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RESEARCH ARTICLE

Comparative studies on in vitro anti-diabetic activities of saponin and flavonoid extracts of *Jatropha gossipifolia*

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ABSTRACT

Diabetes mellitus is the most important disease involving the endocrine pancreas. It is a medical disorder characterized by varying or persistent hyperglycaemia with or without glycosuria. Diabetes is also a leading factor in causing heart disease, stroke and kidney damages. The recommendation of WHO about the diabetes has led to a rise in investigations of medicinal plants for hypoglycaemic agents. The main purpose of this research was to compare the in vitro anti-diabetic activity of saponin and flavonoid extracts of *Jatropha gossipifolia*. The quantitative phytochemical analysis of *J. gossipifolia* aqueous extract was determined using spectrophotometric method. The alpha-glycosidase inhibitory activity of *J. gossipifolia* flavonoid and saponin extracts was also deduced using standard in vitro inhibition assay and metformin was used as a positive control. The quantitative phytochemical analysis recorded highest (276.15±134.57 mg/100 g) and lowest (43.56 ± 0.02 mg/100 g) concentrations in flavonoid and saponin respectively. In in vitro anti-diabetic assay, maximum enzyme inhibition (93.07±0.92%) was recorded in flavonoid extract at highest concentration (100 ug/mL) with IC_{50} of 11.83 ± 0.14 µg/mL which was found to be more active than the standard drug (metformin). However, there was no significant difference ($p>0.05$) in the % inhibition at 60 ug/mL between the extracts and the standard (metformin). This study suggests that, the presence of bioactive compounds could be responsible for the extract to exhibit the dose-dependent action by increasing the inhibitory effects of alpha-glycosidase. Therefore, *J. gossipifolia* flavonoid and saponin extracts could be used as alternative herbal drug in reducing the level of glucose in diabetic patients.

Keywords: *Jatropha gossipifolia*, anti-diabetic, alpha-glucosidase, Metformin

1. INTRODUCTION

Diabetes mellitus is the most important disease involving the endocrine pancreas. The word 'diabetes' is derived from the Greek word 'diabainein' which means 'to pass through' and the word 'mellitus' means 'honey sweet'. It is characterized by an excess of sugar in the blood and urine, hunger, thirst and gradual loss of weight [1]. Diabetes mellitus is a medical disorder characterized by varying or persistent

hyperglycemia with or without glycosuria [2]. Diabetes is one of the leading factor in causing heart disease, stroke, kidney damage as well as nerve complications, pregnancy complications and birth defects in children born to diabetic mothers [3]. The number of people suffering from diabetes worldwide is increasing at an alarming rate with a projected 366 million people likely to be affected by the year 2030 as against 191 million estimated in the year 2000 [4]. India leads the world with largest number of diabetic subjects earning the dubious distinction of being termed the "diabetes capital of the world" with 41 million Indians having diabetes. Every fifth diabetic in the world is an Indian [5].

The World Health Organization Expert Committee on diabetes has recommended that traditional medicinal herbs be further investigated for hypoglycaemic agents [6]. There are numerous plants which have the ability to reduce the glucose production, stimulate the utilization of glucose and combat with secondary complications. Out of an estimated 250,000 plants, less than 1% have been pharmacologically evaluated and only a fraction of these for diabetes. The most commonly used drugs of contemporary medicine such as aspirin, anti-malarial, anticancer, digitalis etc. originated from plant sources. Therefore, it is practical to look for options in herbal medicine for diabetes. On the basis of this report which lists the impending effectiveness of medicinal plant against diabetes, it can be believed that phytochemicals can play a vital role in the management of diabetes [7]. *Jatropha gossypifolia* L. is an erect, branched shrub, about 1.8 meters high, native of Brazil but now widespread throughout the tropical world. It has naturalized in many parts of India and listed as a weed. The plant is traditionally used in leprosy, diarrhoea, dysentery, anaemia, vertigo, malaria, toothache, as antidote for snake bite, antibiotic, insecticidal, blood purifier, purgative, stimulant, stomachic, febrifuge, emetic, emmenagogue, to treat wound, sores, boils, carbuncles, eczema, itches, ulcers, to reduce pain and to stop bleeding from skin and nose [8]. Decoction prepared from roots of *Jatropha gossypifolia* L. is used in the treatment of diabetes [9]. Various parts of the plant have been shown to possess anti-cancer, antihypertensive, anti-malarial, antimicrobial, anti-oxidant, anti-tubercular, anti-viral, haemostatic and molluscicidal activity [10]. This study will emphasize on comparative studies on in vitro anti-diabetic activities of saponin and flavonoid extracts of *Jastropha gossipifolia*.

2. METHOD AND MATERIAL

2.1. Sample preparation

The *Jatropha gossipifolia* leaves were washed and dried at room temperature for 8 days and after drying it was grinded into powder using electric blender. The grounded powder was stored in refrigerator before extraction process [11].

2.2. Preparation of aqueous extract

Twenty gram (20 g) of powdered *Jatropha gossipifolia* sample was added to 200 ml of distilled water at room temperature. The mixture was filtered after 24 hours using cheese

cloth and whatman filter paper (no 1). The filtrate was then concentrated using freeze-dryer [12].

2.3. Extraction of crude saponin

Crude saponin from *Jatropha gossipifolia* was extracted by heating 20 g of powdered sample for 4 hours at 55 °C with 100 ml of 20% ethanol. The extract was filtered and residue was re-extracted with 200 ml of 20% ethanol. The extract was concentrated on water bath till the volume reduced to 40 ml, which was mixed with 20 ml diethyl ether in a separating funnel. The mixture was vigorously shacked and then the separating funnel was fixed in a stand till the development of aqueous and diethyl layer. Aqueous portion was collected while the diethyl ether portion was discarded. To the aqueous layer, 60 ml of n-butanol was added and properly mixed by vigorous shaking. The n-butanol extract was treated with 10 ml of 5% NaCl solution. The resultant solution was concentrated on a water bath and the cured saponins were dried in an oven [13].

2.4. Extraction of crude flavonoid

Powdered (40 g) *Jatropha gossipifolia* was mixed with 200 ml of twice 80 % ethanol and 1 ml of concentrated HCl was added in ratio 1:80:20 and left in cold maceration for 72 hours followed by filtration with muslin cloth and the mixture was then concentrated to about 40 ml. 40 ml of the extract and 20 ml of n-hexane was mixed in a separation funnel, the n-hexane fraction was collected after few minutes and it was discarded followed by addition of 60 ml of ethyl acetate into the separation funnel. After 20 minutes, the ethyl acetate layer was collected and this process was repeated three times. However, the ethyl acetate and extract layers were collected and concentrated in water bath at 35 °C [14].

2.5. Quantitative phytochemical analysis of J. gossipifolia aqueous extract

2.5.1. Determination of total phenol

About 2 g of the sample were defatted with 100ml of diethyl other using a soxhlet apparatus for 2 hr. The fat free sample was boiled with 50 ml of petroleum ether for the extraction of the phenolic component for 15 min. 5 ml of the extract was pipette into a 50 ml flask, then 10ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5ml of concentrated amylalcohol were also added. The samples were made up to mark and left to react for 30min for colour development and was measured at 505 nm. Tannic acid was used to establish the calibration curve [15].

2.5.2. Determination of total flavonoid

Total flavonoid was determined using aluminum chloride colorimetric method [16]. Quercetin was used to establish the calibration curve. Exactly 0.5 ml of the diluted sample was added into test tube containing 1.5 ml of methanol. 0.1 ml of 10% AlCl3 solution and 0.1ml sodium acetate ($NaCH_3COO^-$) were added, followed by 2.8 ml of distilled water. After incubation at room temperature for 30min, the absorbance of the reaction mixture was measured at 415 nm. The amount of 10% $AlCl_3$ was substituted by the same amount of distilled water in blank [16].

2.5.3. Determination of total alkaloids

About 0.5 g of the sample was dissolved in 96% ethanol 20% H_2SO_4 (1:1). 1 ml of the filtrate was added to 5 ml of 60% tetraoxosulphate (VI), and allowed to stand for 5 min. Then; 5 ml of 0.5% formaldehyde was added and allowed to stand for 3 h. The reading was taken at absorbance of 565 nm. The extinction coefficient (E_{296}, ethanol {ETOH} = 15136 $M^{-1}cm^{-1}$) of vincristine was used as reference alkaloid [17].

2.5.4. Determination of saponins

About 0.5 g of the sample was added to 20 ml of 1NHCl and was boiled for 4 h. After cooling it was filtered and 50 ml of petroleum ether was added to the filtrate for ether layer and evaporated to dryness. 5 ml of acetone ethanol was added to the residue. 0.4 mls of each was taken into 3 different test tubes. 6 ml of ferrous sulphate reagent was added into them followed by 2 ml of concentrated H_2SO_4. It was thoroughly mixed after 10 min and the absorbance was taken at 490 nm. Standard saponin was used to establish the calibration curve [17].

2.5.5. Determination of tannins

About 0.2 g of sample was measured into a 50 ml beaker. 20 ml of 50% methanol was added and covered with para film and placed in a water bath at 77-80^{o}C for 1hr. it was shaken thoroughly to ensure a uniform mixture. The extract was quantitatively filtered using a double layered whatman No. 41 filter paper into a 100 ml volumetric flask, 20ml water added, 2.5ml Folin-Denis reagent and 10ml of Na_2CO_3 were added and mixed properly. The mixture was made up to mark with water mixed well and allowed to stand for 20 min for the development of a bluish-green color. The absorbance of the tannic acid standard solutions as well as samples was read after color development on a UV-spectrophotometer model 752 at a wavelength of 760 nm [18].

2.5.6. Quantitative test for terpenoids

Dried plant extract 100 mg was taken and soaked in 9 mL of ethanol for 24 hour. The extract after filtration, was extracted with 10 mL of petroleum ether using separating funnel. The ether extract was separated in pre-weighed glass vials and waited for its complete drying (W_f) and ether was evaporated. The yield (%) of total terpenoids contents was measured by the equation 1

$$Wi - \frac{wf}{wi} * 100 \qquad \text{...eq. 1}$$

2.6. Inhibition of alpha-glycosidase enzyme

Inhibitory activity of Alpha-Glycosidase was determined by incubating a solution of starch substrate (2 % w/v maltose or sucrose), 1 ml with 0.2 M Tris buffer and various volume (0.2, 0.4, 0.6, 0.8 and 1.0 ml) of plant extract for 5 minutes at 37 °C. The reaction initiated by adding 1 ml of alpha-glycosidase enzyme (IU/ml) to it followed by incubation for 40 minutes at 35 °C. However, the reaction was terminated by addition of 2 ml of 6N HCl and the intensity of the color was measured at 540 nm [19-20].

Moreover, calculation of 50% inhibitory concentration (IC_{50}) of the plant extract required to scavenge 50% of the radicals was done using the percentage scavenging activities

at five different levels of the extract. Percentage inhibtion (I %) was calculated by equation 2

$$I\% = \frac{Ac-As}{Ac} * 100 \quad \text{...eq. 2}$$

Where:
Ac= Absorbance of the control
As= Absorbance of the sample

3. RESULTS AND DISCUSSION

3.1. Percentage yield of J. gossipifolia extracts

The results depict the percentage yield of *J. gossipifolia* (table 1). 0.4 g of extract was gotten for flavonoid extraction of *J. gossipifolia* and 0.6 g for saponin extraction. So this represents 1 % and 3 % of extract yield respectively.

The aqueous extract of *J. gossipifolia* was 0.325% from 40 g of powdered *J. gossipifolia*. However, flavonoid extract yield was 0.4 g which is 1 % while saponins extract was 0.6 g which is 3%. The above method of extractions of crude saponin and flavonoid was described by Komolafe *et al*., (2021) who reported that the percentage yield of flavonoid and saponin to be 2.5% and 1.2% respectively [21]. The variation in yield might be due to difference in *J. gossipifolia* specie, climatic factor and differences in planting.

3.2. Quantitative phytochemical analysis of extract

The results below shows the quantitative phytochemical analysis of *J. gossipifolia* aqueous extract (table 2). The result showed the highest percentage (29.03%) in flavonoid with concentration of 276.15±134.57 mg/100 g. Therefore, there was significant difference ($p<0.05$) in the parameter screened with exception in tannins and steroids.

The result showed highest concentration in flavonoid (276.15±134.57^{c} mg/100g) with 29.03% while lowest concentration was recorded in steroid (43.56±0.02^{a} mg/100 g) with 4.58%. There was no significant difference ($p<0.05$) in saponin and phenol concentrations. Also, terpenoids and steroids showed no significant difference ($p>0.05$) between the two. N'Djetchi *et al*., (2017) reported highest concentrations in flavonoid with 250.09±0.03 mg/100 g concentration in *A. sativum* [22]. However, this is related to the work of Komolafe *et al*., (2021) who reported the presence of saponin in *A. indica* and absence of phenol in the sample [21]. The study elucidated the use of phytochemicals like phenols, flavonoids and saponins to reduce the oxidative damages by decreasing the free radicals produced [23]. The difference in the concentration might be due to infection (pest and disease) to the plant during cropping time, due to lack of fresh water in cropping and probably mode of planting.

3.3. In vitro anti-diabetic activities of flavonoid and saponin extracts

The results show the in vitro anti-diabetic activities of flavonoid and saponin extracts (table 3). The results showed highest percentage inhibition in flavonoid extract at 100 ug/ml. And the IC_{50} recorded was 11.83±0.14 ug/ml in flavonoid extract. The result showed IC_{50} of 17.18±0.00^{b}, 11.83±0.14^{c}, and 41.40±0.01^{a} in metformin (standard), flavonoid and saponin with lowest % inhibition

Table 1. Yield extracts of *J. gossipifolia* in percentage (%)

Plant Extracts	Powdered *J. gossipifolia* (g)	Extract yield (%)
Aqueous	40.00 (0.13)	0.32
Flavonoid	40.00 (0.40)	1.00
Saponin	20.00 (0.60)	3.00

Table 2. Quantitative phytochemical analysis of *J. gossipifolia* aqueous extract

Phytochemicals	Results (mg/100 g)	Percentage (%)
Flavonoids	276.15 ± 134.57^{c}	29.03
Alkaloids	235.42 ± 0.11^{bc}	24.75
Saponins	83.78 ± 0.21^{ab}	8.81
Tannins	155.53 ± 0.02^{abc}	16.35
Terpenoids	57.03 ± 0.01^{a}	5.99
Phenols	99.57 ± 0.01^{ab}	10.48
Steroids	43.56 ± 0.02^{a}	4.58

Values are Mean ± Standard Error Mean of determination of three replicates. Values with different superscript on same column are $p<0.05$ (significantly different)

Table 3. *In vitro* Anti-diabetic activities of flavonoid and saponin extracts

Concentrations (ug/ml)	Metformin	% Inhibition Flavonoids	Saponins
20	25.68 ± 2.91^{b}	16.86 ± 1.63^{a}	33.43 ± 0.54^{c}
40	45.90 ± 10.71^{ab}	33.35 ± 6.98^{a}	62.88 ± 0.48^{b}
60	60.82 ± 8.09^{a}	46.79 ± 0.16^{a}	62.09 ± 2.35^{a}
80	72.65 ± 3.65^{a}	87.05 ± 1.87^{b}	66.06 ± 0.53^{a}
100	83.43 ± 0.31^{b}	93.07 ± 0.92^{c}	72.61 ± 0.38^{a}
IC_{50}	17.18 ± 0.01^{b}	11.83 ± 0.14^{c}	41.40 ± 0.01^{a}

Values are Mean ± Standard Error Mean of determination of three replicates. Values with different superscript on same row are $p<0.05$ (significantly different)

recorded in flavonoid extract (16.86 ± 1.63^{a} %). Among the two extracts (flavonoid and saponin), there was a better anti-diabetic activities in flavonoid than the saponin because the lower the IC_{50} the better the activities. This work is related to the work of Harneet *et al.*, (2013) who reported anti-hyperglycaemic effects of alloxan-induced *in vivo* diabetic mice [24]. The more pronounced effects of insulin in diabetic patient may be due to the partial or compromised effect of insulin in diabetic condition and conversely a greater and more direct role of the hypoglycaemic principle present in the extract. Several hypoglycaemic

principle have been reported which effect hypoglycaemic [26].

Furthermore, Hammad *et al*., (2016) reported, *J. gossipifolia* plant showed alpha-glycosidase activity with a better and highest % inhibition at 200 ug/ml [27]. Several synthetic glycosidase inhibitors are used as treatment for diabetes but their prices are high and side effects occurs [28]. Further studies may be undertaken in order to elucidate the probable mechanism of action and to isolate bioactive phytoconstituents from the plant.

4. CONCLUSION

In the light of these findings, it can be concluded that the saponin and flavonoid extract screened showed inhibitory activities against the enzyme alpha-glycosidase and both can be considered as anti-diabetic drug and for further studies in the management of diabetes and ulcer as natural remedy.

It is recommended that further works on the isolation of various active constituents on different plants through bioassay-directed fractionation is encouraged to ascertain the most potent anti-diabetic agents among the various medicinal plants.

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6. CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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