International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 07-06-2021, Accepted: 22-06-2021, Published: 08-07-2021
Volume 6, Issue 4, 2021, Page No. 77-80

Efficacy of *Ruellia prostrata* leaves extract on antiinflammatory and anti-diabetic properties

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Abstract
Medicinal plants are important in the treatment of human diseases. Phytochemicals that possess anti-diabetic and anti-inflammatory activity, and modulate pathways involved in obesity and glucose metabolism. In folk medicine, a single plant may be used to treat various diseases owing to the presence of phytochemical varieties in plants. The qualitative analysis showed that the preliminary phytochemical analysis of *Ruellia prostrata* revealed presence of tannin, saponins, flavonoids, steroids, terpenoids, triterpenoids, anthroquinones and polyphenol while emodins, anthocyanins were absent in both extract. Alkaloids and glycosides were present only ethanol extract. Coumarins were present only aqueous extract. The *in vitro* anti-diabetic potential of plant extract was confirmed through α-amylase and α-glucosidase inhibition methods. Anti-inflammatory activity of *Ruellia prostrata* was also confirmed.

Keywords: *Ruellia prostrata*, phytochemicals, anti-diabetic activity, anti-inflammatory activity

Introduction
India is widely known as the botanical garden of the world since it is the largest producer of medicinal herbs (Shariff *et al.*, 2006) [1]. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Tiwari *et al.*, 2011) [2]. The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China, India and the Near east, but it is doubtless an art as old as mankind. Herbal medicine has been practiced worldwide and is now recognized by WHO as an essential building block for primary healthcare (Chopra *et al*., 1956) [3].

Diabetes mellitus is a metabolic disorder characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Different types of reported diabetes mellitus can be classified under following two categories: Type 1 is insulin dependent diabetes mellitus (IDDM), in which the body does not produce any insulin (Kirti *et al*., 2008) [4]. Type 2 is noninsulin-dependent diabetes mellitus (NIDDM), in which the body does not produce enough. Many research and investigation of oral anti-hyperglycemic agents of natural plant origin were used in traditional medicine have been studied and many of them have been found to possess the positive activity (Balaraman *et al*., 2010) [5].

Inflammation is a multifaceted response mediated by the activation of cells of immune system in response to the invasion by a foreign body. Inflammation can also accelerate cancer and chronic inflammation which is regarded as an essential factor for the progression of the neoplastic process (Ganeshpurkar *et al.*, 2012) [6]. For chronic diseases such as osteoarthritis and rheumatoid arthritis, lifelong dependency on anti-inflammatory drugs is necessary. The most widely used non-steroidal anti-inflammatory drugs (NSAID's) cause several side effects. Also it is well known that the incidence of diabetes mellitus is high all over the world, especially in Asia (Wiseman and Halliwell, 1996) [7].

Rullia prostrata is an indigenous medicinal plant, which present in moist shady places throughout India. It is widely distributed in Arica, Srilanka, Pakistan and throughout India (Akthar *et al.*, 1992) [8]. The plant is commonly known as bell weed (Anonymous, 1959) [9] and black weed (Palanisamy *et al*., 2012) [10]. The objective of this study was therefore to investigate the leaf extracts of the *Rullia prostrata* for anti-diabetic and anti-inflammatory activities as well as to determine the phytochemical contents.

Material and Methods
Plant materials
The whole plant of *Ruellia prostrata* were collected from Kathattipatti (Palaiyapatti North) Thanjavur, Tamil Nadu, India from a herb. The plant were identified and authenticated by Dr. S. John Britto, The Director, the Rapinat Herbarium and center for molecular systematics, St. Joseph's college Trichy-Tamil Nadu. India. A Voucher specimen (JJVS 001) has been deposited at the Rapinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil Nadu, India.

Preparation of extracts
The collected *Ruellia prostrata* leaves were washed several times with distilled water to remove the traces of impurities from the leaves. The leaves were dried at room temperature and coarsely powdered. The powder was extracted with ethanol and aqueous for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The *Ruellia prostrata* leaves extract (RPLE) was stored in refrigerator until used.

Phytochemicals screening

Chemical tests were carried out on the alcoholic extract using standard procedures to identify the preliminary phytochemical screening following the methodology of Sofowara (1993) [11], Trease and Evans (1989) [12] and Harborne (1973) [13].

***In vitro* Anti-diabetic and anti-inflammatory activity**

In vitro Anti-diabetic (Alpha-amylase and Alpha-glucosidase) activity was carried out by the method of Apostolidis (2007) [14]. Anti-inflammatory activity evaluated by protein denaturation (Egg albumin and Bovine serum albumin) method as described by Padmanabhan and Jangle (2012) [15].

Results and Discussion

Phytochemicals screening

The qualitative analysis showed that the preliminary phytochemical analysis of *Ruellia prostrata* revealed presence of tannin, saponins, flavonoids, steroids, terpenoids, triterpenoids, anthroquinones and polyphenol while emodins, anthocyanins were absent in both extract. Alkaloids and glycosides were present only ethanol extract. Coumarins were present only aqueous extract.

Adewole *et al.*, (2015) [16] screening of the leaf and flower showed the presence of flavonoid, tannin, saponin, alkaloid and glycoside. From the quantitative evaluation of the leaf and flower of this plant, this has really confirmed the local use in the treatment of eczema in human body when the liquid is being extracted and the plant is a reservoir of many novel compounds which can be of immense use to the pharmaceutical world.

***In vitro* anti-diabetic activity**

Medicinal plants are ties of most effective plants were in part explained by the ability of the phytoconstituents to increase glucose transport and metabolism in muscle and/ or to stimulate insulin secretion (Edwards *et al.*, 1987) [17]. These can be an important strategy in management of blood glucose. The *in-vitro* α-amylase and α-glucosidase inhibitory studies demonstrated that *Ruellia prostrata* well anti-diabetic activity. The percentage inhibition at 100, 200, 300, 400 and 500µg/ml concentration of crude plant extracts shown concentration dependent reduction in percentage inhibition.

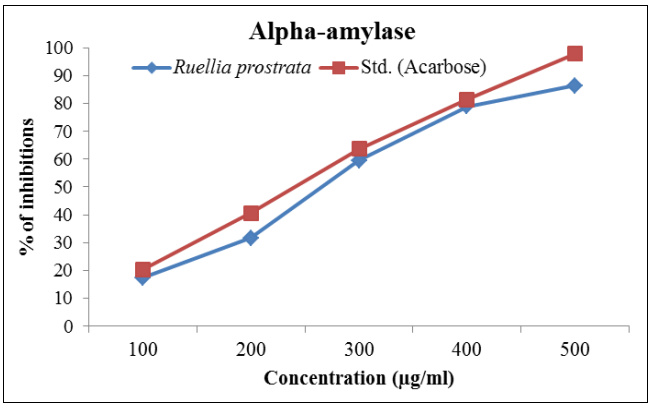

Fig 1: Alpha-amylase Anti-diabetic activity of *Ruellia prostrata* leaves extract

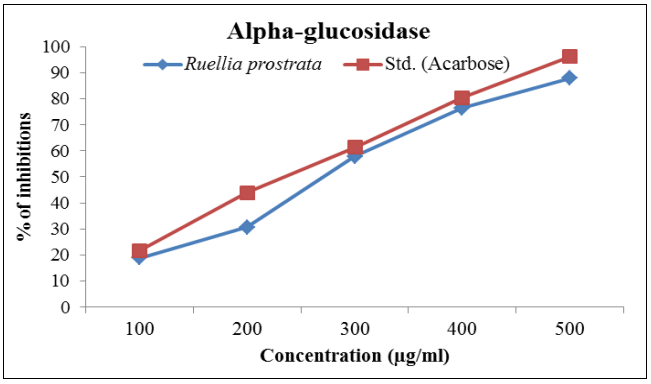

Fig 2: Alpha-glucosidase Anti-diabetic activity of *Ruellia prostrata* leaves extract

Inhibition of α-amylase assay was used to evaluate the in vitro anti-diabetic activity of the *Ruellia prostrata*. The activity was investigated through the inhibition of αamylase, an enzyme that made the digestion of starch and so reduced the glucose absorption. Similar results were obtained on the anti-diabetic activity *in vitro* studies on *Amaranthus caudatus* seeds (Filomena Conforti *et al.*, 2005) [18]. Acarbose is complex oligosaccharides that delay the digestion of carbohydrates. It inhibits the action of pancreatic amylase in breakdown of starch. The reaction mechanisms involved in inhibition of α-amylase enzyme by plant protein inhibitors (Narkhede *et al.*, 2011) [19].

***In vitro* anti-inflammatory activity**

Anti-inflammatory activity of extract of leaves of *Ruellia prostrata* by protein denaturation investigated. The extract at different concentrations was incubated with egg albumin and bovine albumin in controlled experimental conditions and subjected to determination of absorbance to assess the anti-inflammatory property. The effect of *Ruellia prostrata* (500µg/ml) was found to be close to diclofenac sodium. From the present study it can be concluded that *Ruellia prostrata* showed marked *in vitro* anti-inflammatory effect against the denaturation of protein.

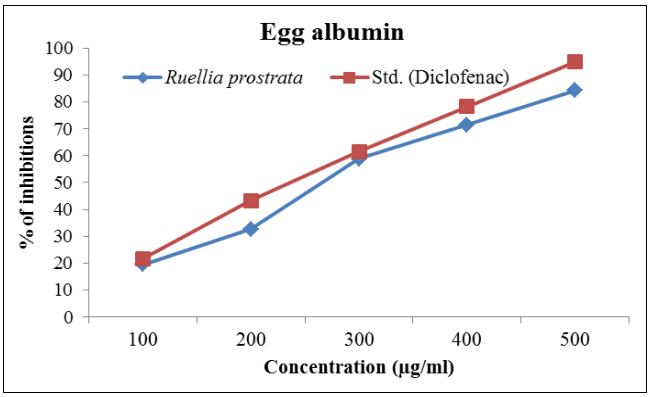

Fig 3: Egg albumin Anti-inflammatory activity of *Ruellia prostrata* leaves extract

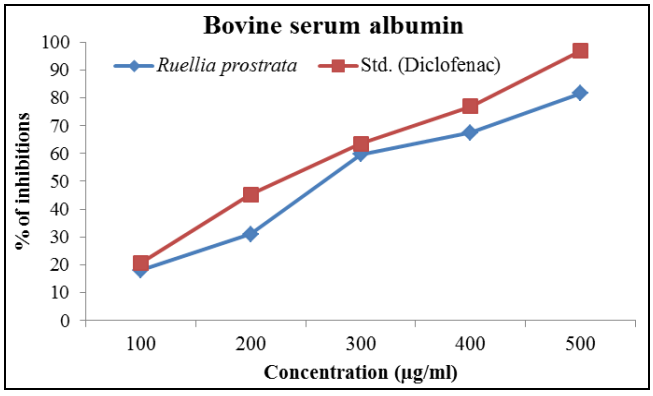

Fig 4: Bovine serum albumin Anti-inflammatory activity of *Ruellia prostrata* leaves extract

The increments in absorbance of *Cissus vitiginea* with respect to control indicated stabilization of protein denaturation by and reference diclofenac sodium (Jagtap *et al.,* 2011) [20]. Alhakmani *et al.* (2013) [21] reported that *Moringa oleifera* flower are rich in phenolic compounds and evauated it's *in vitro* anti-inflammatory activity. Reduction in protein denaturation is another method of showing the anti-inflammatory capacity. Protein denaturation is a pathological process by which the proteins lose their configuration and become functionless (Opie, 1962) [22\]. It was previously reported that leukocytes proteinase play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors (Das and Chatterjee, 1995) [23].

Conclusion

The present study reveals that *Ruellia prostrata* stem and leaves contains major phytochemicals including tannin, saponins, flavonoids, steroids, terpenoids, triterpenoids, anthroquinones and polyphenol which may contribute to the anti-diabetic and anti-inflammatory activities which occur in a dose-dependent manner. It may be concluded that the plant extracts can be with the constituents of biopharmaceutical importance.

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