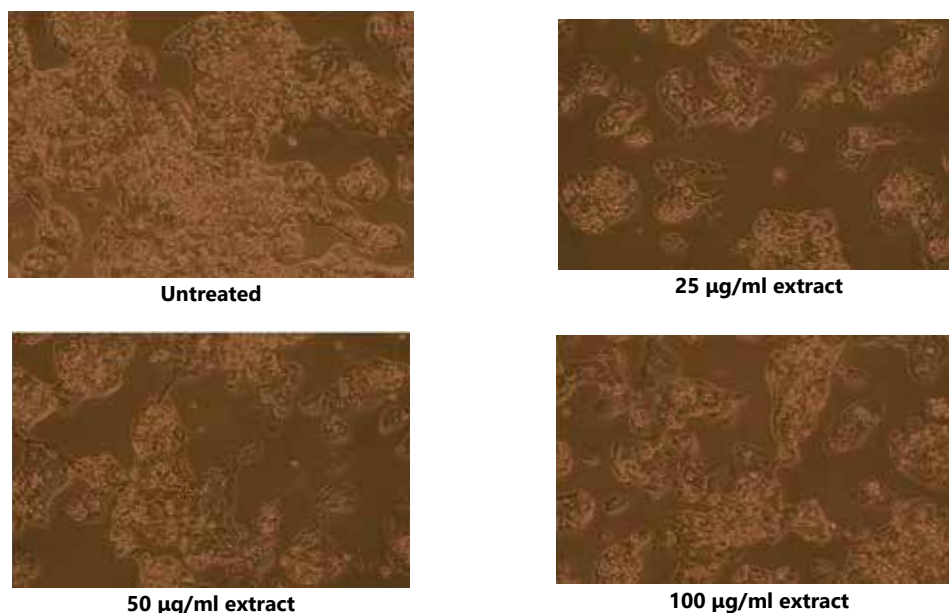


Table 3:
Hepatoprotective activity of the methanolic root extract of *Merremia tridentata*

Sample MTCR	Percentage cell viability
Untreated	100
H ₂ O ₂ (73 µM) + 25 µg/ml	51.2
H ₂ O ₂ (73 µM) + 50 µg/ml	64.8
H ₂ O ₂ (73 µM) + 100 µg/ml	73.2

The IC₅₀ value of methanolic root extract of *M. tridentata* was found to be 10.7µg/ml (Table 3). The result showed that the cell viability was increasing with the concentration of plant extract, which shows its hepatoprotective activity (Fig. 3 & 4).

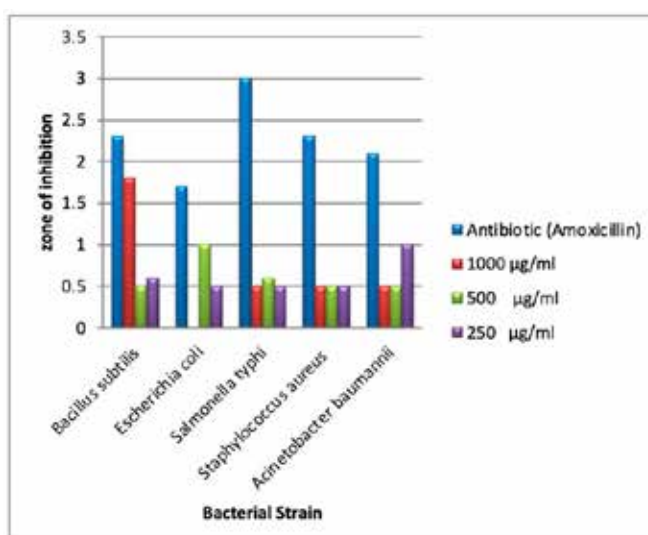
Fig. 4
Cell Viability of untreated and root extract on chang liver cells



Antibacterial activity

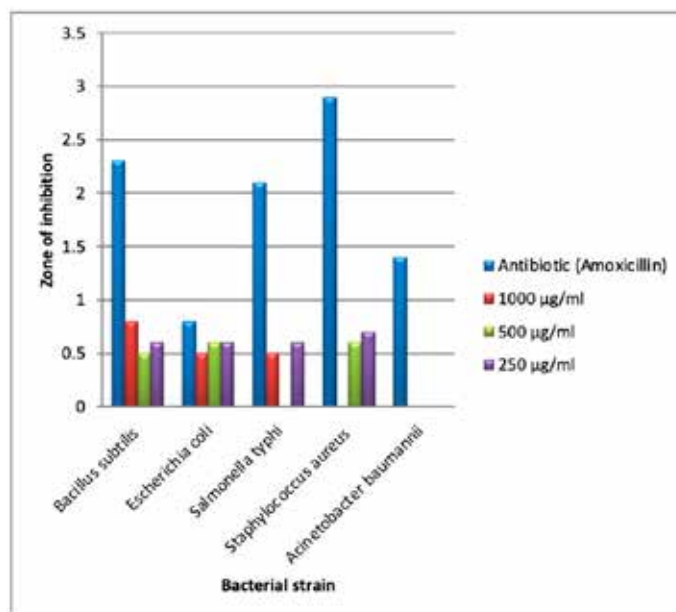
In this study, maximum inhibition was found against *Bacillus subtilis*, *Escherichia coli*, *Acinetobacter baumannii*, whereas *Salmonella typhi*, *Staphylococcus aureus* were least susceptible microbes to the shoot extracts (Fig. 5).

Fig. 5
Antibacterial activity of cold methanolic shoot extract of *Merremia tridentata* against selected bacterial strains



In the case of root extracts, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus* showed the least zone of inhibition, whereas *Acinetobacter baumannii* does not exhibit any kind of inhibition (Figure 6).

Fig. 6
Antibacterial activity of cold methanolic root extract of *Merremia tridentata* against selected bacterial strains



Discussion

The present study was designed to evaluate the phytochemical, antioxidant, hepatoprotective and antibacterial activity of *Merremia tridentata*. The qualitative phytochemical analysis of the methanolic extract of shoot and root of *M. tridentata* revealed the presence of carbohydrates, proteins, fats and oil, gums and mucilage, flavonoids, phenolics, tannins, terpenoids, alkaloids, saponins, glycosides and phytosterols. Most secondary metabolites were identified on methanolic root extract rather than methanolic shoot extract of this plant through the preliminary qualitative screening. The quantitative studies convey that the methanolic cold extract of *M. tridentata* possesses more activity than hot extract (Fig. 1). Therefore, cold extracts were taken for further studies. The medicinal properties of *M. tridentata* were attributed due to the presence of these phytochemicals.

The antioxidant property of extract was determined by DPPH free radical scavenging assay. DPPH (2,2-diphenyl-1-picryl-hydrazyl hydrate) is a simple and efficient method to analyse antioxidant capacity based on electron transfer (Huang et al., 2005). Antioxidants neutralize the excess of free

radicals and protect from degenerative diseases. Free radicals are molecules that contain one or more unpaired electrons that cause damage to our cells, which negatively affect our immune system. Low or moderate levels of free radicals are vital for human health. The induction of mitogenic response is one of the beneficial roles (Pham-Huy et al., 2008).

The efficiency of scavenging was measured in terms of IC_{50} . It is the concentration of a molecule required to inhibit the formation of free radical by 50%. A lower IC_{50} indicates good antioxidant capacity. The root extract of *M. tridentata* exhibited good antioxidant activity than shoot extract in a concentration-dependent manner. The antioxidant property of the root extract of *M. tridentata* might be due to the high amount of phenolic content.

Some traditional medications like Ayurveda, Siddha, Unani, and Chinese predominantly use plant material that has the ability to protect hepatic cells against toxic chemicals. In recent years several plant components such as phenolics, alkaloids, terpenes, and glycosides have been discovered with hepatoprotective properties

(Bhawna et al., 2009). This study elucidates the possible contribution of hepatoprotective activity as the root extract showed a potent effect against Chang liver cell line. The flavonoid diosmin was proven to have good cytoprotective activity against Chang cell line (Sangeetha et al., 2016). Interestingly, there is a link between antioxidant and hepatoprotective activity. Several studies have indicated that an important mechanism of hepatoprotective effects may be associated with their capacity to transfer hydrogen to free radicals that activate antioxidant enzymes and inhibit oxidases (Huang et al., 2018). The hepatoprotective property of the *M. tridentata* is due to the flavonoids and high amount of phenolics and saponin content (Fig:1)

Recently, drug resistance to human pathogenic bacteria has been commonly reported from all over the world (Mulligan et al., 1993). With the regular use of antibiotics, microorganisms have become resistant to those antibiotics. In addition to this complication, antibiotics are sometimes associated with adverse effects on hosts which include hypersensitivity, immunosuppressant and allergic reactions (Lopez et al., 2001). This has created immense clinical problems in the treatment of infectious diseases (Davis, 1994). Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases; one approach is to screen local medicinal plants for possible antimicrobial properties. Phenolic agents present in this plant may be responsible for both antioxidant and antimicrobial activity.

The phytochemicals such as saponins, steroids, phenols, and tannins are found to be more predominant and, therefore, may be responsible for the antimicrobial action (Sule et al., 2010). The Antibacterial effect was more significant in the shoot extract than root extract due to the presence of a higher amount of saponins in shoot extract (Fig:1).

Conclusion

Preliminary qualitative phytochemical analysis of methanolic extract of shoot and root of *M. tridentata* revealed the presence of carbohydrates, proteins, fats and oil, gums and mucilage, flavonoids, phenolics, tannins, terpenoids, alkaloids, saponins, glycosides, and phytosterols. The quantitative study confirmed that the methanolic cold extracts of *M. tridentata* possess more activity than hot extracts. *Merremia tridentata* possess antioxidant, hepatoprotective, and antibacterial properties. Only a few research

works have been done on *Merremia tridentata* and this study opens a door for treating hepatic diseases. Still, many pharmacological activities of this plant remain to be explored. So further research is needed to explore its components and for *in vivo* and *in vitro* regeneration methods, so maximum utilization of plants can be done for human welfare. These investigations can lead to the development of therapeutic drugs in the future.

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Received: 5 November 2020

Revised and Accepted: 2 December 2020

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