RESEARCH PAPER

Pharmacological Evaluation of Polyherbal Extract for in-vitro anti-oxidant and anti-diabetic potential

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ABSTRACT
Bryophytes are emerging as valuablesources of structurally diverse bioactive compounds with significant pharmacological Diabetes mellitus is a metabolic disease marked by blood glucose levels that are abnormally elevated. Pharmacological evaluation of polyherbal extract for in-vitro anti-oxidant and anti-diabeticpotential. The freshleavesof Azadirachta Indica, Ocimum Sanctum, Hibiscus rosa sinensis L., and fresh flowers of Clitoria ternatea L. were collected from the Prayagraj region, UP East, India. There were authenticated by the botanist at Botanical Survey of India, Allahabad, UP with the reference no. 2023-24/534. The dried leaves of Azadirachta indica, Ocimum sanctum and Hibiscus rosa sinensis L. were rendered into fine powder and extracted using hydroalcoholic (distilled water and ethanol; 1:1) solution through cold maceration process. The leaves of different plants were soaked in a beaker containing hydroalcoholic (distilled water and ethanol; 1:1) solution for 15 days with gradual stirrings, separately. Similarly, dried flowers of Clitoria ternatea L. was rendered into fine powders then soaked into a beaker containing hydroalcoholic (distilled water and ethanol; 1:1) solution for 15 days with gradual stirrings and thus extracted-out. Standardization of herbal extracts was performed. Isolation of phytochemicals was done through TLC and HPTLC. The antioxidant and anti-diabetic potentials were evaluated through DPPH scavenging assay, oxygen radical absorbance capacity assay and alpha amylase inhibitory assay. In results, the antioxidant and anti-diabetic potentials were found in decreasing order as DM-03> DM-02> DM-01> DM-04. Thus, among all the polyherbal formulations, DM-03 exhibited the highest pharmacological properties including 71.40% (DPPH antioxidant activity), at 200µg/ml, 3% (Oxygen Radical Absorbance Capacity Assay), at 1000µg/ml, 89.39±0.67% α-amylase inhibition. In conclusion, the antioxidant and antidiabetic response of the dried leaves of Azadirachta indica, Ocimum sanctum, flowers of Clitoria ternatea and leaves of Hibiscus rosa sinensis may be attributed to lower and sustain the cellular oxidation processes and its ability to enhance the responsiveness of tyrosine kinase to insulin .

Keywords: Azadirachta indica, Ocimum sanctum, Clitoria ternatea, Hibiscus rosa sinensis, Antioxidant, Anti-diabetic activity

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INTRODUCTION

Diabetes mellitus is a metabolic disease marked by blood glucose levels that are abnormally elevated. It is clear from looking at historical documents that Apollonius of Memphis used the term "diabetes" for the first time between 250 and 300 BC. At the University of Toronto, Banting et al. isolated insulin from the pancreas of cows in 1922, leading to the creation of a workable diabetes treatment (Arumugam et al. 2013). Glucose intolerance and diabetes mellitus are commonlyobserved in individuals with various endocrinopathies (Yasuda et al.2008). Since the disease can sometimes be caused by a combination of factors, the aetiology of diabetes mellitus may be unclear. Hyperglycemia on its own can decrease the quantity of insulin generated by the pancreatic beta-cells and affect their function (Sapra Amit and Bhandari, 2023). Insulin resistance arises from an excessive presence of fatty acids and cytokines (Umpierre et al., 2011).

Neem belongs to the Meliaceae family. Bangladesh, Nepal, India, and Pakistan are among the tropical and semitropical regions where it is often produced. The tree may reach a height of 20□23 meters and has a straight trunk that is 4-5 feet wide. It grows quickly. Complex and imparipinnate, each leaf comprises five to fifteen leaflets. Its greenish drupes become golden yellow when it ripens (Alzohairy, 2016). Fresh neem leaves were used to refine quercetin and ß-sitosterol, flavonoids (polyphenolic), which showed antibacterial and antifungal properties (Govindachari et al. 1998). Meanwhile, seeds include significant components including gedunin and azadirachtin (Hossain et al. 2011). Tulsi refers an aromatic plant with many branches; 30-60 cm tall, fragrant plant. The oval leaves have a basic green or purple color, a gently serrated or dented edge, and a blade length of 5 cm. The blooms have a short, hairy stalk and are violet. The plant produces reddish-yellow seeds and little fruit. It has a harsh and caustic taste (Prajapati et al. 2003). The two kinds of flavonoids that were isolated from the aqueous leaf extract were orientin and vicenin (Singh et al. 1996).

Clitoria ternatea is belongs to Fabaceae family(Pulok et al. 2008). In the past, the root was used to induce abortions, and the paste is frequently used to treat mucous issues, sore throats, and stomach inflammations. Nootropic, anticonvulsant, anti-inflammatory, and other properties have been demonstrated for C. ternatea (Jain et al. 2003). It

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increases acetylcholine levels in rats and enhances memory (Taranalli et al. 2003).

Hibiscus rosa-sinensis leaf sizes range from 3.5-12 cm in length and 2-5.5 cm in width. The leaves are either oblong or oval in shape. The leaves are whole at their stalks and coarsely serrated at their tips. Flavor is sticky and thick. Flowers have an actinomorphic shape, are pedicellate, have five meristematic parts, and are fully formed. The Corolla has five red petals. and a diameter of around 3 inches; it is widely accessiblein manyclimates where it may thrive. The 3 primary parts of stems are teraxyl acetate, beta-sitosterol, and malvalic acid (Goutam et al. 2018).

MATERIALS AND METHODS

Experimental requirements

Fresh leaves of *Azadirachta Indica, Ocimum Sanctum,* and *Hibiscus rosa sinensis L.*, Fresh flowers of *Clitoria ternatea L.* Wistar rats, diethyl ether, Glibenclamide, streptozotocin, D-glucose, hematoxylin, eosin stains, ethanol, distilled water, Soxhlet apparatus.

Collection and authentication of plants materials

The fresh leaves of *Azadirachta Indica, Ocimum Sanctum, Hibiscus rosa sinensis L.,* and fresh flowers of *Clitoria ternatea L.* were collected from the Prayagraj region, UP East, India. There were authenticated by a botanist at BSI, Allahabad, UP with the reference no. 2023-24/534. The plant materials were washed, shade dried and crushed into coarse powders.

Extraction process

Leaves of *Azadirachta indica, Ocimum sanctum* and *Hibiscus rosa sinensis L.* were rendered into fine powder and extracted using hydroalcoholic (distilled water and ethanol; 1:1) solution through cold maceration process. The leaves of different plants were soaked in a beaker containing hydroalcoholic (distilled water and ethanol; 1:1) solution for 15 days with gradual stirrings, separately. Similarly, dried flowers of *Clitoria ternatea L.* was rendered into fine powders then soaked into a beaker containing hydroalcoholic (distilled water and ethanol; 1:1) solution for 15 days with gradual stirrings. Each beaker was mounted with aluminium foil. After the due time, each beaker□s aluminium foil was removed and filtered using the cotton plug and finally with Whatman filter paper. The obtained slurry was made concentratedthroughevaporation using rotatory evaporator. Thus, the herbal extract was found in powder form and weight to calculate the % yield. All the extracts were kept in desiccator to keep the extract moisture free (Khan *et al.* 2020).

Standardization of herbal extracts

Organoleptic characteristics

The organoleptic characteristics of the diverse herbal extract were determined through physical examination i.e., physical appearance, color and odor.

Physicochemical evaluations

The followingphysicochemical parameters were performed of the diverse herbal extracts:

Loss on drying

In a 100 ml beaker that has already been weighed, precisely weigh 2-4 g of each herbal extract. It is then cooked for five hours at 105 degrees Celsius in an oven. The weight is taken once the beaker has cooled in the desiccator. Until a consistent weight was achieved, the process was repeated (Chauhan et al. 2018).

Formula:

$$\text{Loss of drying (\%)} = \frac{\text{weight loss}}{\text{weight of sample}} \times 100$$

Total ash

In a Silica dish that has been previously lit and tarred, 2-4g of each herbal extract are precisely weighed. The material is evenly spread and then burnt in a muffle furnace at 600ºC until it turns white that confirms the absence of carbon. If carbon free ash is not extracted, the residue on the cold dish is wet with water (2ml) or a saturated solution of ammonium nitrate, dried on a water bath, then burnt in the muffle furnace until it reaches the constant weight (Chauhan et al. 2018).

Formula:

$$\text{Total ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Acid insoluble ash

45ml of 1:5 hydrochloric acid, divided into three pieces of 15 ml each, is added to the dish with the complete ash, gently heated for five minutes, and then filtered. After gathering the insoluble material on Whatman filter paper devoid of ash, the residue is cleaned of acid using distilled water. After being moved to the original dish, the filter paper holding the insoluble material is dried and burned to a consistent weight. The dish is weighed after it has cooled in desiccators (Chauhan et al. 2018).

Formula:

$$\text{Acid insoluble ash (\%): Weight of acid insoluble residue} \times 100$$

Weight of sample

Water soluble ash

25 milliliters of water are combined with the ash that is produced by the entire ash process. After filtering the mixture, the mixture that remains on the filter paper is gathered and weighed. The weighed amount of insoluble materials is subtracted from the weighed amount of ash to determine the water-soluble ash value. This weighted amount is used to calculate the percentage of water-soluble ash value (Chauhan *et al.* 2018).

$$\text{Water soluble ash (\%): Weight of water-soluble residue} \times 100$$

Weight of sample

pH value

10g of each herbal extract is added into water (100ml), stirred, and filtered. pH meter utilized for the measurement of pH.

Alcohol soluble extractive value

3g of each herbal extract is weighed accurately in a cork fitted flask. To this 100ml of absolute alcohol is added and shacked occasionally for 6 hours. After keeping for 18 hours, it is filtered. 25ml of this filtrate is pipette out in a pre-weighed 100ml beaker and evaporated to dryness on a water-bath, after which it is kept it in an hot air oven at 105°C for 6 hours. Once the flask is cool-down in a desiccator, weight is taken (Bysani *et al.* 2017).

Formula:

Alcohol-soluble extractive value (%) = Weight of herbal extract ×100

25 × weight of sample

Water soluble extractive value

A glass stoppered flask is filled with precisely weighed 4g of each plant extract. This is mixed with 100 milliliters of distilled water and shaken periodically for six hours. It is filtered after 18 hours of storage. 25 ml of this filtrate is pipetted out into a 100 ml beaker that has been previously weighed. It is then dried on a water bath and placed in an air oven set at 105°C for six hours. Weighing the flask after it has cooled in desiccators (Bysani *et al.* 2017).

Formula:

Water-soluble extractive value (%) = Weight of herbal extract ×100

25×weight of sample

Sulphated ash value

The sulphated ash value in each herbal extract is determined by a gravimetric method. A known weight of the extract is placed in a crucible, treated with sulfuric acid to aid in complete combustion, and then incinerated in a furnace. The remaining residue, the sulphated ash, is weighed, and its percentage is calculated. This value indicates the inorganic mineral content of the extract.

Preliminary phytochemical investigation

Preliminary phytochemical investigation of leaves extract of *Azadirachta indica*, *Ocimum sanctum* and *Hibiscus rosa sinensis L.* is carried out by qualitative chemical test and chromatographic technique. Similar procedure was applied for the dried flowers of *Clitoria ternatea L.* (Devi and Kottai, 2014; Alaekwe *et al.* 2015).

Alkaloids

Before filtration, each extract was individually treated in diluted HCl.

Mayer□s Test: We used Mayer's reagent, potassium mercuric iodide, to run the filtrates. When a yellowish precipitate forms, it means that alkaloids are present.

Wagner□s Test: Potassium mercuric iodide, often known as Wagner's reagent, was used to run the filtrates. Alkaloids are present when a yellow precipitate forms.

Glycosides

Fehling□s test: Fehling mixed distilled water with his solutions A and B and then heated them for a minute. Eight drops of plant extract were added to this clear solution. Then, it was mixed with one milliliter of Fehling's solution and cooked in a water bath for five minutes. The presence of glycosides is shown by a brick-red precipitate.

Saponins

Foam test: Aconsistent, long-lasting froth was achieved by rapidly shaking 10ml of distilled water with around 2g of the plant extract. There are saponins present when froth appears.

Tannins

Ferric chloride test: Twenty milliliters of water were used to boil 0.5 grams of the dried powdered material in a test tube, and then the mixture was filtered. Following the addition of a few drops of 0.1% FeCl3, the coloration was examined for blue-black or brownish green-black.

Lead acetate test: Two milliliters of plant extract and two milliliters of distilled water were combined. The liquid was well shaken after 0.01g of lead acetate was added. When precipitate and white turbidity form, tannins are present.

Terpenoids

Following the addition of 2.0 ml of chloroform and 5 ml of the aqueous plant extract, the mixture was heated with 3 ml of concentrated H2SO4 and evaporated on the water route. When the terpenoids formed, they took on a gray color.

Steroids

Two milliliters of chloroform and concentrated H2SO4 were mixed with five milliliters of aqueous plant crude extract. The presence of steroids was shown by the redness that appeared in the bottom layer of chloroform.

Tests for reducing sugar & carbohydrate

Fehling's test: It is used to identify sugars that are declining. Dissolve 34.6g of copper sulfate in 500ml of distilled water (solution A). To make Solution B, mix 50g of sodium hydroxide and 17.3g of potassium sodium tartrate with 50ml distilled water. Before using, mix two solutions in the same amount. You should boil Fehling's A and B solutions together for one minute. You should add equal amounts of the test solution. For five to ten minutes, put in a pot of boiling water. The color was originally yellow, and later brick red.

Flavonoids

H_2SO_4 test: A portion of the extract was subjected to treatment with concentrated H_2SO_4, resulting in the appearance of an orange hue.

Chromatographical analysis

TLC analysis

The plant extracts (after dissolving in respective solvents) were placed to the precoated TLC plate in the form of dots using a fine capillary. The top of the plate had identification markings. Chromatography tests were performed in rectangular glass vessels. Asmooth sheet of filter paper was inserted in the TLC chamber and left in the developing solvent to prevent insufficient chamber saturation and the unwanted edge effect. Anisaldehyde-sulphuric acid was sprayed on the plate, and then it was heated at 115 degrees Celsius for 5 minutes. The solvent system utilised was chloroform: ethyl acetate: acetone (7:1.5:1.5). Plates were developed, allowed to air dry, and then analysed for spot count, colour, and Rf values. Agents used for spraying anisaldehyde and sulfuric acid (Kumar *et al.* 2013).

$$Rf = \frac{\text{Distance traveled by the compound}}{\text{Distance traveled by the solvent front}}$$

HPTLC Analysis

The stationary phase is typically a thin layer of silica gel or other adsorbents. The choice of stationaryphase dependson the nature of the sample components (polar or non-polar). The mobile phase can be a mixture of solvents, depending on the solubility of the analytes. Common mobile phasecan be a mixture of polar and non-polar solvents, which allow for optimal separation of components.

Sample preparation

Prepare the sample by dissolving the analyte in a suitable solvent. Filtration might be necessary to remove any particular matter. The sample is applied to the TLC plate in

small volumes using a micropipette or a syringe. Sample application shouldbe uniform and concentrated in one area.

Chromatographic Separation

The plate is placed in a developing chamber, where the mobile phase will move upward due to capillary action. Components of the sample will travel at different rates based on their interaction with the stationary phase, leading to separation. After development, the plate is dried and observed under UV light or through staining techniques for colorimetric analysis. Densitometric scanning (measurement of intensity of bands) will also be performed to quantity the separated components.

Evaluation of anti-oxidant and anti-diabetic potential

Estimation of antioxidant capacity (DPPH method)

The in vitro measurement of the antioxidant activity was conducted using the DPPH technique developed by Tariq et al. (2015). The experiment involved mixing 1.6ml of 0.135mM DPPH dissolved in 100% v/v methanol with 0.4ml of different concentrations. The reaction mixture was vigorously mixed and then placed in a lightless environment at the ambient temperature for a duration of 30 minutes. The mixture's absorbance was measured at a wavelength of 517nm after a duration of 2 minutes. Rutin, obtained from Sigma-Aldrich, with a purity of at least 94% and of high-performance liquid chromatography (HPLC) quality, was employed as a reference medication at the same doses as the plant extracts.

Formula:

DPPH scavenging capacity (%) = [(Absorbance(C)−Absorbance (S)/ (Absorbance (C)] × 100

Oxygen Radical Absorbance Capacity (ORAC) Assay

Preparation of Reagent

Phosphate buffer (pH 7.0).

AAPH: To make a working solution of 240 mM, 10 ml of buffer was added to 648.8 mg of AAPH. This was kept on ice until it was time to use it for tests. 8 hours of stability.

Trolox (MW 250.3): Astock solution (20mM) was prepared by dissolving 50mg Trolox in 10ml buffer (aliquot and store in dark at –80oC, stable for up to 4 months). Use calibration curve (6.25, 12.5, 25 and 50 µM) in each set of assay.

Fluorescein (MW 332.31): To prepare stock of 4.2 mM dissolve 13.9 mg in 10 ml buffer (aliquot and store in dark at –80oC, stable for up to 2 months). Working solution 0.08 µM: Take 1 µl of stock and makeup to 50 ml with buffer (Prepare a fresh dilution, keep in dark in an ice bath before the assay).

Preparation of Test sample (Extract)

Weigh 20mg of extract in 2 ml Eppendorf tube and add 2 ml of ice-cold 50% acetone. Sonicate for 15 minutes. Centrifuge samples (4.5g for 30 min at 4ºC) and collect the supernatant in fresh 2-ml tubes. Use dilutions of samples (20,40,80,100,200,400).

Test Protocol

The microplates were placed in a microplate incubator at 37°C for 10 minutes without shaking. Choose the brand of the plate that goes in the instrument. Every 90 seconds after incubation, fluorescence measurements (Ex. 485 nm, Em. 520 nm) were made to find the background signal. After three cycles, a multi-channel pipette was used to introduce 25 µl (240 mM) of AAPH by hand. You need to do this as soon as feasible because the ROS generator starts working right away after you install it. The test started again, and fluorescence readings were taken for up to 90 minutes (Nishimura *et al.* 2011).

Alpha amylase inhibitory assay

The alpha-amylase assay was conducted following the procedure outlined by Odeyemi (Ratnaningtyas *et al.* 2022). In summary, 15 microliters of the different plant extracts at various doses dissolved in a phosphate buffer, were combined with 5 microliters of porcine pancreatic enzyme solution in a 96-well plate. The reaction was begun by adding 20µl of starch solution after 10 minutes of incubation at 37°C. It was then incubated for an additional 30 minutes at thesame temperature. The reactionwas halted by introducing 10µl of 1M HCl to each well, followed by the addition of 75µl of iodine reagent. Each test sample did not include an enzyme control or a starch control. The absorbance was conducted at a wavelength of 580nm, and the calculation of the percentage inhibitory activity was performed using the subsequent equation:

Inhibition (%) = 100 − % reaction (min)

Where, % reaction = Maltose in sample (mean)× 100
Maltose in control (mean)

RESULTS AND DISCUSSION

Percentage yield

The percentage yield of dried leaves of *Azadirachta indica*, *Ocimum sanctum*, flowers of *Clitoria ternatea* and leaves of *Hibiscus rosa sinensis* were found as 47.26%, 52.64%, 49.34% and 54.12%, respectively when extracted using hydro-alcoholic solvent.

Standardization of herbal extracts

Table 1. Organoleptic characteristics

Hydro-alcoholic Leaves Extract	**Organoleptic characteristics** **Appearance** **Odor**		**Color**
Azadirachta indica	Powder	Dark green	Characteristics
Ocimum sanctum	Powder	Dark green	Characteristics
Clitoria ternatea	Powder	Dark green	Characteristics
Hibiscus rosa sinensis	Powder	Dark green	Characteristics

Physicochemical evaluations

Table 2. Physicochemical evaluations

Parameters	**Hydro-alcoholic Leaves Extract** *A. indica* *O. sanctum* *C. ternatea* *H. rosa sinensis*

Loss on drying (%)	8.4	9.2	8.1	8.7
Total ash (%)	9.6	4.9	4.3	5.3
Acid insoluble ash (%)	0.42	0.36	0.32	0.39
Water soluble ash (%)	0.79	0.68	0.63	0.74
Alcohol soluble extractive value (%)	23.53	20.34	18.21	22.75
Water soluble extractive value (%)	17.45	15.28	14.16	16.31
Sulphated ash value (%)	3.27	2.14	2.06	2.63
pH value	5.8	6.4	6.2	6.5

Preliminary phytochemical investigation

Table 3. Phytochemical investigation of polyherbal extracts

Phytochemical	**Hydro-alcoholic Leaves Extract**			
	A. indica	*O. sanctum*	*C. ternatea*	*H. rosa sinensis*
Alkaloids	++	++	+	+
Phlobatannins	-	++	++	++
Tannins	++	++	++	+
Glycosides	+	+	+	++
Saponins	+	++	++	+
Terpenoids	++	++	++	+
Steroids	+	++	+	+
Flavonoids	++	+	++	+
Phenols	+	+	++	+
Carbohydrate	++	++	+	++
Proteins	++	++	+	+

++: Abundance, +: Moderate; -: Absent

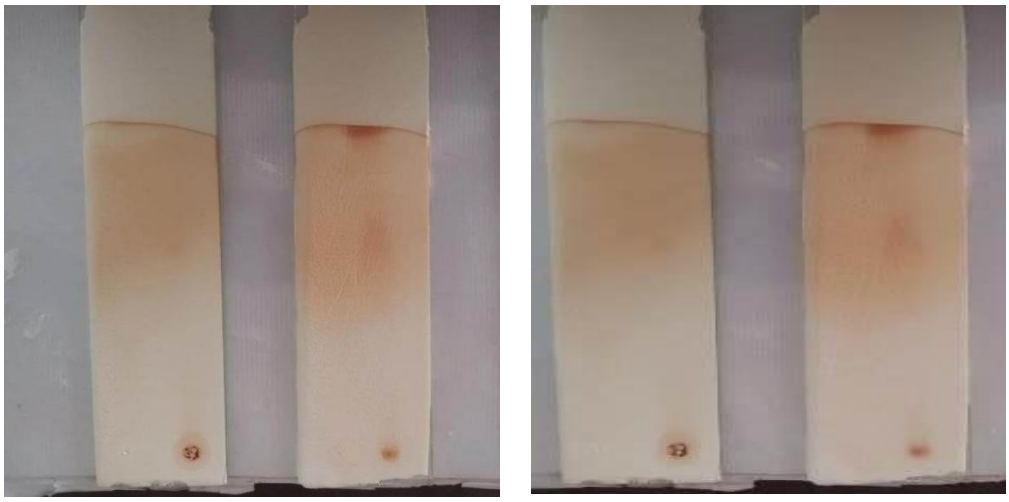
Chromatographical analysis

TLC analysis of herbal leaves extract

In *A. indica* leaves extract, the Rf values were observed as 0.72, 0.76 and 0.69 in chloroform: ethyl acetate: acetone (70:15:15), chloroform: ethyl acetate: acetone (60:20:20), and Chloroform: ethyl acetate: acetone (70:20:10) fractions, respectively. The *O. sanctum* leaves extract showed the highest Rf value (0.86) in Chloroform: ethyl acetate: acetone (60:20:20) fraction. In *H. rosa* sinensis, chloroform: ethyl acetate: acetone (60:20:20) fractions exhibited the Rf value as 0.74. Therefore, it can be said that among all 3 fractions, chloroform: ethyl acetate: acetone (60:20:20) was found more efficient in isolation of plant constituents.

4. TLC analysis of hydroalcoholic leaves extract of *A. indica*, *O. sanctu* *rosa* sinensis

Herbal extract	Solvent system	R_f Value
A. indica	Chloroform: ethyl acetate: acetone (70:15:15)	0.72
	Chloroform: ethyl acetate: acetone (60:20:20)	0.76
	Chloroform: ethyl acetate: acetone (70:20:10)	0.69
O. sanctum	Chloroform: ethyl acetate: acetone (70:15:15)	0.83
	Chloroform: ethyl acetate: acetone (60:20:20)	0.86
	Chloroform: ethyl acetate: acetone (70:20:10)	0.81
C. ternatea	Chloroform: ethyl acetate: acetone (70:15:15)	0.73
	Chloroform: ethyl acetate: acetone (60:20:20)	0.82
	Chloroform: ethyl acetate: acetone (70:20:10)	0.66
H. rosa sinensis	Chloroform: ethyl acetate: acetone (70:15:15)	0.68
	Chloroform: ethyl acetate: acetone (60:20:20)	0.74
	Chloroform: ethyl acetate: acetone (70:20:10)	0.71



a) b) c) d)

Fig 1. TLC Plates: a) *A. indica,* b) *O. sanctum,* c) *C. ternatea,* d) *H. rosa sinensis*

HPTLC Analysis

Table 5. HPTLC analysis of herbal extract

Herbal extract	Solvent system	R_f Value	Expected compounds
A. indica	Chloroform: ethyl acetate: acetone (70:15:15)	0.72	Phenol, Flavonoids & Tannins
	Chloroform: ethyl acetate: acetone (60:20:20)	0.76	
O. sanctum	Chloroform: ethyl acetate: acetone (70:15:15)	0.83	Phenol, Flavonoids & Terpenoids
	Chloroform: ethyl acetate: acetone (60:20:20)	0.86	
C. ternatea	Chloroform: ethyl acetate: acetone (70:15:15)	0.73	Phenol, Flavonoids & Terpenoids
	Chloroform: ethyl acetate: acetone (60:20:20)	0.82	
H. rosa sinensis	Chloroform: ethyl acetate: acetone (70:15:15)	0.71	Phenol, Flavonoids & Steroids
	Chloroform: ethyl acetate: acetone (60:20:20)	0.74	

Pharmacological evaluation

Estimation of antioxidant capacity (DPPH method)

Table 6. Estimation of antioxidant capacity of polyherbal formulations

PHF	DPPH Scavenging capacity (%)
DM-01	58.36
DM-02	66.21
DM-03	71.40
DM-04	52.29

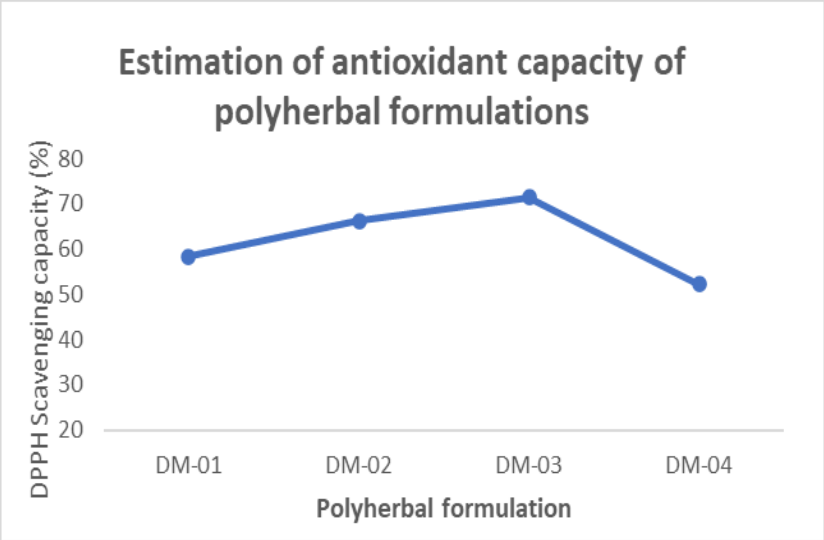


Fig 2. Estimation of antioxidant capacity of polyherbal formulations

Oxygen Radical Absorbance Capacity Assay

Table 7. ORAC assay of DM-01, DM-02, DM-03, DM-04

Conc. (µg/ml)	Area Under Curve (AUC)				
	DM-01	DM-02	DM-03	DM-04	Trolox (control)
25	1.3	1.2	1.4	1.1	1.9
50	1.7	1.8	1.9	1.6	2.5
100	2.4	2.3	2.5	2.2	3.1
150	2.5	2.4	2.7	2.6	3.2
200	2.8	2.9	3	2.9	3.4

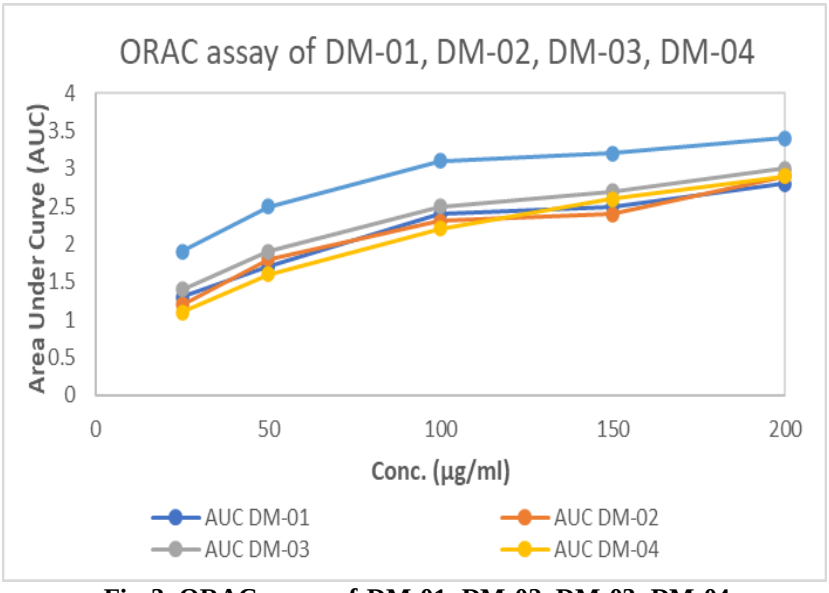


Fig 3. ORAC assay of DM-01, DM-02, DM-03, DM-04

Alpha amylase inhibitory assay

Table 8. Estimation of % α-amylase inhibition of polyherbal formulations

Conc. (µg/ml)	% α-Amylase inhibition				
	DM-01	**DM-02**	**DM-03**	**DM-04**	**Acarbose**
50	16.34±0.27	24.23±0.41	29.12±0.17	21.26±0.23	53.27±0.69
100	23.12±0.35	34.29±0.26	38.23±0.56	27.34±0.16	64.32±0.56
200	29.23±0.56	42.54±0.35	49.74±0.23	38.46±0.25	73.29±0.37
400	38.35±0.23	53.16±0.34	61.30±0.12	46.24±0.45	85.16±0.46
600	47.19±0.25	62.30±0.54	69.57±0.23	56.17±0.56	92.34±0.54
800	56.54±0.21	71.27±0.67	78.13±0.46	64.34±0.17	95.56±0.23
1000	69.34±0.10	83.15±0.56	89.39±0.67	75.20±0.45	97.62±0.29

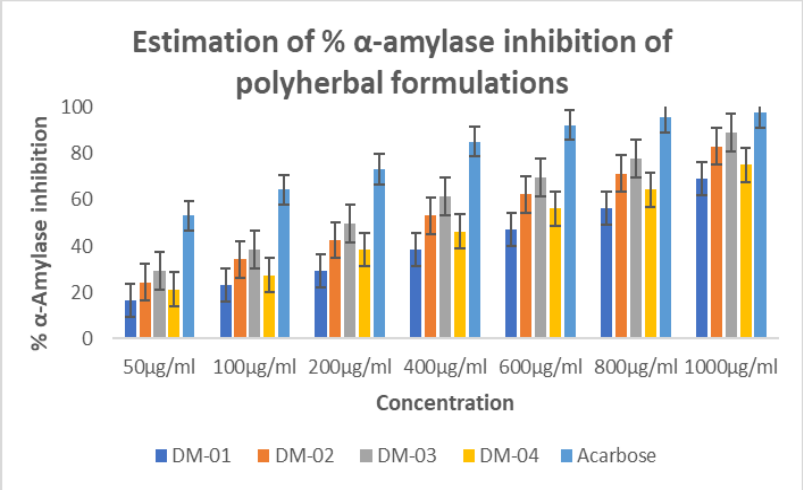


Fig 4. Estimation of % α-amylase inhibition of polyherbal formulations

A study has found that *Artemisia afra* has hypoglycaemic effect in diabetic rabbits. This activity is attributed to the presence of Saponins, which may stimulate the production of insulin via repairing pancreatic beta cells (Alexandru *et al.* 2007). Additionally, some of the bioactive components in this study may enhance the activity of glycolytic and glyconeogenic enzymes either synergistically or independently (Wolde *et al.* 2016). Acommonly recognized fact is that the decrease in postprandial hyperglycaemia can be accomplished by impeding the action of intestinal α-glucosidase and pancreatic α-amylase. exploration for novel medicines derived from natural resources, particularly medicinal plants, is an appealing strategy for addressing postprandial hyperglycaemia. The ethyl acetate solvent fraction exhibited the highest level of inhibition (54.23%), whereas the aqueous fraction demonstrated the lowest level of effectiveness (26.18%). The ethyl acetate extract is highly likely to contain semi-polar compounds that exhibit α amylase inhibiting activity (Aloulou *et al.* 2012).

It is advisable to do further investigationandisolate thepure active compounds. These are prominent polyphenolic chemicals known for their ability to block α-amylase. The phytochemical examination of the extracts indicated a high concentration of polyphenolic compounds, indicating that the bioactive substance responsible forinhibiting α-amylase may be present in all plant extracts. The *H. abyssinica* extract exhibitedantioxidant activity that was dependingon the dosage.

Furthermore, it was observed that these extracts did not cause any weight loss in diabetic mice. Dried leaves of

Azadirachta indica, *Ocimum sanctum*, flowers of *Clitoria ternatea* and leaves of *Hibiscus rosa sinensis* demonstrated a significant antioxidantand anti-diabeticpotential in all the parameters studied. These herbal extracts were taken in different proportionate to prepare the polyherbal formulations which named as DM-01, DM-02, DM-03 and DM-04. Additional research is required to determine the specific mechanism by which this plant exhibits its antidiabetic properties.

CONCLUSION

A variety of phytochemicals i.e., phenolics, saponins and flavonoids etc. were identified which are responsible for these biological properties. The antioxidant and anti-diabetic potentials were found in decreasing order as DM-03> DM-02> DM-01> DM-04. Thus, among all the polyherbal formulations, DM-03 exhibited the highest pharmacological properties including 71.40% (DPPH antioxidant activity), at 200µg/ml, 3% (Oxygen Radical Absorbance Capacity Assay), at 1000µg/ml, 89.39±0.67% α-amylase inhibition. There polyherbal formulations may serve as a promising natural reservoir of antioxidant and hypoglycaemic potential suitable for application in the domains of nutrition and pharmaceuticals. Nevertheless, additional assessment of their bioactive components and antioxidant properties in living models is necessary.

In conclusion, the antioxidant and antidiabetic response of the dried leaves of *Azadirachta indica*, *Ocimum sanctum*, flowers of *Clitoria ternatea* and leaves of *Hibiscus rosa sinensis* may be attributed to lower and sustain the cellular oxidation processes and its ability to enhance the responsiveness of tyrosine kinase to insulin. In order to confirm the MOA, it requires molecular investigations to determine the specific receptor subtypes these polyherbal formulations (DM-01, DM-02, DM-03, DM-04) target and to explore methods for enhancing its binding efficiency..

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