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PHARMACOLOGICAL SCREENING & EVALUATION OF LEAF OF *TRIUMFETTA RHOMBOIDEA* & LEAF OF *CHLOROXYLON SWIETENIA* FOR ANTIDIABETIC ACTIVITY"

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ABSTRACT

Diabetes mellitus is a combination of heterogeneous disorders commonly presenting with episodes of hyperglycaemia and glucose intolerance, as a result of lack of insulin, defective insulin action, or both. Such complications arise due to derangements in the regulatory systems for storage and mobilization of metabolic fuels, including the catabolism and anabolism of carbohydrates, lipids and proteins emanating from defective insulin secretion, insulin action, or both. Leaf of *Triumfetta Rhomboidea* and leaf of *Chloroxylon Swietenia* is widely used as an herbal complementary & alternative herbal oregano-medicine. Various activities of *Triumfetta rhomboidei and Chloroxylon Swietenia* as an herbal extract have reported in pre-clinical studies.

KEYWORDS: Diabetes mellitus, Insulin, metabolic, secretion.

INTRODUCTION

Diabetes mellitus is a combination of heterogeneous disorders commonly presenting with episodes of hyperglycaemia and glucose intolerance, as a result of lack of insulin defective insulin action, or both (Sicree *et al.*, 2006). Such complications arise due to derangements in the regulatory systems for storage and mobilization of metabolic fuels, including the catabolism and anabolism of carbohydrates, lipids and proteins emanating from defective insulin secretion, insulin action, or both.[1] Classification of diabetes mellitus is based on its etiology and clinical presentation. As such, there are four types or classes of diabetes mellitus viz; type 1 diabetes, type 2 diabetes, gestational diabetes, and other specific types.[2] Type 1 diabetes is said to account for only a minority of the total burden of diabetes in a population although it is the major type of the diabetes in younger age groups at majority of well-to-do countries. The incidence of type 1 diabetes is increasing in both rich and poor countries. Furthermore, a shift towards type 1 diabetes occurring in children at earlier ages is imminent.[3]

Symptoms of Type 1 Diabetes

1. Frequent urination
2. Unusual thirst
3. Extreme hunger
4. Unusual weight loss
5. Extreme fatigue and Irritability

There is a reason why diabetes is termed the silent killer. It is important to bear in mind that these symptoms may be mistaken for an ailment in themselves or for some other disease. The best method to diagnose this condition is to have a blood test taken. And if you have already noticed this symptom, you should see a doctor at the earliest.

Symptoms of Type 2 Diabetes

1. Excessive Urination and Thirst
2. Increased Hunger
3. Unexplained Weight Gain
4. Irritability and Fatigue
5. Blurred Vision
6. Warning Signs of Diabetes

a) Decelerated Healing
b) Skin and Yeast Infections plus Frequent Gum and Bladder

Treatment of Diabetes mellitus

The major components of the treatment of diabetes are:

A) Drug treatment for diabetes
B) Non drug treatment for diabetes

The basic aim of the study is Pharmacological Screening & Evaluation of Leaf of *Triumfetta Rhomboidea* & Leaf of *Chloroxylon Swietenia* for Antidiabetic Activity.

MATERIAL AND METHODS

Plant collection and Authentication

The plant *Triumfetta rhomboidea* & *Chloroxylon swietenia* was collected from local area of Bhopal (M.P.). The botanical identity was authenticated by expert and specimen was submitted.

Determination of Total Ash value

Accurately weighed 2 grams of each selected powdered drug in a silica crucible, previously ignited and weighed. Then powder was incinerated by gradually increasing the heat to temperatures not exceeding 450 0C for 4 hours, until free from carbon in muffle furnace. Crucible was cooled and weighed.

Determination of water-soluble Ash value

The ash was boiled for 10 min. with 25 ml of water for 5 minutes. It was filtered through an ashless filter paper, residue was washed twice with hot water. Filter paper with residue was placed together into the crucible and heated gently until vapours ceases to be evolved. Then it was cooled in a desiccator and weighed. The percentage of water soluble ash was calculated with reference to the air- dried drug using following formula.

Determination of acid insoluble ash value

The ash was boiled for 10 min. with 25 ml of 2 M dil. HCl for 5 minutes. It was filtered through an „ashless" filter paper, residue was washed twice with hot water. Filter paper with residue was placed together into the crucible and heated gently until vapours ceases to be evolved. Then it was cooled in a desiccator and weighed.

The percentage of acid insoluble ash was calculated with reference to the air- dried drug using following formula.

Extractive values[4]

Determination of water-soluble extractive value

5 gm of each selected powdered drug was macerated with 100 ml of distilled water in a stoppered flask and kept for 24 hrs with intermittent shaking. Rapidly filtered the macerate through filter paper. 25 ml of water extract was evaporated to dryness in a tarred dish at 105^0 C and weighed it. Then the percentage of alcohol soluble extractive value with reference to the air-dried drug was calculated.

Determination of alcohol soluble extractive value

5 gm of each selected powdered drug was macerated with 100 ml of alcohol (90% v/v) in a stoppered flask and kept for 24 hrs with intermittent shaking. Rapidly filtered the macerate through filter paper, taking necessary precaution to avoid excess loss of alcohol. 25 ml of alcoholic extract was evaporated to dryness in a tarred dish at 105^0 C and weighed it. Then the percentage of alcohol soluble extractive value with reference to the air-dried drug was calculated.

Determination of Loss on drying

Accurately weighed quantity (1.5 g) of powdered drug was taken in a porcelain china dish. The sample was kept in the oven at a temperature 105°C for 2 hrs. Then it was cooled in a desiccator to room temperature, the procedure was repeated till constant weight is observed.

Extraction procedure[4]

Following procedure was adopted for the preparation of methanol extracts from the shade dried and powdered herb

Extraction by maceration process[5]

Dried powdered *Triumfetta rhomboidea* & *Chloroxylon swietenia* has been extracted with methanol solvent using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40^0C.

Quantitative Estimation of Bioactive compounds

Total phenolic content estimation[6]

Principle: The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method.

Preparation of Standard: 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50µg/ml was prepared in methanol.

Preparation of Extract: 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol.

Procedure: 2 ml of methanolic extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Total flavonoids content estimation[6]

Principle: Determination of total flavonoids content was based on aluminium chloride method

Preparation of standard: 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol.

Preparation of extract: 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of flavonoids.

Procedure: 1 ml of 2% $AlCl_3$ solution was added to 3 ml of methanolic extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

***In vitro* Antioxidant Activity (DPPH Method)**

It is a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecules do not dimerise. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color.

Procedure

DPPH scavenging activity was measured by the spectrophotometer. Stock solution (1.5 mg/ml in methanol) was prepared such that 75 µl of it in 3 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10- 100 µg/ml) was noted after 15 minutes. 75 µl of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 75 µl of DPPH and 50 µl of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally the mean was taken. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm. for the estimation of enzyme inhibition.

RESULT AND DISCUSSION

Collection of Plant Material the Selected plants powder was collected from Local Area of Bhopal in the month of December.

Table No 01: Collection of Plant material.

S. No	Name of plant	Part of plant	Source
1.	*Triumfetta Rhomboidea*	Leaf	Bhopal
2	*Chloroxylon Swietenia*	Leaf	Bhopal

Authentication of Plant Material

The plant material was then authenticated by expert and the certificate with **Reference no.: NU/Nip/2023/524 was provided.**

Table No 02: Authentication of Plant Material.

S. No	Name of plant	Authenticated by	Reference no.
1.	*Triumfetta Rhomboidea*	Pharmacognosist	**NU/Nip/2023/524**
2	*Chloroxylon Swietenia*	Pharmacognosist	**NU/Nip/2023/524**

Table No. 03: Physical Parameters of Selected Plant.
Results of physiochemical properties *of selected drugs*

S. No	Parameters	*Triumfetta Rhomboidea* Value % w/w	*Chloroxylon Swietenia* Value % w/w
1.	Moisture content	2.87	2.99
2.	Total Ash value	4.42	5.39
3.	Acid Insoluble Ash value	1.57	1.92
4	Water insoluble Ash	2.50	2.21
5	Determination of foreign matter	**Nil**	Nil

Extractive values

Extractive value determinations tell us the amount of phytoconstituents which is present in the medicinal plant. Under a given sent of conditions theses values varies within a narrow limit and hence can be set as an in-house standard for routinely used drugs. These values can also tell us about the adulteration of crude drug with already exhausted drug as it will yield low extractive values. The results of the extractive values are shown. The results of Extractive value shown in Table 04.

Table No. 04: Extractive value of Selected Plants.

S. No	Plant	Water soluble extractive value (Value % w/w)	Alcohol soluble extractive value (Value % w/w)
1.	*Triumfetta Rhomboidea*	6.83	**6.84**
2	*Chloroxylon Swietenia*	**18.4**	**8.16**

Extraction

The selection of solvent depends on the fact that how much it dissolved the required Phyto-constituents. Plant materials were extracted by maceration Process with methanol as a solvent which shown in Table 07.

Table No. 05: Extraction Process of Selected Plants.

S. No	Plant Name	Part used	Solvent used	Procedure
1.	*Triumfetta Rhomboidea*	Leaves	Methanol	Maceration
2	*Chloroxylon Swietena*	Leaves	Methanol	Maceration

Phytochemical Evaluation

Plants are known to contain various primary metabolites like sugar, fats which are used by animals and humans. They also contain many secondary metabolites which show certain physiological effects. Qualitative phytochemical test were perform on all two selected plants which shown presence of various metabolites. Performed phytochemical tests were proving that all two selected plants contain flavonoids, Alkaloids and Steroids which enhance Anti Diabetics activity.

Table No. 06: Qualitative Phytochemical Test.

S. No	Test	*Triumfetta Rhomboidea*	*Chloroxylon Swietena*
1	**Steroids**	+	+
a	salkowaski Test	+	+
2	**Glycosides**	+	+
a	Bontrager	+	+
b	kellar killiani	+	+
c	Legals	+	+
3	**Saponin**	+	-
a	Foam test	+	-
b	Haemolysis Test	+	-
4	**Cabohydrates**	+	+
a	Molisch Test	+	+
b	Barfirds Test	+	+
c	Fehling Test	+	+
5	**Alkaloids**	-	-
a	Mayer Test	-	-
b	Wahners Test	-	-
c	Dragondroff Test	-	-
d	Haggers Test	-	-
6	**Flavonoids**	+	+
a	Shinoda Test	+	+
b	Lead acetate Test	+	+
c	Pew's Test	+	+
d	NAOH Test	+	+
7	**Tannins**	+	+
a	Ferric choloride	+	+
b	Gelatin Test	+	+
8	**Protein**	-	-
a	Precipitation test	-	-
b	Xanthoproteic	-	-
9	**Amino acid**	-	-
a	Ninhydrine	-	-

Determination of Percentage Yield

Yield of Extraction: The crude extracts so obtained after the maceration extraction process, extract was further concentrated on water bath evaporation the solvents completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from different samples using Pet ether and methanol as solvent is depicted in the table 07.

Table No 07: % Yield of Selected Plants.

S. No.	Solvent	*Triumfetta Rhomboidea* % Yield (w/w)	*Chloroxylon Swietena* % Yield (w/w)
1	Methanol	3.4%	12.86%

Estimation of Total Phenolic and Flavonoids Contents

Total Phenolic Content Estimation (TPC)

The Total phenolic content (TPC) was expressed as mg/100mg of Gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: **Y = 0.011X+0.011, R^2= 0.998,** where X is the Gallic acid equivalent (GAE) and Y is the absorbance.

Table No. 08: Preparation of calibration curve of Gallic acid.

S. No	Concentration	Absorbance
1	0	0
2	10	0.135
3	20	0.247
4	30	0.364
5	40	0.474
6	50	0.581

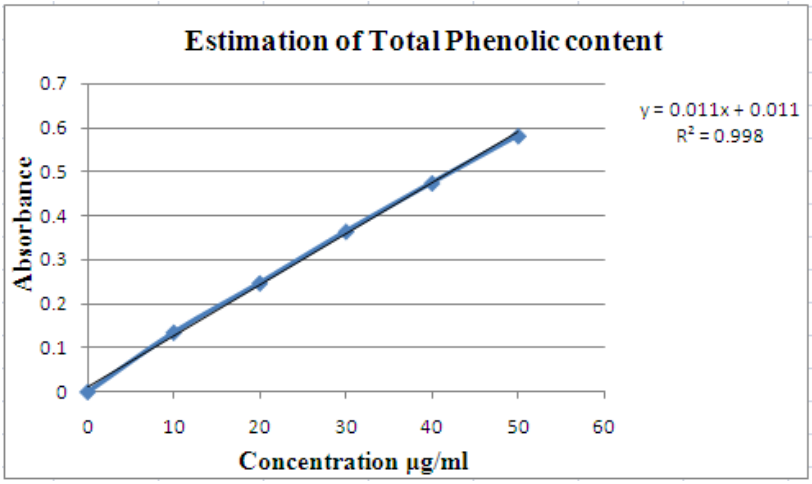

Figure 01: Graph of Estimation of Total Phenolic Content.

Total Flavonoids Content Estimation (TFC)

Total flavonoids content was calculated as Quercetin equivalent (mg/g) using the equation based on the calibration curve: **Y=0.040X + 0.009, R^2=0.999**, where X is the Quercetin equivalent (QE) and Y is the absorbance.

Table No. 09: Preparation of calibration curve of Quercetin.

S. No	Concentration	Absorbance
1	0	0
2	5	0.216
3	10	0.425
4	15	0.625
5	20	0.815
6	25	1.021

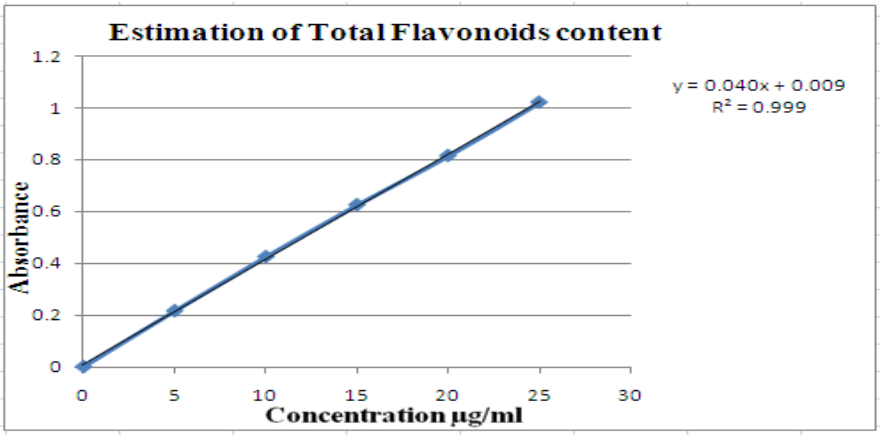

Figure 02: Graph of Estimation of Total Flavonoids Cont.

Estimation of Total Phenolic and Total Flavonoids Content

Table No. 10: Total Phenolic and Total Flavonoids Content

S. No	Extract	Total Phenolic content (mg/100mg of dried extract)	Total Flavonoids content (mg/ 100 mg of dried extract)
1	*Triumfetta Rhomboidea*	7.81	1.94
2	*Chloroxylon Swietena*	21.3	15.1

Antioxidant Activity of *Triumfetta Rhomboidea* using DPPH Method

Table No. 11: % Inhibition of Methanolic extract of *Triumfetta Rhomboidea* and Ascorbic Acid using DPPH method.

S. No.	Concentration	% Inhibition	
		Ascorbic acid	*Triumfetta Rhomboidea*
1	20	46.58	34.56
2	40	58.98	38.98
3	60	68.87	43.35
4	80	82.25	57.45
5	100	89.98	63.25
IC 50		24.89	66.59

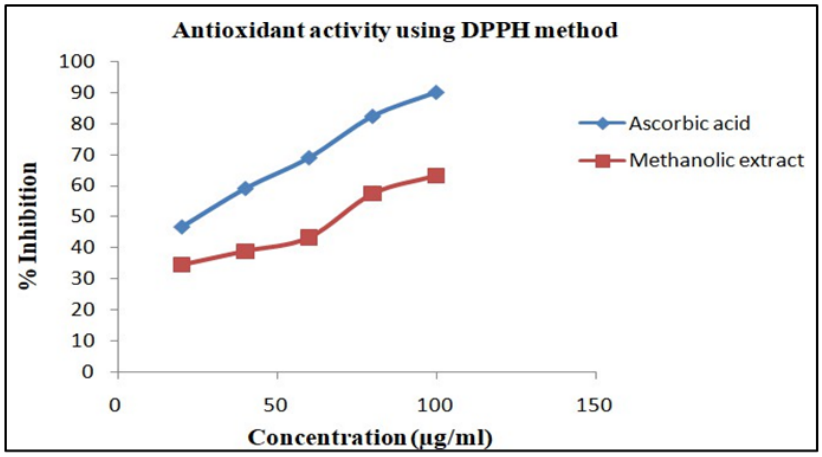

Figure 03: Graph of Antioxidant Activity using DPPH method.

Antioxidant Activity of *Chloroxylon Swietena* using DPPH method

DPPH radical scavenging abilities of *Chloroxylon Swietena* Methanolic extract were recorded in fig 04, high reduction of DPPH is related to the high scavenging activity performed by sample. Methanolic extract of *Chloroxylon Swietena* have strong DPPH scavenging activity.

Table No. 12=2: % Inhibition of Methanolic extract of *Chloroxylon Swietena* and Ascorbic Acid using DPPH method.

S. No.	Concentration	% Inhibition	
		Ascorbic acid	*Chloroxylon Swietena*
1	20	40.00	20.00
2	40	61 .98	28.98
3	60	78.87	45.35
4	80	82.25	67.45
5	100	89.98	73.25
IC 50		27.03	43.05

In vitro* Anti Diabetic Studies of *Triumfetta Rhomboidea

Table No. 13: Results of *In vitro* Anti Diabetic Studies *Triumfetta.*

S. No	Acarbose		Plant Extract	
	Conc.	% Inhibition	Conc.	% Inhibition
1	10	35.45	10	55.58
2	20	45.58	20	62.56
3	30	50.25	40	68.78

4	40	65.45	60	70.15
5	50	72.25	80	78.89
IC_{50} (µg/ml)			**IC_{50}**	92.25

In vitro* Anti Diabetic Studies of *Chloroxylon Swietena

S. No	**Acarbose**		**Plant Extract**	
	Conc.	**% Inhibition**	**Conc.**	**% Inhibition**
	Conc.	**% Inhibition**	**Conc.**	**% Inhibition**
1	10	35.34	10	54.58
2	20	45.34	20	60.56
3	30	50.10	40	67.78
4	40	65.24	60	69.15
IC_{50} (µg/ml) 52.54			50	71.25

CONCLUSION

A plant is the basic source of knowledge of modern medicine. The relatively lower incidence of adverse reactions to plant preparations, compared to modern conventional pharmaceuticals, coupled with their reduced cost is encouraging both the consuming public and national health care institutions to consider plant medicines as alternative to synthetic drugs.

Today herbal drugs are prescribed widely even when their biologically active compounds are unknown because of their effectiveness and minimal side effect in clinical experience large numbers of plants belonging to different families have been studied for their therapeutic properties. The investigation aimed to scientifically explore the important medicinal uses of study plant. Today there is an increase in research interest to identify new medications from plant parts. These criteria promoted us to formulate a new phytomedicine from the study plant with strict scientific protocol *Triumfetta rhomboidea* (Jacq.) belongs to Tiliaceae have many medicinal properties.

The results of the present study instigate that the Ethanolic extract of *Triumfetta rhomboidei* & ***Chloroxylon Swietena*** of α-amylase showed maximum antidiabetic activity. Hence the extract may be useful as better therapeutic agent especially for the treatment of diabetes mellitus.

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