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Evaluation of Anti-hyperglycemic Potential of an Ethno Medicinal Plant *Grewia bracteata* B. Heyne ex Roth

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Abstract

Grewia bracteata is an ethno medicinal shrub used by many traditional users for various illnesses. The phytochemicals present in *Grewia bracteata* ethyl acetate, ethanol and chloroform solvent extracts are screened by standard methods and its anti-hyperglycemic activity by enzymatic methods at various concentrations (59.37, 118.75, 237.5, 475, 950 ug/ml). The phytochemical analysis exhibited the various secondary metabolites like alkaloids, flavonoids, saponins, terpenoids, tannins, steroids and glycosides are present in *Grewia bracteata*. Both the enzymatic assays for anti-hyperglycemic assay exhibits a significant inhibitory effect at highest concentration of 950 ug/ml in all the solvents used. The ethanol extract exhibited the strongest activity in both the assays α-amylase and α-glucosidase with a percentage inhibition of 85.2±0.27 and 55.46±0.01 (IC50, 116.24±1.16 and 400.03±1.09 µg/ml) respectively followed by ethyl acetate and chloroform In Conclusion, this study has revealed that *Grewia bracteata* leaves possess noticeable in vitro α-amylase and α-glucosidase inhibitory activities due to the presence of its phytochemicals.

***Key words*:** Acarbose, Anti-hyperglycemic activity, *Grewia bracteata*, Organic solvents, Phytochemicals

Plants are integral part of human civilization, which have one or more of its organs containing substances that can be used for the therapeutic purpose. The evolution of human civilization has been greatly influenced by medicinal plants [4]. Many contemporary medications are made directly from plants, which are also the basis of many ancient remedies [6]. The effects of medicinal plants have drawn more attention within the last ten years. Conventional herbal remedies originating from many ecosystems and regions may offer a fresh approach to wound care and infection prevention [5]. In recent times, focus on plant research has increased all over the world and a large body of evidence was collected to show immense potential of medicinal plants used in various traditional systems [7]. Because, the herbal preparations are complementary and alternative medicine (CAM) and the search for the discovery of novel compounds derived from natural sources like herbs or plants is growing mainly due to acquired resistance, side effects, and adverse events (AE) of allopathic medication [1], [15]. Diabetic mellitus (DM) is the condition arising by insulin deficiency, often combined with insulin resistance. It is because of the disruption of a metabolic system of carbohydrates, proteins and fats resulting in complications of the kidneys, eyes and cardiovascular system [13-14]. World Health Organization has also reported that about 171 million people worldwide were suffering from diabetes mellitus in the year 2000, while predicting that this figure will double come the year 2030. This report was corroborated by Wild et al. Diabetic type 2 (non-insulin-dependent diabetes mellitus) is most dominant; almost 90% of all diabetic cases belong to this type in the world [8]. The hypoglycemic effect of several plant extracts and herbal formulations have been confirmed which are being used as antidiabetic remedies and their therapeutic capabilities are investigated intensively. The utilization of traditional herbal medicine to treat diabetes has been accepted by the people who live in the rural area or in the urban/close to modern medical center. The use of medicinal plants reported to be cost effective anti-diabetic agents with fewer reported side effects. Especially, the price is lower for patients who cannot afford to buy medicine at higher price [2]. Medicinal plants have always been an exemplary source of drugs and which are particularly used by the traditional users since the ancient time but they do not have much scientific data [10]. Hence considering the mentioned points, the present study was therefore undertaken to investigate the antidiabetic activity of the ethnomedicinal plant *Grewia bracteata.*

MATERIALS AND METHODS

Grewia bracteata is Wight's Gross berry, a shrub and which is found in dry deciduous forests in Andhra Pradesh, Karnataka, Tamil Nadu, Kerala and Sri Lanka. The plant sample was collected and identified by Rapinat Herbarium, Center for molecular systematics, St. Joseph's College, Tiruchirappalli.

Extraction of plant material

The plant material was thoroughly washed and well dried in shade. Then the dried plant material was ground to the fine

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powder, which is used to make extraction by using soxhlet apparatus. 20 g leaf powder has soaked separately in 250 mL of solvents such as ethyl acetate, ethanol, and chloroform for 24 h. Next to that, the extract of ethyl acetate, ethanol, and chloroform was concentrated in a rotary vacuum evaporator (Evator) under reduced pressure at 40 °C until solid residues were obtained. Then the dried extracts were collected and stored at 20 °C use for in further studies.

Table 1 Phytochemicals present in *Grewia bracteata* leaf extracts

Phyto constituents	Chemical tests	Ethyl acetate	Ethanol	Chloroform
Carbohydrates	***Molisch's test*** (2-3ml extract + Naphthol + Sulphuric acid → Violet colour ring)	+	+	+
Proteins	***Million's test*** (2ml extract + Million's reagent → Red colour precipitation formed)	+	+	+
Amino acids	***Ninhydrin test*** (Filterate + lead acetate solution + Spotted + ninhydrin spray → violet spots appeared)	+	+	+
Steroids	***Salkowski test*** (Extract, chloroform and con. H_2SO_4 (2 ml of each) → shaking for 5 minutes → stand for 5 minutes → Red colour layer formed)	+	+	-
Alkaloids	***Mayer's test*** (Extract + Mayer's reagent → coloured creamy precipitate formed)	+	+	+
Phenolic compounds	***Lead acetate test*** (Extract + few drops lead acetate solution → Formation of white precipitate)	+	+	-
Saponins	***Foam test*** (2ml extract + diluted dist. H_2O 20ml equivalent + stirred for 20 minutes → formation of foam layer)	-	-	-
Glycosides	***Keller-kilani test*** (2ml extract + 1ml glacial acetic acid + 1 drop 5% ferric chloride + 1ml Conc. HCl → Reddish brown colour appeared)	-	-	-
Tannins	***Gelatin test*** (extract 2ml + gelatin solution + shake well→ white precipitate formed)	+	-	+

Preliminary phytochemical analysis

The phytocompounds present in the yield of crude extracts were examined by using various biochemicals by standard techniques [16] (Table 1). Three solvent extracts *viz.* ethyl acetate ethanol and chloroform extracts of *G.bracteata* were used for the anti-hyperglycemic studies.

α-amylase inhibitory activity

The α-amylase with the *Grewia bracteata* leaf extracts at various concentrations (59. 37- 950 µg/ mL) was premixed 0.5 % starch (substrate) was added to start the reaction. At 37 °C the reaction was incubated for 5 min and terminated by the addition of 2mL of DNS (3, 5- dinitrosalicylic acid) reagent and incubated. The reaction mixture was heated at 100 °C for 15 min and diluted with 10 ml of distilled water in an ice bath [18]. By measuring the spectrum at 540nm the α-amylase inhibitory activity was measured and the following formula was used for calculating the α-amylase inhibitory activity:

$$\% \text{ Inhibition} = [(\text{Abs Control} — \text{Abs Samples}) / \text{Abs Control}] \times 100$$

α-glucosidase inhibitory activity

The *Grewia bracteata* leaf extracts at various concentrations (50-200 µg/ mL) was premixed with α-glucosidase. As a substrate 3mM p-nitrophrnyl glucopyranoside was added (pNPG) to start the reaction [18]. Incubated the reaction mixture for 30 min at 37 °C and stop by added 2 mL of Na_2CO_3. P-nitrophenol released from pNPG was measured at 400 nm for determined the α-glucosidase activity and the inhibitory activity was calculated by the following formula:

$$\% \text{ Inhibition} = [(\text{Abs Control} — \text{Abs Samples}) / \text{Abs Control}] \times 100$$

RESULTS AND DISCUSSION

Preliminary phytochemical analysis

Phytochemicals are non-nutritive plant chemicals with protective or disease-prevention properties. Such compounds are found in plants to protect themselves, but current research suggests that they can also defend humans and animals from many diseases and infections [11]. The inhibitory activity of plant extract may vary according to the nature of active ingredients in the plant. Successful prediction of bioactive components from plant materials is largely dependent on the type of solvents used in the extraction procedure [9]. The phytochemicals present in *Grewia bracteata* leaf extracts (Ethyl acetate, ethanol and chloroform) were determined by various tests. The results revealed that the presence of carbohydrates, proteins, alkaloids, flavonoids, tannins, terpenoids, phenolic compounds and glycosides were present (Table 1).

Anti-diabetic activity

In vitro anti-diabetic activity of *G. bracteata* leaf extracts were assessed by the enzymatic method viz. α-amylase and α-glucosidase assays by the Xavier *et al*. [18] protocol.

α - Amylase inhibition activity

In vitro assay of α- Amylase inhibition activity was performed to determine the anti-diabetic activity of the *G. bracteata* (Fig 1). Here, α-amylase enzyme converts starch into glucose. When α-amylase, glucose and plant extract are taken together as a solution, the extract causes inhibition of enzyme activity [17]. The present study reveals that the inhibitory effect of α-amylase on *G. bracteata* ethanol extract showed the highest percentage of inhibition (85.2±0.27) at 950 ug/ml concentration followed by ethyl acetate extract (82.92±0.15) at same concentration (Table 2). The IC_{50} values of ethanol, ethyl acetate and chloroform are 116.24±1.16, 244.02±0.57 and

309.84±0.67 µg/ml. which is defined as the sample concentration required to triggering 50% inhibition in which the higher inhibition was noted as lower IC_{50} value (Table 4). While (Fig 1) showed *Grewia bracteata* extracts exhibits comparatively similar level of inhibitory effect with standard Acarbose. Whereas, Kumar *et al.* [12] showed that the best inhibition activity of *Solanum torvum* and *Solanum trilobatum* obtained using the water extract of these plants. When, the concentration increased the percentage of inhibition of α-amylase is also increased.

Table 2 α-amylase activity of *Grewia bracteata* in percentage

Concentration (ug/ml)	Ethanol	Ethyl acetate	Chloroform	Acarbose
59.37	39.17±0.27	36.25±0.41	30.95±0.27	44.47±0.41
118.75	52.69±0.41	44.38±0.27	38.08±0.27	51.59±0.15
237.5	60.82±0.27	57.07±0.15	49.4±0.15	57.80±0.27
475	69.22±0.15	63.92±0.31	66.11±0.31	70.95±0.27
950	85.2±0.27	82.92±0.15	81.55±0.15	83.01±0.27

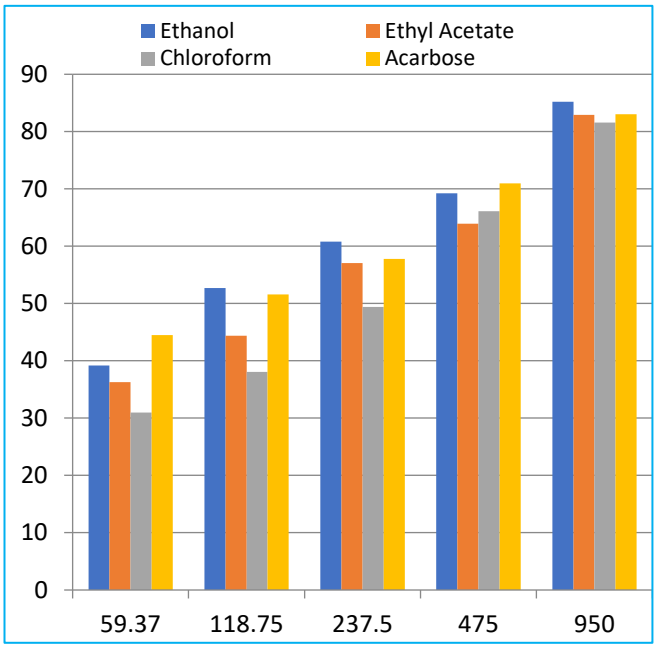

Fig 1 α-amylase activity of *Grewia bracteata* in percentage

α - Glycosidase activity

In vitro assay of α- glucosidase inhibition activity was performed to determine the anti-diabetic activity of the *Grewia bracteata.* The results of α - glucosidase inhibitory activity on ethyl acetate, ethanol and chloroform extract was mentioned with IC_{50} of 400.03±1.09, 455.49±1.38 and 495.72±1.67 µg/ml respectively (Table 3). Here, α - glucosidase inhibitory effect is lower than the standard Acarbose with IC_{50} of 88.90±1.42 µg/ml (Fig 3). Comparatively, Egharevba *et al.* (2019) reported the ability of *G. bracteolata* to inhibit α - glucosidase activity which is similar to the present findings. Likewise, the percentage of α - amylase activity of *G. bracteata* extracts were mentioned in (Table 3) [3]. The ethanol extract of the *G. bracteata* with the maximum percentage of inhibition (55.46±0.01) at 950µg/ml was shown in the (Fig 2), followed by chloroform and ethyl acetate at the same concentration (Table 2). Similarly, Selvi and Yogananth [15] documented their findings the concentration of the plant extract increases, the potential inhibitory action percentage also increased. When compared with both enzyme activities, α-amylase activity on an ethanol extract of *G. bracteata* leaf extract has the highest inhibitory activity and this is the first study for the evaluation of the enzymatic activity of *G. bracteata.*

Table 2 α - glucosidase activity of *Grewia bracteata* in percentage

Concentration (ug/ml)	Ethanol	Ethyl acetate	Chloroform	Acarbose
59.37	44.59±0.01	48.54±0.01	46.56±0.02	45.78±0.02
118.75	47.96±0.01	48.85±0.02	47.62±0.02	46.97±0.02
237.5	49.27±0.01	49.00±0.01	48.65±0.00	49.54±0.02
475	50.99±0.01	50.17±0.00	50.61±0.01	54.61±0.01
950	55.46±0.01	51.84±0.08	52.61±0.02	61.28±0.02

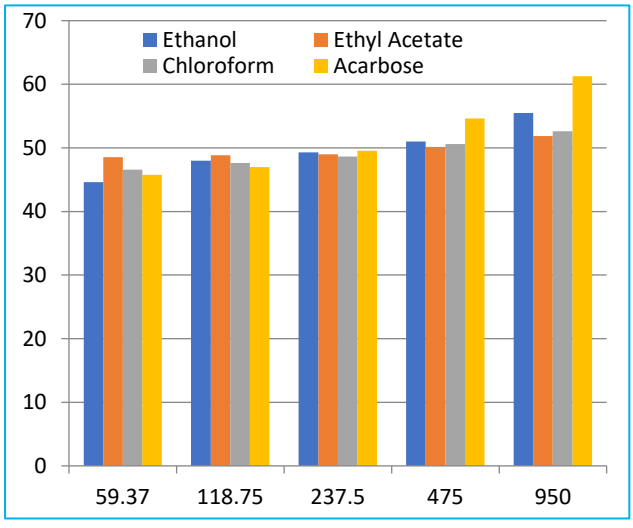

Fig 2 α - Glucosidase activity of *Grewia bracteata* in Percentage

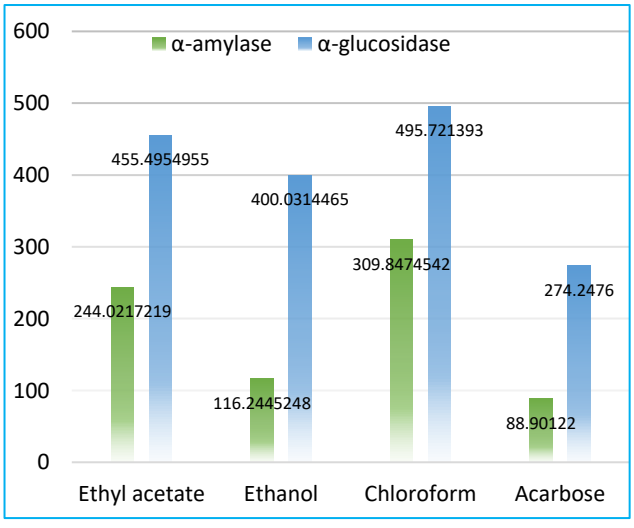

Fig 3 IC_{50} value of anti-diabetic activity of *Grewia bracteata*

Table 3 IC_{50} value of anti-diabetic activity of *Grewia bracteata*

Solvents used and control	IC_{50} Value (µg / ml)	
	α-amylase	α-glucosidase
Ethyl acetate	244.02±0.57	455.49±1.38
Ethanol	116.24±1.16	400.03±1.09
Chloroform	309.84±0.67	495.72±1.67
Acarbose	88.90±1.42	274.24±1.24

CONCLUSION

In conclusion with this study exhibited the highest polarity solvents like ethyl acetate, ethanol, and chloroform extracts of *Grewia bracteata* leaves about the preliminary phytochemicals, in vitro anti-diabetic activities. The result of the determination of phytochemicals revealed that the secondary metabolites like proteins, alkaloids, flavonoids, tannins, carbohydrates, amino acids, phenolic compounds were present in both ethanol and ethyl acetate extracts. Saponins and glycosides were absent in chloroform extract. In enzymatic assays the best inhibition was noted on the α-amylase enzyme in all the extracts used when compare with α-glucosidase enzyme. So, the *Grewia bracteata* leaf extracts has the potential of high antidiabetic property due to the presence of various phytocompounds.

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