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Extraction Of Phytochemicals From *Hemidesmus Indicus* Leaves: Evaluation Of *In Vitro* Antioxidant, Antidiabetic And Antifungal Properties

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Abstract:
This study investigates the in vitro antioxidant, antidiabetic, and antifungal properties of *Hemidesmus indicus* leaf extracts using various solvents, including benzene, chloroform, methanol, ethanol, and aqueous solutions. The antioxidant activity was assessed using DPPH and FRAP assays, while the antidiabetic potential was evaluated through α-amylase and α-glucosidase inhibition assays. The antifungal activity was tested against *Candida albicans*, *Aspergillus fumigatus*, and *Aspergillus niger* using the well diffusion method. Among the extracts, the methanolic extract demonstrated the highest efficacy, with IC*50* values of 42.88 µg mL^{-1} (DPPH), 29.62 µg mL^{-1} (FRAP), 28.55 µg mL^{-1} (α-amylase inhibition), and 31.64 µg mL^{-1} (α-glucosidase inhibition). It also exhibited effective antifungal activity, particularly against *Candida albicans*. The chloroform extract showed effective activity, especially in the FRAP assay and enzyme inhibition tests, along with moderate antifungal properties. In contrast, the benzene, ethanol, and aqueous extracts demonstrated moderate to lower efficacy across all assays, though the aqueous extract showed significant antifungal activity against *Aspergillus niger*. These findings represents the significant potential of *Hemidesmus indicus* as a source of natural antioxidant, antidiabetic, and antifungal agents, with the methanolic extract showing the most promise.

Keywords: *Hemidesmus indicus*, phytochemistry, antioxidant, antidiabetic, antifungal property.

1. INTRODUCTION:

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by high blood glucose levels due to either insufficient insulin production or ineffective insulin utilization. It is a significant global health concern, with its prevalence nearly doubling since 1980. The disease is primarily categorized into Type 1, Type 2, and gestational diabetes, each with distinct etiologies and management strategies. The increasing prevalence of diabetes is closely linked to lifestyle factors, and it poses substantial risks for various complications, including cardiovascular diseases, neuropathies, and renal failure [1,2]. Oxidative stress results from an imbalance between the production of reactive oxygen species (ROS) and the body's ability to detoxify these reactive intermediates or repair the resulting damage. This imbalance is particularly pronounced in diabetes, where hyperglycemia leads to increased production of ROS, contributing to the progression of the disease and its complications [3].

Inhibition of α-amylase and α-glucosidase enzymes is a promising strategy for managing diabetes, as these enzymes play crucial roles in carbohydrate digestion and glucose absorption. Various natural compounds and plant extracts have been studied for their potential to inhibit these enzymes, offering alternative or complementary options to conventional antidiabetic drugs like acarbose [4]. Phytochemicals, naturally occurring compounds in plants, play a significant role in inhibiting alpha-amylase and alpha-glucosidase enzymes, which are crucial in carbohydrate metabolism, and exhibit potent antioxidant activities. These properties make them valuable in managing diabetes and oxidative stress-related conditions [5].

Hemidesmus indicus, commonly known as Indian Sarsaparilla, is a medicinal plant with a rich history in traditional Ayurvedic medicine. It is renowned for its diverse pharmacological properties, which have been validated through various experimental and clinical studies. This plant is utilized for its anxiolytic, anti-inflammatory, antioxidant, and blood-purifying properties, among others [6].

This plant has demonstrated significant anxiolytic activity in rodent models. The ethanolic extract of the plant (EEHI) was shown to reduce anxiety-like behaviors in tests such as the Elevated Plus Maze, Open Field Test, and Light-Dark Box test [7]. The extracts of *Hemidesmus indicus* was found to inhibit hyperalgesia and lower blood sugar levels in diabetic rats, indicating its neuroprotective effects [8]. The plant also shown promising antibacterial activity, validating its use in various Ayurvedic formulations for blood purification [9]. The ethanolic extract of *Hemidesmus indicus* roots

was effective in reducing crystal nucleation and growth, thereby preventing renal stone formation in animal models. It also demonstrated potent diuretic activity, supporting its traditional use in treating urolithiasis [10].
According to an extensive literature review, no study has been conducted on the *in vitro* antioxidant and antidiabetic activity of *Hemidesmus indicus* leaves. As a result, the objectives of the present study are: (a) sequential extraction of phytochemicals from *Hemidesmus indicus* leaves. (b) qualitative determination of phytochemicals; (b) *in vitro* antioxidant activity; and (d) *in vitro* antidiabetic activity.

2. Materials and Methods:

2.1. Sequential extraction of phytochemicals using Soxhlet method:

The collected leaves of *Hemidesmus indicus* were shaded dried under sunlight for 3 days and made into fine powder using an electrical blender. 25 grams of leaf powder were packed and kept in a soxhlet apparatus. The soxhlet method was used to extract the compounds using 300 mL of non-polar and polar solvents (benzene, chloroform, methanol, ethanol, and distilled water) and continued up to 7 cycles. The extracted solutions were collected, dried, and weighed separately.
The extracted phytochemicals further subjected to qualitative phytochemical screening and determination of total polyphenol content (TPC).

2.2. Qualitative Phytochemical Screening:

The qualitative phytochemical screening of all extracts was conducted to identify the secondary metabolites, including flavonoids, phenols, tannins, alkaloids (Mayer's and Wagner's), terpenoids, anthraquinones, saponins, quinones, coumarins, glycosides, steroids, and reducing sugars using standard protocols [11].

2.3. Total Polyphenol Content (TPC) estimation:

Total phenolic content (TPC) was determined using the Folin-Ciocalteu (FC) reagent procedure. The TPC was determined by dissolving 1 mg mL^{-1} of dried extracts in methanol and filtering it through Whatman No. 1 filter paper. Test tubes were filled with 0.02 mL of either extract or standard solution, along with 1 mL of FC reagent in a ratio of 1:10 (volume to volume). The solution was combined with 1 mL of 7.5% sodium carbonate and allowed to incubate for a maximum duration of two hours. The absorbance at a wavelength of 765 nm was measured using a UV-Vis Spectrophotometer (Shimadzu UV 2600). The results were expressed as gallic acid equivalents (GAE/g) in milligrams per gram of dry weight extract [12].

2.4. *In vitro* antioxidant activity:

The *in vitro* antioxidant activity in the present study was determined using 2,2-diphenyl-1-picryl hydrazyl (DPPH) free radical scavenging activity and ferric reducing antioxidant power (FRAP) assay [13].
The free radical scavenging activity of the phytochemicals extracted from the leaves of *Hemidesmus indicus* was assessed using the DPPH assay. Various concentrations of the each extract, ranging from 40 to 200 µg mL^{-1}, were prepared in methanol. A 3 mM solution of DPPH in methanol was then added to each concentration of the extract, and the mixtures were incubated in the dark for 1 hour at room temperature. Ascorbic acid was used as a standard reference, and a control was prepared by adding DPPH with methanol. The absorbance of the samples and the control was measured at 517 nm using a UV-Vis spectrophotometer.
In FRAP assay, Various concentrations of the extract, ranging from 40 to 200 µg mL^{-1}, were prepared in acetate buffer (pH 3.6). The FRAP reagent was prepared by mixing acetate buffer, $FeCl_3$, and TPTZ in a proportion of 10:1:1 (v/v/v). The extract was then added to the FRAP reagent, and the mixture was incubated in the dark at room temperature for 4 hours. After incubation, the samples were centrifuged at 5000 rpm for 5 minutes at 4°C. Ascorbic acid was used as a standard reference, and the control consisted of FRAP reagent with acetate buffer. The absorbance of the samples and the control was measured at 593 nm using a UV-Vis spectrophotometer.
The percentage (%) of activity for both activities was carried out according to the equation 1 and the Inhibitory concentration 50 (IC_{50}) was calculated using equation 2.

2.5. *In vitro* antidiabetic activity:

The inhibition of α-amylase and α-glucosidase enzymes by the phytochemicals extracted from the leaves of *Hemidesmus indicus* was evaluated using standard enzyme inhibition assays.
For the α-amylase inhibition assay, the extract with various concentrations (40 to 200 µg mL^{-1}) was diluted in 0.1 M acetate buffer (pH 6.9) and incubated with a solution of α-amylase (1U mL^{-1}) enzyme at 37°C for 10 minutes. After this incubation, a starch solution (1% w/v) was added to the reaction mixture, which was further incubated at 37°C for an additional 10 minutes. The enzymatic reaction was then terminated by adding dinitrosalicylic acid (DNS) reagent, and the mixture was heated in a boiling water bath for 5 minutes to develop the color indicative of starch hydrolysis. After cooling the reaction mixtures to room temperature, the absorbance was measured at 540 nm using a UV-Vis spectrophotometer. A control sample was prepared without the plant extract, and acarbose was used as a standard inhibitor.

Similarly, the α-glucosidase inhibition assay was conducted using the same concentrations of extracts (40 to 200 µg mL^{-1}). The extract was prepared in 0.1 M phosphate buffer (pH 6.8) and incubated with α-glucosidase enzyme (1U mL^{-1}) at 37°C for 10 minutes. Following this incubation, p-nitrophenyl-α-D-glucopyranoside (pNPG) was added as a substrate, and the mixture was further incubated at 37°C for 30 minutes. The reaction was stopped by adding 100mM sodium carbonate solution, and the absorbance was measured at 405 nm using a UV-Vis spectrophotometer. As in the alpha-amylase assay, a control sample without the extract was used, and acarbose served as the standard inhibitor.
The percentage (%) of inhibition for both activities was carried out according to the equation 1 and the Inhibitory concentration 50 (IC_{50}) was calculated using equation 2.

$$\textbf{Activity (\%)} = \frac{\textbf{Absorbance of the control} - \textbf{Absorbance of the sample}}{\textbf{Absorbance of the control}} \qquad \textbf{Eqs…1.}$$

$$\boldsymbol{IC_{50}} = Y = \frac{Max}{1+\left(\frac{X}{IC_{50}}\right)^{Hill\ coefficient}} \qquad \textbf{Eqs…2.}$$

2.6. Antifungal activity:

The antifungal activity of *Hemidesmus indicus* leaf extracts was evaluated using the well diffusion method on three fungal species: *Candida albicans*, *Aspergillus fumigatus*, and *Aspergillus niger*. Muller Hinton Agar (MHA) was used as the growth medium for the assay. The agar plates were first prepared by pouring a uniform layer of MHA into sterile Petri dishes and allowing them to solidify. Each fungal strain was cultured separately and standardized to achieve a turbidity equivalent to 0.5 McFarland standards, which corresponds to approximately 1.5 x 10^8 CFU mL^{-1}. After inoculating the surface of the MHA plates with the fungal cultures using a sterile swab, wells were carefully punched into the agar using a sterile cork borer. Each well was then filled with 100 µL of *Hemidesmus indicus* leaf extracts at a concentration of 1000 µg mL^{-1}. As a positive control, 100 µL of Amphotericin B at a concentration of 500 µg mL^{-1} was added to separate wells. The plates were incubated at 35-37°C for 24-48 hours, depending on the growth rate of the fungal species. Following the incubation period, the antifungal activity was assessed by measuring the diameter of the inhibition zones around each well, which indicated the efficacy of the plant extract in inhibiting fungal growth. The results were compared with the standard antifungal agent Amphotericin B to evaluate the relative potency of the *Hemidesmus indicus* extracts against the tested fungal species.

3. Result and Discussion:

Soxhlet extraction is a hot continuous extraction process that allows for the complete extraction of compounds using a minimal amount of solvent. This method is advantageous for its efficiency in extracting a wide range of phytochemicals, including phenolics, flavonoids, and alkaloids. The method involves repeated washing of the plant material with a solvent, which is continuously evaporated and condensed, ensuring thorough extraction of the desired compounds [14]. The phytochemicals extracted sequentially using soxhlet method were further subjected to qualitative phytochemical screening.

3.1. Qualitative phytochemical screening:

The phytochemical investigation of *Hemidesmus indicus* leaves involved analyzing different extracts using various solvents, including benzene, chloroform, methanol, ethanol, and aqueous solutions, to identify the presence of various bioactive compounds and results were represented in table 1. The results indicated that tannins were moderately present in the methanol extract, with no detectable levels in the benzene, ethanol, or aqueous extracts, while chloroform extract showed a mild presence. Both Mayer's and Wagner's tests for alkaloids yielded negative results across all solvent extracts, indicating the absence of alkaloids in the plant. Saponins were also absent in all the solvent extracts tested. In contrast, cardiac glycosides were strongly present in the benzene, chloroform, and methanol extracts but were not detected in the ethanol and aqueous extracts. Steroids were found in moderate amounts in the methanol and aqueous extracts, while they were absent in the benzene, chloroform, and ethanol extracts. Terpenoids showed moderate presence in the methanol and aqueous extracts, a mild presence in the ethanol extract, but were absent in both the benzene and chloroform extracts. Flavonoids were detected in all extracts except the aqueous extract, with a strong presence in the methanol and ethanol extracts and a moderate presence in the benzene and chloroform extracts. However, when tested using Shinoda's test, flavonoids were absent across all solvent extracts. Phlobatannins, anthraquinones, and reducing sugars were not detected in any of the solvent extracts. Carbohydrates, on the other hand, were present in the ethanol extract and showed a strong presence in the aqueous extract, while they were absent in the benzene, chloroform, and methanol extracts. This comprehensive analysis highlights the varied distribution of phytochemicals in *Hemidesmus indicus* depending on the solvent used for extraction, with methanol and aqueous extracts showing the broadest range of phytochemical constituents.

Table 1: Qualitative phytochemical screening of various extracts from leaves of *Hemidesmus indicus*.

Phytochemical screening	Benzene	Chloroform	Methanol	Ethanol	Aqueous
Tannins	-	+	++	-	-
Alkaloids (Mayer's)	-	-	-	-	-
Alkaloids (Wagner's)	-	-	-	-	-
Saponins	-	-	-	-	-
Cardiac glycosides	++	++	++	-	-
Steroids	-	-	+	-	++
Terpenoids	-	-	++	+	++
Flavonoids	+	+	++	++	-
Flavonoids (Shinoda's test)	-	-	-	-	-
Phlobatannins	-	-	-	-	-
Anthraquniones	-	-	-	-	-
Reducing sugars	-	-	-	-	-
Carbohydrates	-	-	-	+	++

Moderately present (+), Abundant (++), Absent (-).

3.2. Estimation of total polyphenol content (TPC):

The total polyphenol content of *Hemidesmus indicus* leaf extracts, expressed as mg of gallic acid equivalents (GAE) per 100 mg of extract, varied across different solvents. The benzene extract exhibited the highest polyphenol content, measuring 45.83 ± 3.53 mg GAE/100 mg, followed closely by the methanol extract with 43.66 ± 0 mg GAE/100 mg. The ethanol extract also demonstrated a substantial polyphenol content of 41.922 ± 2.38 mg GAE/100 mg. In contrast, the chloroform extract had a slightly lower polyphenol content at 36.202 ± 0.78 mg GAE/100 mg. The aqueous extract showed the lowest polyphenol content among all the tested solvents, with a value of 34.58 ± 1.55 mg GAE/100 mg. These results suggest that benzene and methanol are the most effective solvents for extracting polyphenols from *Hemidesmus indicus* leaves. Polyphenols are ubiquitous in the plant kingdom, with over 10,000 different types identified. They are synthesized in all plant cells and play a critical role in plant defence against biotic and abiotic stressors [15]. Polyphenols have shown potential in preventing and treating various diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders, due to their antioxidant and anti-inflammatory effects [16].

3.3. *In vitro* antioxidant activity:

The antioxidant activity of *Hemidesmus indicus* leaf extracts was evaluated using DPPH and FRAP assays across concentrations ranging from 40 to 200 µg mL^{-1}, with the results represented in Figure 1. The methanolic extract exhibited the highest DPPH radical scavenging activity, ranging from 31.33% to 81.25%, indicating its strong antioxidant potential. This was followed by the benzene extract, which showed a DPPH activity range of 12.47% to 62.5%. The aqueous and chloroform extracts demonstrated moderate antioxidant activity, with ranges of 11.2% to 53.27% and 8.4% to 51.48%, respectively. The ethanolic extract displayed the lowest DPPH activity, with a range of 2.5% to 21.79%, suggesting it was the least effective in scavenging free radicals among the extracts tested.

In the FRAP assay, the methanolic extract again showed the highest antioxidant activity, with values ranging from 30.2% to 83.47%, highlighting its superior ferric reducing ability. The chloroform extract followed closely, with FRAP activity ranging from 32.5% to 81.67%, indicating its effectiveness as a reducing agent. The aqueous and ethanolic extracts displayed moderate FRAP activity, with ranges of 7.9% to 41.76% and 7.62% to 42.5%, respectively. In contrast, the benzene extract exhibited the lowest FRAP activity, with values ranging from 1.68% to 12.48%, suggesting its limited capacity to reduce ferric ions.

The results indicate that the methanolic extract of *Hemidesmus indicus* leaves possesses the strongest antioxidant activity, as demonstrated by both DPPH and FRAP assays. This may be attributed to the higher polyphenol content in the methanolic extract, as polyphenols are known to contribute significantly to antioxidant activity. The chloroform extract also showed substantial antioxidant potential in the FRAP assay, possibly due to the presence of other reducing agents in this solvent. The lower activity observed in the ethanolic and benzene extracts suggests that these solvents are less effective in extracting compounds with strong antioxidant properties from *Hemidesmus indicus* leaves.

The antioxidant properties of *Hemidesmus indicus* have been linked to various health benefits, including anti-cancer, anti-diabetic, and anti-inflammatory effects. Its ability to mitigate oxidative stress makes it a promising candidate for developing functional foods and health supplements [17]. The antioxidant mechanism involves the donation of hydrogen atoms or electrons to neutralize free radicals, thereby preventing cellular damage. The presence of phenolic compounds in *Hemidesmus indicus* contributes to its ability to act as a singlet oxygen quencher, further enhancing its antioxidant efficacy [18].

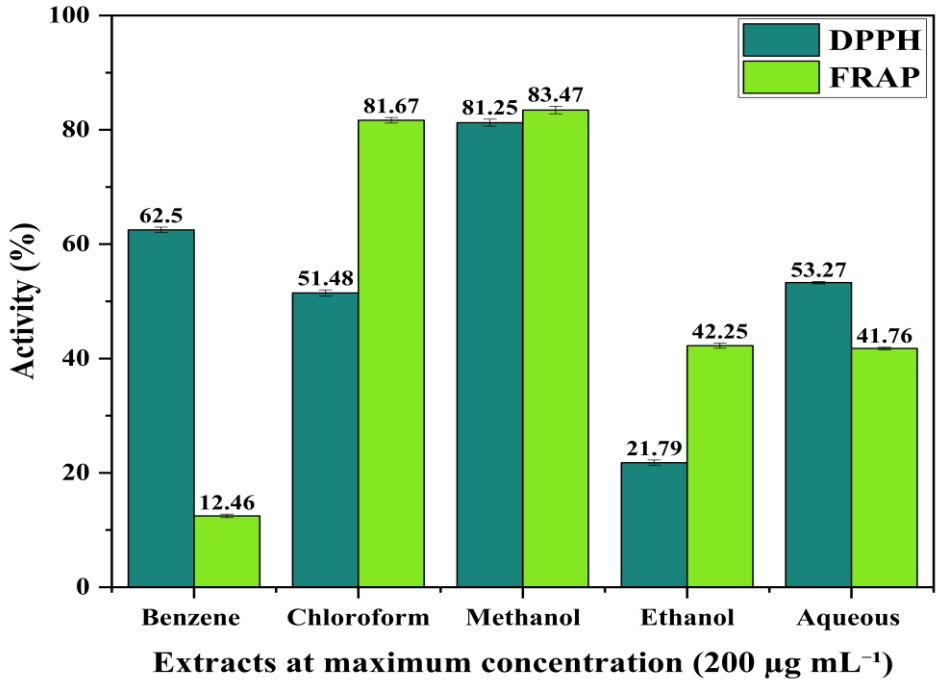

Figure 1: *In vitro* antioxidant activity of *Hemidesmus indicus* leaf extracts.

3.4. *In vitro* antidiabetic activity:

The antidiabetic activity of *Hemidesmus indicus* leaf extracts was assessed through their inhibitory effects on α-amylase and α-glucosidase enzymes at concentrations ranging from 40 to 200 µg mL^{-1}, with results presented in Figure 2. The methanolic extract exhibited the highest α-amylase inhibition, with a range of 25.97% to 89.54%, indicating its strong potential to interfere with carbohydrate digestion and subsequent glucose absorption. This was followed by the aqueous extract, which showed inhibition ranging from 13.67% to 61.54%, and the ethanolic extract with inhibition values from 12.55% to 61.28%. The chloroform extract also demonstrated significant α-amylase inhibitory activity, ranging from 9.4% to 61.57%, whereas the benzene extract displayed the lowest inhibition, with values between 3.4% and 17.14%.

For α-glucosidase inhibition, the methanolic extract again demonstrated the highest inhibitory activity, with a range of 41.44% to 87.67%. This suggests that the methanolic extract is particularly effective in reducing postprandial hyperglycemia by inhibiting the enzyme responsible for breaking down carbohydrates into glucose. The chloroform extract showed substantial inhibition, with values ranging from 12.78% to 59.76%, followed by the aqueous extract with a range of 8.8% to 58.14%. The ethanolic extract displayed moderate inhibitory activity, ranging from 10.54% to 52.33%, while the benzene extract had the lowest α-glucosidase inhibition, with values between 4.7% and 19.54%.

These results suggest that the methanolic extract of *Hemidesmus indicus* leaves is the most effective among the tested solvents in inhibiting both α-amylase and α-glucosidase enzymes, which is indicative of its strong antidiabetic potential. The high inhibitory activity observed in the methanolic extract could be attributed to the presence of bioactive compounds, such as polyphenols and flavonoids, which are known to contribute to enzyme inhibition. The chloroform and aqueous extracts also showed considerable inhibitory effects, making them potential candidates for managing diabetes. The lower activity observed in the benzene extract suggests that it is less effective in extracting compounds with strong antidiabetic properties from *Hemidesmus indicus* leaves.

The methanolic extract of *Hemidesmus indicus* roots has shown significant inhibitory activity against carbohydrate hydrolytic enzymes α-amylase and α-glucosidase, which are crucial in managing postprandial blood glucose levels. This inhibition is competitive, suggesting that the extract can effectively reduce glucose absorption in the intestines [19]. *In vivo* studies using streptozotocin (STZ)-induced diabetic models have demonstrated that *Hemidesmus indicus* extracts can significantly lower blood glucose levels and improve lipid profiles. These effects are attributed to the modulation of oxidative stress and enhancement of antioxidant enzyme activities [20]. While the antidiabetic potential of *Hemidesmus indicus* is promising, further research is needed to fully understand its mechanisms and optimize its use in clinical settings. This includes identifying more active phyto-constituents and elucidating their structure-activity relationships.

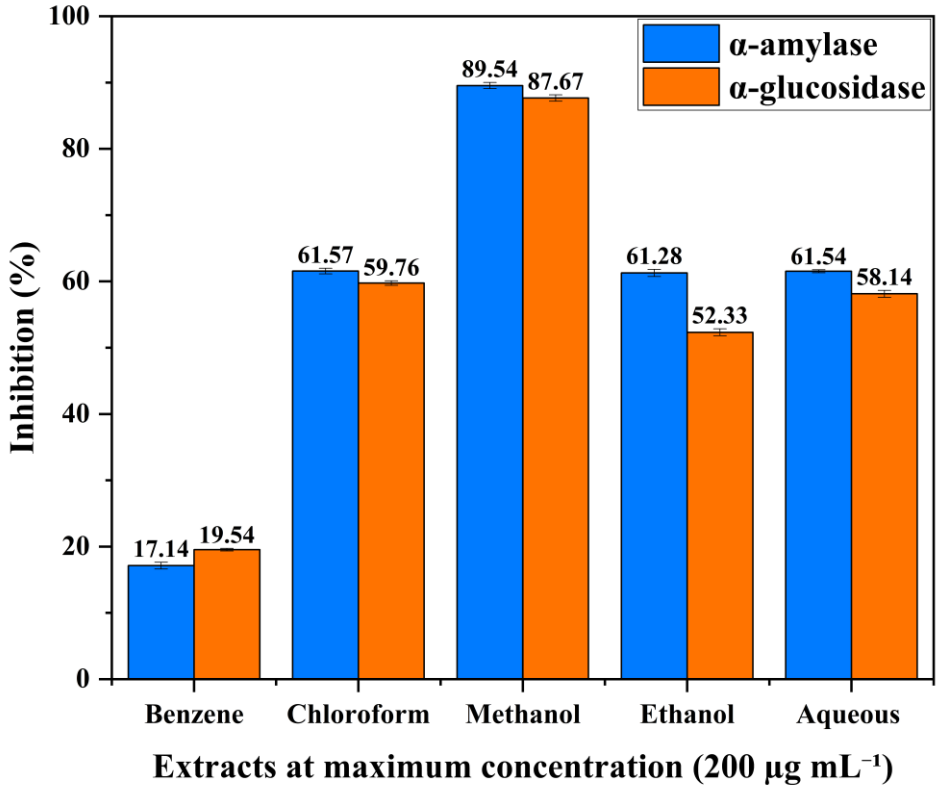

Figure 2: *In vitro* antidiabetic activity of *Hemidesmus indicus* leaf extracts.

3.5. Inhibitory Concentration 50 (IC_{50}):

The IC_{50} values for antioxidant and antidiabetic activities of plant extracts are crucial in determining their efficacy in neutralizing free radicals and inhibiting enzymes related to diabetes. These values provide insight into the potential of various plant extracts as therapeutic agents for managing oxidative stress and diabetes. The inhibitory concentration 50 (IC_{50}) values of *Hemidesmus indicus* leaf extracts were determined for their antioxidant and antidiabetic activities, revealing significant differences across the various solvent extracts shown in table 2. In the DPPH assay, the methanolic extract exhibited the lowest IC_{50} value of 42.88 µg mL^{-1}, indicating its strong free radical scavenging activity, which was comparable to the standard ascorbic acid with an IC_{50} of 12.47 µg mL^{-1}. The aqueous extract followed with an IC_{50} of 144.0 µg mL^{-1}, while the benzene, chloroform, and ethanol extracts had significantly higher IC_{50} values of 162.45, 287.91, and 362.22 µg mL^{-1}, respectively, indicating lower antioxidant potency.

In the FRAP assay, the methanolic extract again showed superior activity with an IC_{50} of 29.62 µg mL^{-1}, close to the standard ascorbic acid (IC_{50} of 10.80 µg mL^{-1}). The chloroform extract also demonstrated strong ferric reducing power with an IC_{50} of 32.60 µg mL^{-1}. In contrast, the aqueous extract had a moderate IC_{50} value of 215.74 µg mL^{-1}, and the benzene and ethanol extracts displayed much weaker activity with IC_{50} values of 358.17 and 196.69 µg mL^{-1}, respectively.

Regarding antidiabetic activity, the methanolic extract exhibited the most potent inhibitory effects on both α-amylase and α-glucosidase enzymes, with IC_{50} values of 28.55 µg mL^{-1} and 31.64 µg mL^{-1}, respectively. These values are relatively close to the standard acarbose, which had IC_{50} values of 7.92 µg mL^{-1} for α-amylase and 7.54 µg mL^{-1} for α-glucosidase, suggesting that the methanolic extract is particularly effective in inhibiting these enzymes. The chloroform extract also showed considerable inhibitory activity, with IC_{50} values of 96.45 µg mL^{-1} for α-amylase and 162.25 µg mL^{-1} for α-glucosidase. The aqueous extract demonstrated moderate inhibition with IC_{50} values of 87.79 µg mL^{-1} for α-amylase and 144.51 µg mL^{-1} for α-glucosidase. However, the benzene extract had the highest IC_{50} values for both enzymes (187.72 µg mL^{-1} for α-amylase and 207.32 µg mL^{-1} for α-glucosidase), indicating the least effectiveness among the extracts. Similarly, the ethanol extract exhibited higher IC_{50} values (84.11 µg mL^{-1} for α-amylase and 158.01 µg mL^{-1} for α-glucosidase), suggesting moderate antidiabetic potential.

These results justify the methanolic extract of *Hemidesmus indicus* as the most effective in both antioxidant and antidiabetic assays, likely due to its higher content of bioactive compounds such as polyphenols and flavonoids. The chloroform extract also shows promise, particularly in the FRAP assay and enzyme inhibition, while the benzene and

ethanol extracts appear less effective, especially in antioxidant assays. The aqueous extract demonstrates moderate activity across all assays, reflecting its potential as a natural source of antidiabetic and antioxidant agents, albeit less potent than the methanolic extract.

A study on polyherbal formulations revealed that the formulation S28, which includes *Trillidium goanum*, *Centorium tenuiflorum*, *Morchella conica*, and *Rubinia pseudoacacia*, exhibited potent antioxidant activity with IC_{50} values of 7.6±0.36 µg/mL for phenols and 8.17±1.69 µg/mL for tannins [21]. *Bauhinia variegata and Syzygium cumini* showed strong antioxidant activities in ABTS and superoxide radical scavenging assays, with EC_{50} values of 31.19 ± 4.15 and 28.82 ± 4.42 for *B. variegata*, and 13.64 ± 10.39 and 30.19 ± 6.82 for *S. cumini*, respectively [22].

Table 2: Inhibitory Concentration 50 (IC_{50}) values.

Extract (s)	**Inhibitory Concentration 50 (IC_{50}) (µg mL^{-1})**			
	DPPH assay	**FRAP assay**	**α-amylase inhibition assay**	**α-glucosidase inhibition assay**
Standard (Ascorbic acid/Acarbose)	12.47	10.80	7.92	7.54
Benzene	162.45	358.17	187.72	207.32
Chloroform	287.91	32.60	96.45	162.25
Methanol	42.88	29.62	28.55	31.64
Ethanol	362.22	196.69	84.11	158.01
Aqueous	144.0	215.74	87.79	144.51

3.6. Antifungal activity:

The antifungal activity of *Hemidesmus indicus* leaf extracts was evaluated against *Candida albicans*, *Aspergillus fumigatus*, and *Aspergillus niger* using the well diffusion assay, with results presented in Table 3 and Figure 3. The standard antifungal agent, Amphotericin B, exhibited the highest zone of inhibition across all three fungal species, with zones measuring 26 ± 0.051 mm for *Candida albicans*, 24 ± 0.047 mm for *Aspergillus fumigatus*, and 26 ± 0.053 mm for *Aspergillus niger*, confirming its strong antifungal efficacy. Among the *Hemidesmus indicus* extracts, the aqueous extract demonstrated the most potent antifungal activity, showing inhibition zones of 18 ± 0.051 mm for *Candida albicans*, 18 ± 0.054 mm for *Aspergillus fumigatus*, and 22 ± 0.051 mm for *Aspergillus niger*. This suggests that water-soluble compounds in the aqueous extract are particularly effective against these fungal pathogens.

The ethanol extract also exhibited significant antifungal activity, with inhibition zones of 16 ± 0.051 mm for *Candida albicans*, 16 ± 0.021 mm for *Aspergillus fumigatus*, and 14 ± 0.047 mm for *Aspergillus niger*. The methanol extract showed moderate activity, especially against *Candida albicans* with a zone of 16 ± 0.047 mm, but lower activity against the other two species, with zones of 10 ± 0.023 mm for *Aspergillus fumigatus* and 10 ± 0.051 mm for *Aspergillus niger*. The chloroform extract displayed moderate antifungal activity, particularly against *Candida albicans* (14 ± 0.032 mm), while showing slightly lower inhibition for *Aspergillus fumigatus* and *Aspergillus niger* (12 ± 0.051 mm and 12 ± 0.047 mm, respectively). The benzene extract was the least effective, showing minimal inhibition across all tested fungi, with a consistent zone of 6 ± 0 mm or slightly more, indicating limited antifungal properties.

These results suggest that the aqueous and ethanol extracts of *Hemidesmus indicus* are the most promising candidates for further investigation as natural antifungal agents. The relatively high antifungal activity of these extracts compared to others could be attributed to the presence of more effective bioactive compounds in polar solvents, which may enhance their ability to disrupt fungal growth. The lower efficacy of the benzene extract suggests that non-polar compounds in *Hemidesmus indicus* leaves may not significantly contribute to antifungal activity.

The antifungal activity of plant extracts presents a promising avenue in combating antimicrobial resistance, particularly against resistant fungal strains. Plant-derived compounds, known for their diverse chemical structures and mechanisms, offer a natural alternative to synthetic antifungal agents [23].

Table 3: Antifungal activity of *Hemidesmus indicus.*

Extract (s)	**Zone of Inhibition (mm) (mean±SD)**		
	Candida albicans	***Aspergillus fumigatus***	***Aspergillus niger***
Amphotericin B	26 ± 0.051	24 ± 0.047	26 ± 0.053
Benzene	6 ± 0	6 ± 0.047	6 ± 0.021
Chloroform	14 ± 0.032	12 ± 0.051	12 ± 0.047
Methanol	16 ± 0.047	10 ± 0.023	10 ± 0.051
Ethanol	16 ± 0.051	16 ± 0.021	14 ± 0.047
Aqueous	18 ± 0.051	18 ± 0.054	22 ± 0.051

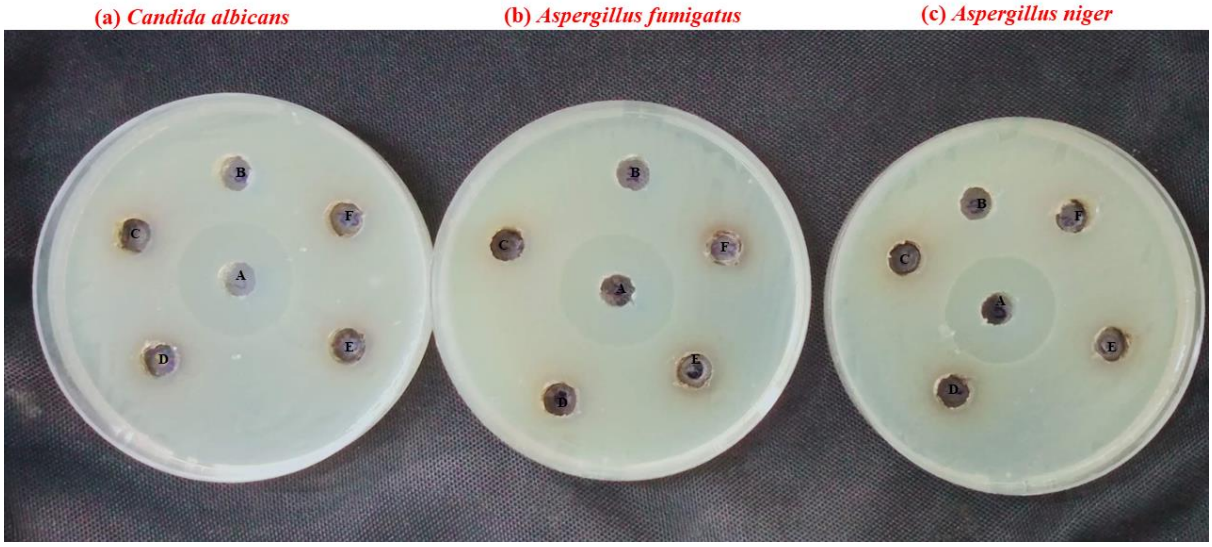

Figure 3: Antifungal activity of *Hemidesmus indicus* (A) Amphotericin B, (B) Benzene, (C) Chloroform, (D) Methanol, (E) Ethanol and (F) Aqueous.

Conclusion and future perspectives:

The present study demonstrates that *Hemidesmus indicus* leaf extracts possess significant antioxidant, antidiabetic, and antifungal activities, with the methanolic extract emerging as the most potent across these assays. The methanolic extract exhibited the lowest IC_{50} values, with 42.88 µg mL^{-1} for the DPPH assay, 29.62 µg mL^{-1} for the FRAP assay, 28.55 µg mL^{-1} for α-amylase inhibition, and 31.64 µg mL^{-1} for α-glucosidase inhibition, indicating its strong potential as a natural source of bioactive compounds for managing oxidative stress and hyperglycemia. The methanolic extract showed efficient antifungal activity, particularly against *Candida albicans*, reinforcing its broad-spectrum bioactivity. The chloroform extract also showed considerable activity, particularly in the FRAP assay (IC_{50} = 32.60 µg mL^{-1}) and enzyme inhibition assays (IC_{50} = 96.45 µg mL^{-1} for α-amylase and 162.25 µg mL^{-1} for α-glucosidase), as well as moderate antifungal properties. In contrast, the benzene, ethanol, and aqueous extracts displayed higher IC_{50} values, reflecting lower potency in comparison to the methanolic extract, though the aqueous extract demonstrated significant antifungal activity, particularly against *Aspergillus niger*.

Future research should focus on the isolation and characterization of specific bioactive compounds responsible for the observed antioxidant and antidiabetic activities in *Hemidesmus indicus* leaf extracts. Advanced chromatographic and spectrometric techniques could be employed to identify the key polyphenols, flavonoids, and other phytochemicals that contribute to these effects. Additionally, *in vivo* studies are essential to evaluate the efficacy and safety of these extracts in animal models, followed by clinical trials to determine their potential therapeutic benefits in humans. The potential for synergistic effects among these bioactive compounds should also be explored, along with the development of functional foods or nutraceuticals incorporating these extracts. Expanding research to other parts of the plant could provide a more comprehensive understanding of the medicinal value of *Hemidesmus indicus*, paving the way for its broader application in natural health products.

Declarations

Conflict of interest:

Authors declare no conflict of interest.

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