Exploring the potential of *Rhynchosia heynei* Wight & Arn whole plant extracts for *in vitro* anti-diabetic and anti-TB activities

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Abstract

This study's goal was to investigate possible anti-TB and anti-diabetic properties of various extracts of *Rhynchosia heynei*, a plant traditionally used in Ayurveda and Unani medicine. Preliminary phytochemical analysis was performed to determine the composition of the extracts. *In vitro* assays were carried out to evaluate the potential anti-diabetic activity by measuring glucose uptake and alpha-amylase inhibition, as well as anti-TB activity by determining the minimum inhibitory concentration (MIC) against *Mycobacterium kansasii* strain.In comparison to the control group, the findings demonstrated that all the extracts increased glucose uptake in a dose-related manner. Furthermore, the aqueous extract demonstrated alpha-amylase inhibition at 617.6 µg/ml, while the ethanol and ether dilutions had IC50 values of 103.9 and 135.1 µg/ml, respectively, indicating potential anti-diabetic activity. The study's anti-TB findings revealed that the hexane extract had the lowest IC50 (1.01 µg/ml) followed by the ethanol extract (7.599 µg/ml), ether extract (32.7 µg/ml), and aqueous extract (793.3 µg/ml). The presence of phytochemical compounds such as alkaloids, tannins, saponins, steroids, flavonoids,terpenoids, and phenolic compounds in the extracts of *Rhynchosia heynei* may explain the observed pharmacological activities of the plant. In conclusion, these extracts have the potential to be developed as natural anti-diabetic and anti-TB agents.

Keywords: *Rhynchosia heynei*, anti-diabetic, anti-TB, alpha-amylase inhibition, *Mycobacteriumkansasii*.

1. Introduction

Diabetes and tuberculosis are two major global public health issues that impact millions of people each year.A chronic metabolic condition called diabetes mellitus causes increased blood

glucose because ofinadequate insulin release or insulin resistance. It is a growing global health problem, with approximately 463 million people being affected worldwide in 2019 and projected to reach 700 million by 2045 [1]. Diabetes mellitus is linked to several challenges, such as amputations, neuropathy, retinopathy, kidney disease, and cardiovascular disease, which can greatly reduce quality of life and raise healthcare costs. [2]. The management of diabetes involves lifestyle changes such as weight loss, dietary changes, and regular exercise, as well as pharmacological interventions including insulin and oral hypoglycemic agents. However, these therapies have limitations and can be associated with adverse effects, highlighting the need for new and effective treatments [3].

On the other hand, the infectious disease tuberculosis (TB), which typically affects the lungs and can have severe health consequences if untreated, is brought on by the bacterium Mycobacterium tuberculosis. It is a major health problem in the world, with an estimated 10 m cases and 1.4 mdeaths worldwide in 2019 [4]. The current treatment for TB involves a combination of multiple drugs, including rifampin, ethambutol, isoniazid,and pyrazinamide, taken for at least a duration of six months [5]. However, the emergence of drug-resistant strains of M. tuberculosis, extensively drug-resistant (XDR) and particularly multi-drug-resistant (MDR) TB, has become a significant challenge to the management and control of TB [6]. To address these two pressing issues, there is avital need to develop new and effective anti-diabetic and anti-TB drugs.

Medicinal plants were being used in traditional medicine for hundreds of years and continue to play a significant role in the healthcare systems of many cultures worldwide. Traditional medicine, including the use of medicinal plants, has gained renewed interest in recent years due to its potential as a source of new drugs and as a complementary or alternative treatment option. Indian traditional medicine has a lengthy history and there is evidence that it was practiced during the Vedic era (1500-600 BCE) [7]. Today, traditional medicine remains an important part of the healthcare system in India, with a vast number of medicinal plants being utilized for various ailments [8]. The Eastern Ghats region of India is known for its rich biodiversity and has been recognized as an important area for the conservation and sustainable use of medicinal plants [9]. *Rhynchosia heynei* is a plant species native to the Eastern Ghats region that has been traditionally employed for various medicinal utilities, including the treatment of diabetes. It has gained attention for its chemical composition and pharmacological properties. Bhakshu and Raju (2009) investigated the essential oil of *Rhynchosia heynei* and reported its in vitro antimicrobial activity [10]. Similarly, Soneya et al. (2019) conducted a study on the synthesis of silver nanoparticles using *Rhynchosia heynei* leaf extract and found promising results regarding the antioxidant, antimicrobial, and anticancer potential of the prepared nanoparticles [11]. Rammohan et al. (2020) provided a comprehensive review of the phytochemistry and pharmacological activities of the genus *Rhynchosia*, highlighting its potential in treating various ailments, such as diabetes, inflammation, and cancer [12]. Although the potential of *Rhynchosia* species in treating diabetes and tuberculosis, *Rhynchosia heynei* Wight Arn, a specific species, has not been thoroughly investigated for its anti-diabetic and anti-tuberculosis activities. Further research on this plant species may provide promising results in the development of natural

treatments for these diseases. From our literature review, we came to know *Rhynchosia heynei* is a valuable medicinal herb with promising therapeutic properties, and further investigation is necessary to explore its full potential as a natural remedy for various diseases.

In this context, the present study targets to examine the in vitro anti-diabetic as well as anti-TB activities of *Rhynchosia heynei* Wight & Arn using a glucose uptake and Alpha Amylase enzyme inhibition assay and to evaluate the in vitro anti-TB activity using the anti-microbial activity assay against *M. kansasii*.

2. Materials and Methods

2.1 Chemicals and Reagents:

Ethanol (Merck, Germany), hexane (Merck, Germany), de-mineralized water, diethyl ether (Merck, Germany), 2-Deoxy-D-glucose (Sigma-Aldrich, USA), glucose assay kit (BioVision, USA), alpha-amylase (Sigma-Aldrich, USA), starch (Sigma-Aldrich, USA), iodine solution (Merck, Germany), DMSO (Sigma-Aldrich, USA), and 7H9 broth (BD Biosciences, USA). All the reagents and chemicals used were of analytical reagent grade and no additional purification was done.

2.2 Plant Material and Extraction:

The whole plant of *Rhynchosia heynei* Wight & Arn. was collected from the bioreserve of Eastern Ghats region of India and authenticated by a taxonomist Dr. K Madhav Chetty, Department of Botany, SV university, Tirupati, with a voucher specimen (Accession No. 0791). The plant material was dried, powdered, and was kept in an airtight sample container until further use. The ethanol, diethyl ether, and hexane extracts were obtained by the maceration method using 70% ethanol, diethyl ether, and hexane, respectively. The decoction method was used to make the de-mineralized water extract. Whatman filter paper was used to sift all extracts, and a rotary evaporator was used to concentrate them all under low pressure.

2.3 Preliminary Phytochemical Analysis:

Using conventional techniques, an early phytochemical screening was performed on the extracts (Harborne, 1998) [13]. The tests were performed for the presence of alkaloids, tannins, saponins, phenolic compounds,terpenoids,flavonoids, and steroids.

2.4 In Vitro Glucose Uptake Assay:

In 24-well cell culture plate, cells were seeded at a density of 8×104 cells in each well, and they were then incubated for 24 hours in regular culture media. Cells were cultured in DMEM (glucose-free) for an hour after being washed two times using Krebs-Ringer-phosphate-HEPES (KRPH) solution. The cells were then left to sit in KRPH solution that contained 2% (v/v) BSA for 20 min in with & without 10 mM2-DG. Following that, cells were centrifuged at 500 rpm for 2 min after being rinsed thrice with PBS, extraction buffer used for lysis, later it was frozen, and subjected to heat treatment at 85°C for forty minutes for the degradation of endogenous NADP.Using a GOD-POD Enzyme Assay Kit, the supernatant was gathered and examined for the presence of 2-deoxyglucose-6-phosphate (2-DG6P). The plate was scanned at 505 nm by a microplate scanner. Cell lysates that had not been exposed to 2-DG were analyzed to ascertain the blank number. 2-DG nanomoles were calculated by comparing the outcomes to the standard.

The benchmark was the genuine 2-DG6P. Metformin and Insulin served as the corresponding positive and adverse controls [14].

2.5 Enzyme Inhibition Assay-Alpha Amylase:

An enzyme inhibition assay was performed to determine the inhibitory effect of various sample dilutions on α-amylase activity. Aqueous, ethanol, ether, and hexane dilutions ranging from 0-500 µg/ml were prepared in sodium phosphate buffer. A 96-well plate with specified wells was filled with an enzyme solution containing 20 mg/ml -amylase, then 10 µl of each sample dilution was added. Following a 10-minute incubation period, 50µl of substrate (0.1% soluble starch) was added, and the combination was then incubated for an additional 15 mins. After adding 100 µl of GOD-POD Reagent to halt the reaction, the plate was left to sit at room temperature for 10 minutes. Using a microplate scanner, the absorbance of each sample was determined at 490 nm. (iMark, BioRad). As a positive control, the inhibitor metformin was used at a maximum concentration of 500 µg/ml [15].

2.6 Test Organism and Media:

The *M. kansasii* strain was used to assess the extracts' anti-TB efficacy. Up until the mid-log period, the bacteria were grown in 7H9 broth at 37°C while being shaken at 100 rpm.

2.7 Minimum Inhibitory Concentration Anti-TB:

The Minimum Inhibitory Concentration (MIC) activity against *M. kansasii* was determined using the anti-microbial activity assay [16]. A 0.5 Mc Farland Standard dilution of microbes was used for the study, and 500 µl diluted log cultures of bacteria were added to the micro centrifuge tube and added with 10 µl of prepared treatment dilutions of different concentrations. The treatment dilutions of different concentrations were prepared as mentioned in the excel sheet, and the tubes were incubated for 24 hours. After incubation, all contents were transferred to a 96 well plate, and the reading was taken by Elisa Plate Reader (iMark Biorad) at 490nm and 595 nm. Ciprofloxacin (100µg) was employed as the Positive Control.

2.8 Statistical analysis

The studies were carried out in triplicate, and mean values were used to describe the findings as ± standard deviation (SD).

3. Results

3.1 Plant Material and Extraction:

The yields of ethanol, diethyl ether, hexane, and de-mineralized water extracts were found to be 5.36%, 2.92%, 1.7%, and 2.86%%, respectively.

3.2 Phytochemical Analysis:

An early phytochemical analysis of the extracts was performed., which found the existence of several bioactive substances including alkaloids, tannins, flavonoids, steroids, terpenoids, saponins, and phenolic compounds. **(Table 1).**

Extract of RH	**Alkaloids**	**Tannins**	**Flavonoids**	**Steroids**	**Saponins**	**Terpenoids**	**Phenolics compounds**
Ethanol	+	-	+	-	-	-	+
Diethyl ether	-	-	+	-	-	+	+
Hexane	+	-	+	-	-	+	+
Demineralized Water	-	-	_	-	+	+	+

3.3 In Vitro Glucose Uptake Assay:

The glucose uptake activity was measured at two concentrations, 100 µg/ml and 287.9 µg/ml,10 µg/ml and 76.31 µg/ml ,250 µg/ml and 440 µg/ml, 250 µg/ml and 1000 µg/ml , for the diethyl ether, hexane, ethanol, and aqueous extracts respectively. The outcomes were contrasted with insulin and metformin, two well-known diabetes medications. The data showed in **Figure 1** that the hexane extract had the highest glucose uptake activity at both concentrations tested, with mean values of 27.5 and 25.83 for 10 µg/ml and 76.31 µg/ml, respectively. The ethanol extract also showed noticeable glucose uptake activity, with mean values of 29.17 and 29.5 for 250 µg/ml and 440 µg/ml, respectively. The diethyl ether extract showed little to no activity at both concentrations tested. The aqueous extract showed moderate glucose uptake activity at 250 µg/ml, with a mean value of 25.83, but no significant activity at 1000 µg/ml. In comparison to the standard drugs, insulin and metformin, the extracts showed different levels of glucose uptake activity. The insulin control showed the maximum activity with mean values of 39.17 and 43.33 at 100 µg/ml and 287.9 µg/ml, respectively. Metformin also showed significant activity, with mean values of 30.83 and 35.83 at 250 µg/ml and 500 µg/ml, respectively.

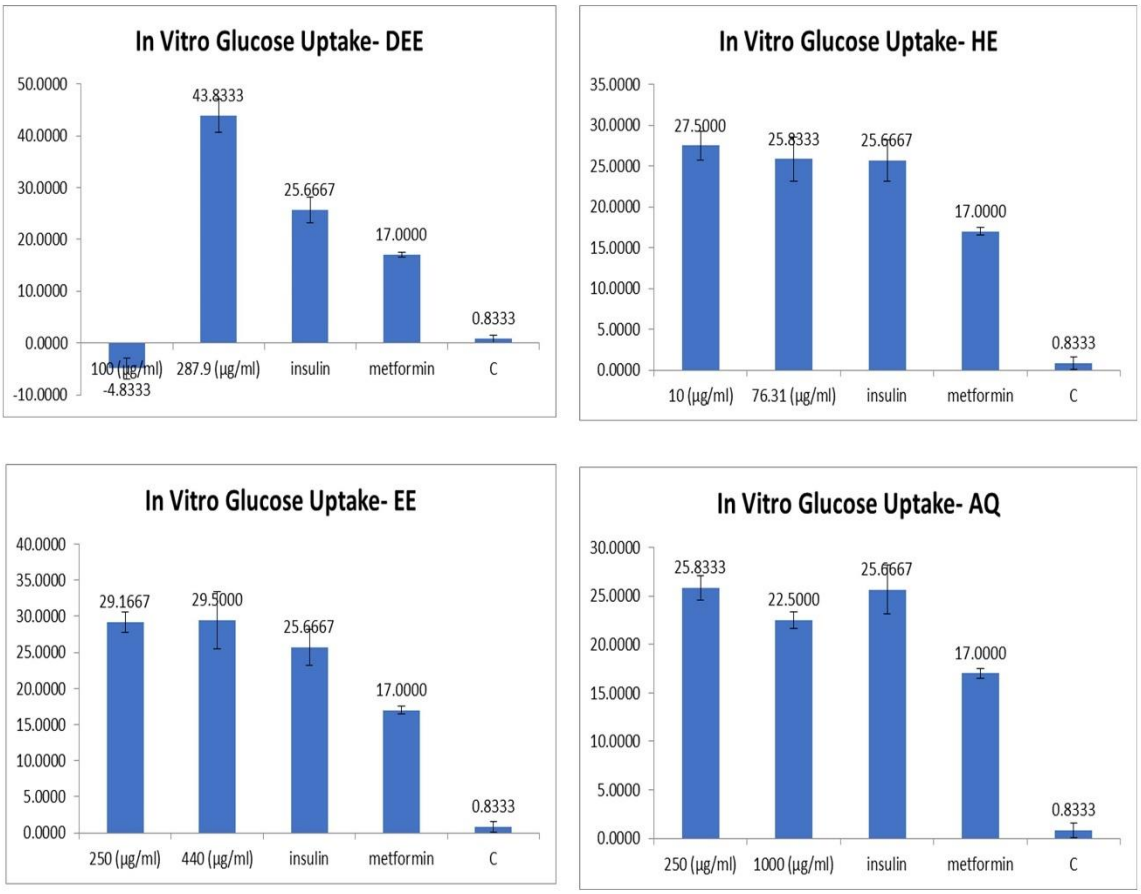

Figure 1. Effect of *Rhynchosia heynei* extracts on glucose uptake of various *Rhynchosia heynei* Wight & Arn whole plant extracts. The figure shows the glucose uptake activity of the diethyl ether, hexane, ethanol, and aqueous extracts of *Rhynchosia heynei* at two concentrations compared to insulin and metformin, known antidiabetic drugs.

3.4 Enzyme Inhibition Assay-Alpha Amylase:

The findings of the α-amylase inhibition assay demonstrated that the IC50 values of the aqueous, ethanol, ether, and hexane dilutions were 617.6, 103.9, 135.1, and not converged, respectively. The positive control, Metformin, revealed a significant reducing effect on α-amylase activity, validating the accuracy of the assay. Among the tested dilutions, the aqueous dilution exhibited the highest inhibitory action on α-amylase activity with an IC50 value of 617.6 µg/ml, followed by the ethanol and ether dilutions with IC50 values of 103.9 and 135.1 µg/ml, respectively. The hexane dilution did not exhibit any noticeable inhibitory effect on α-amylase activity, as the IC50 value was not converged (**Figure2**).

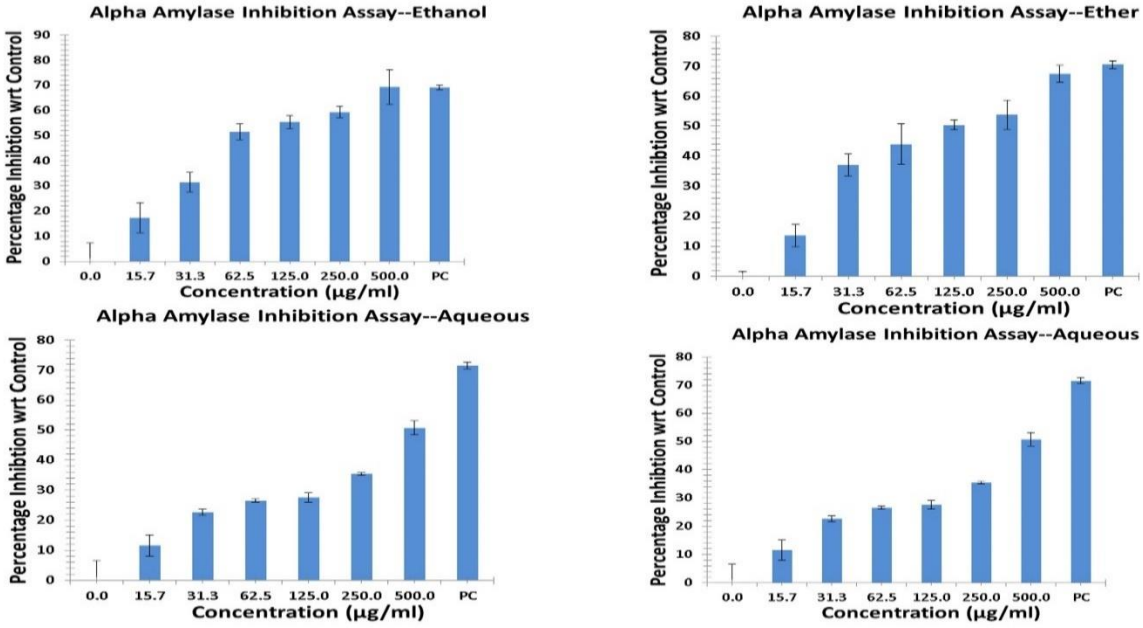

Figure 2. Effect of *Rhynchosia heynei* extracts on alpha-amylase inhibition. The figure displays the IC50 values (µg/ml) for the aqueous, ethanol, ether, and hexane dilutions of *Rhynchosia heynei* extracts in the inhibition of alpha-amylase activity, compared to Metformin, a positive control."

3.5 Minimum Inhibitory Concentration Anti-TB:

The findings of the experimentrevealed that the hexane extract had the lowest IC50 (1.01 µg/ml) followed by the ethanol extract (7.599 µg/ml), ether extract (32.7 µg/ml), and aqueous extract (793.3 µg/ml). The IC50 values represent the amount of extract needed to stop the growth of the microorganisms by 50%. The study revealed that the hexane extract had the maximum antimicrobial activity, then by the ethanol and ether extracts. The aqueous extract had the least antimicrobial activity **(Figure 3)**.

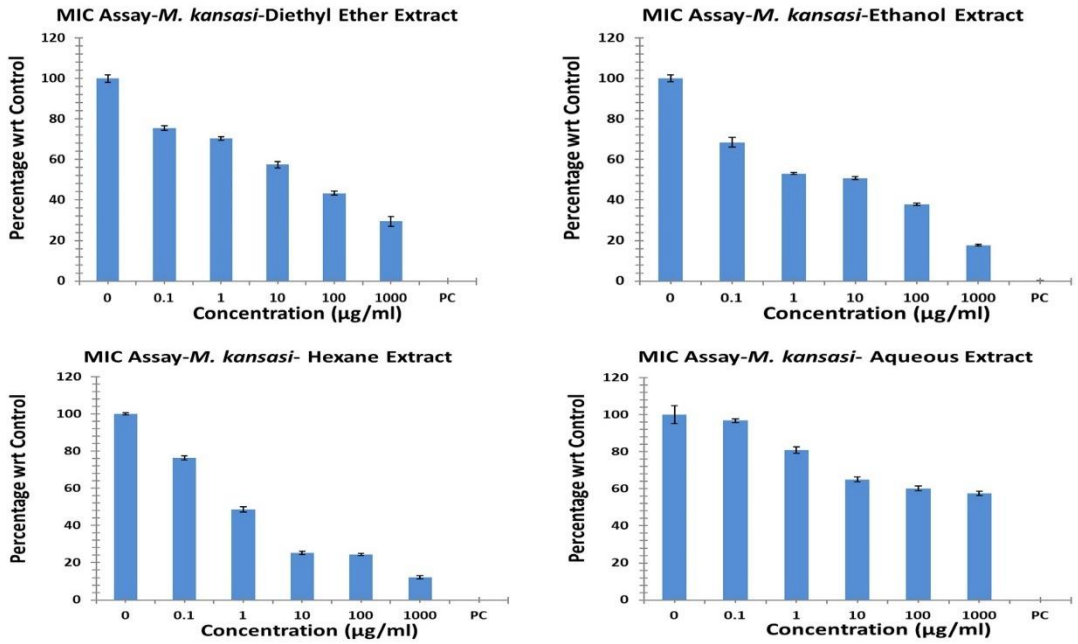

Figure3. Minimum Inhibitory Concentration (MIC) of *Rhynchosia heynei* Extracts against *Mycobacterium kansasii*. The figure displays the IC50 values (µg/ml) for the hexane, ethanol, ether, and aqueous extracts of *Rhynchosia heynei* against *Mycobacterium kansasii*.

Discussion

Rhynchosia heynei is a plant belonging to the Fabaceae family and has been broadlyemployed in Ayurveda and Unani medicine for treating various ailments, including diabetes and tuberculosis. In modern days, there is a growing interest in studying the plant's phytochemical and pharmacological properties.This study targeted to evaluate the potential anti-diabetic and anti-TB activity of different extracts of *Rhynchosia heynei*, as well as their phytochemical composition. The preliminary phytochemical analysis of the various *Rhynchosia heynei* extracts revealed the presence of alkaloids, tannins, phenolic compounds, steroids, saponins, terpenoids, and flavonoids. These bioactive compounds are known for their pharmacological activities, including anti-inflammatory, antioxidant, and antimicrobial effects. Therefore, the observed pharmacological activities of the plant may be linked to the presence of these bioactive constituents.

To investigate the potential anti-diabetic activity of *Rhynchosia heynei* extracts, we conducted an in vitro glucose uptake assay using GOD-POD Enzyme Assay Kit. The results showed that all the extracts increased glucose uptake in a dose-related way compared to the control group. Following the ethanol extract were the diethyl ether, hexane, and aqueous preparations in terms of their effects on glucose uptake. The positive control, insulin, showed the highest glucose uptake, while the negative control, metformin, exhibited the lowest glucose uptake. The increased glucose uptake by the extracts could be attributed to the activation of the insulin

signaling pathway, which is responsible for the uptake of glucose by the cells. Moreover, the bioactive compounds present in the extracts might act on different molecular targets involved in glucose uptake, leading to their synergistic effect on glucose uptake.

The research also looked at how to inhibit alpha-amylase activity, a crucial mechanism for regulating postprandial hyperglycemia, a significant risk factor for developing diabetes. The findings showed that the aqueous, ethanol, and ether dilutions exhibited significant reducing effects on α-amylase activity. Inhibition of α-amylase can lead to a reduction in carbohydrate digestion and absorption, resulting in decreased postprandial glucose levels. The aqueous dilution exhibited the highest inhibitory effect on α-amylase activity, possibly due to the presence of water-soluble active compounds. The ethanol and ether dilutions also showed significant inhibitory effects on α-amylase activity, indicating the presence of active compounds that are soluble in these solvents. However, the hexane dilution did not show significant inhibition, suggesting that the active compounds responsible for α-amylase inhibition may not be soluble in this solvent. To identify the active substances contained in the aqueous, ethanol, and ether dilutions and ascertain their modes of action, additional research is required. Overall, the study suggests that the aqueous, ethanol, and ether dilutions may have potential as α-amylase inhibitors for the management of diabetes and obesity.

In addition, the study evaluated the anti-TB potential of *Rhynchosia heynei* extracts by conducting a minimum inhibitory concentration (MIC) assay against the *Mycobacterium kansasii*. The results of the anti-microbial activity assay showed that the Hexane and Ethanol extracts had the highest anti-microbial activity against *M. kansasii*, with IC50 values of 1.01 µg/ml and 7.599 µg/ml, respectively. The Ether extract had moderate activity with an IC50 value of 32.7 µg/ml, while the Aqueous extract had the lowest activity with an IC50 value of 793.3 µg/ml. The results suggest that the Hexane and Ethanol extracts may contain compounds that are more effective at inhibiting the growth of *M. kansasii*, compared to the compounds in the other extracts. This could be due to differences in the solubility and polarity of the compounds present in the different extracts, which may affect their ability to interact with the bacterial cell membrane and inhibit growth. The plant extracts showed significant anti-diabetic activity by measuring glucose uptake and alpha-amylase inhibition, and significant anti-TB activity against *Mycobacterium kansasii*.

Although the study results are promising, it is important to note that further research is needed to determine the safety and efficacy of using *Rhynchosia heynei* extracts as anti-diabetic or anti-TB agents in experimental animal models. Additionally, more detailed information on the specific bioactive compounds detected in *Rhynchosia heynei* and their potential pharmacological effects is necessary to better understand the observed anti-diabetic and anti-TB effects of the plant extracts.Overall, the study provides evidence for the potential pharmacological activities of *Rhynchosia heynei.*

Conclusion:

In conclusion, our study provides scientific evidence to support the traditional use of *Rhynchosia heynei* in Ayurveda and Unani medicine. The extracts of this plant possess bioactive compounds

that exhibit potential anti-diabetic and anti-TB activity. Further researchis warranted to find the active constituents responsible for these activities and to evaluate their efficacy and safety in vivo".

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Conflicts of interest

The authors declare no conflicts of interest.

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