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ORIGINAL ARTICLE

Phytochemical Investigation and *In vitro* anti-inflammatory activity of aqueous extract of *stevia rebaudiana* leaves

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ABSTRACT
Plant of Stevia genus are recognized source of the various phytochemicals with anti-inflammatory activity, commonly known as sweet leaf or sugar leaf, a plant traditionally used in replacement of sugar in most of the developed countries. However, there is little information related to activities of leves plant extracts. In the present investigation, the leaf extract screening for phytochemical charaterstics, thin layer chromatography and anti-inflammatory activity were performed and its concluded from the investigation, that Stevia rebudiana have the potential to act as as a wellspring of valuable medications and cure the numerous diseases because of the availability of various bioactive compound that apparent to have tremendous action against exhibit human pathogens. The leves plant material The fresh leaves of Stevia were taken beaker, add water, boiled it 30 min. After that filtered the solution and the filtrate were used to carried out the Phytochemical investigation, Thin layer chromatography of leaves extract was carried out by utilizing the solvent system Ethyl Acetate: Ethanol: Water (in 80: 20: 12 proportion). In vitro anti-inflammatory activity of the aqueous extract was evaluated by the protein denaturation method. The consequent of phytochemical screening demonstrated that Stevia rebudiana contains alkaloids, Flavonoids, Tannins, Saponins, glycosides Fat and steroid. Thin layer chromatography of leaves indicated the 7 spots in visual light, was the most antioxidant extract, The leaf extract exhibited the maximum protection of human red blood cells (HRBC) with the leaves extract is, 68.12±1.16% at 200µg/ml as compared with standard drug diclofenac sodium. The minimum protection 12.87±1.03% was observed at the 100µg/ml. These results showed that Stevia rebudiana leaves extract could be a highly effective and potential source of medication , also promising resources of the various phytoconstituents and anti-inflammatory agents.
KEYWORDS: *Stevia rebudiana, Phytochemical, Thin layer chromatography, anti-inflammatory activity*

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INTRODUCTION

Stevia, the nature's delightfull blessing has a place with the family Asteraceae. *Stevia rebudiana* Bertoni, is a normally sweet plant without calorie content, turns into a compelling choice to sugar particularly having a unique concern to diabetic individuals with hyperglycaemia and the eating routine perceptive [1, 2]. Currently, Stevia has been harvest in different countries including Brazil, Korea, Japan, Mexico, US, Indonesia, Tanzania and Canada [3]; for nourishment and pharmaceuticals preparations. *Stevia rebaudiana* is a little bush growing up to 65-80 cm tall, oppositely arranged leaves.

Stevia is a semi-humid subtropical plant that can be effectively developed like other any vegetable yield. The pH required for stevia plant ought to be 6.5-7.5; well-drained red soil and sandy loam soil. There is increment sought after for stevia plant in India for cultivation reason. The item also can be added to tea and coffee, cooked or warmed product, took care of sustenances, pickles, natural item squeezes, tobacco things, confectionary product, sticks and sticks, sugary treats, yogurts, prepared merchandise, gnawing gum and sherbets refreshments [4]. The leaves of Stevia are the wellspring of diterpene, glycosides, stevioside and rebaudioside [5].

Diterpene glycosides are the group of glycoside that have been extracted from Stevia. The leaves of Stevia plants contain 0.3% dulcoside, 0.6% rebaudioside C, 3.8%rebaudioside and 9.1% stevioside. From a large portion of the past examination, Stevia has been accounted for to have no any unfriendly impact on

people [6, 7] *Stevia rebaudiana* has as of late discovered use in the nourishment and pharmaceutical enterprises. Around 80% of the meds of the world rely upon plant-based bioactive segments for relieving different illnesses [8].

Phytochemical analysis have attracted the consideration of plant researchers because of the advancement of new and modern systems. These procedures assumed a noteworthy job in the quest for extra assets of crude material for pharmaceutical industry (phytochemicals) [9].

Inflammation is an ordinary defensive response to tissue harm brought about by physical damage and harmful chemical. The most normally utilized medications for the management of inflammatory conditions is the non-steroidal anti-inflammatory drugs (NSAIDs), which have different antagonistic impacts, particularly gastric irritation, prompting the development of gastric ulcers. The rich wealth of the plant kingdom shows the novel compound with important anti-inflammatory activities [10].

Stevia rebaudiana has been accounted for to have effectiveness against in different activity, hypotensive [11], heart tonic action [12], antidiabetic action [13], antioxidant activity [14], larvicidal, anti-inflammatory action, antimicrobial, antiobesity, anticancerous action [15].

Among therapeutic plants of the world, the biological and therapeutic action of the Stevia rebaudiana similarly less investigated against inflammatory activity. Hence during this study,phytochemical, thin layer chromatograpgy and anti-inflammatory properties were explore more significant.

MATERIAL AND METHODS

PREPRATION OF EXTRACT

The leaves of *Stevia rebaudiana* herbs were collected in March from Gajraula city and authenticated by Dr. Ashok Kumar Botanist, Department of Botany, IFTM University, Moradabad, U.P. A voucher specimen was preserved in the Department for future reference. The plant leaves was freshly collected was stored in an appropriate container at room temperature until required for use.

The fresh leaves of *Stevia* were taken in 200 ml beaker, add water 100 ml, boiled it for 30 min. After that filtered the solution and the filtrate were used to carried out the further research work.

Preliminary phytochemical studies

The aqueous extract was examined for the presence of various phyto-constituents like alkaloids, carbohydrates, tannins, steroids, flavonoids, phenolics, fats and glycosides by employing standard phytochemical tests.

In-vitro anti-inflammatory activity

In vitro protein denaturation was performed by the anti-inflammatory activity of aqueous extract of *Stevia rebaudiana* (leaf) was examined by the protein denaturation method. The reaction mixture (5ml) were consist from the 0.2 ml of egg albumin (from fresh hen's egg), 2.8ml phosphate buffered saline (pH: 6.4) and 2ml of altering concentration of plant extracts. Equal volume of double distilled water were served as control. Then the mixtures were incubated at 37±2 °C in an incubator for 15 minutes and then heated at 70 ºC for 5 minutes. After cooling, their absorbance was measured at 660nm by utilizing the vehicle as blank. Diclofenac at the final concentration of (1mg/ml) was used as reference drug and were treated similarly for the calculation of the absorbance [16].

$$\% \text{ of Inhibition} = 100 \text{ X } \{ \text{ Vt / Vc} - 1 \}$$

Where,

Vt = Mean absorbance of test sample.

Vc = Mean absorbance of control

RESULT

Aromatic and therapeutic plants are very significant wellsprings of the secondary metabolites, which have a variety of utiliztions in management of the mankind and plant maladys, pharmaceutical industry and cosmetics [17].

The recent study revealed the availabilty of various secondary metabolites such as tannins, saponins alkaloids, glycosides, flavonoids, and others from qualitative and quantitative phytochemical analysis of aqueous extract of *Stevia rebaudiana* leaves, which indicate that the leaves extract are the rich sources of bioactive compounds.

Preliminary phytochemical studies

During the current investigation, preliminary phytochemical study of the aqueous extract of *Stevia rebaudiana* leaves was carried out to identify the active constituents like alkaloids, glycosides, carbohydrate, protein, amino acids, flavonoids, glycosides, saponins, fat, tannins and phenolic compound. The entire major groups of phytochemicals constituents were found to be available in the sample as evidenced by the various chemical analyses as detailed out in Table 1.

Table 1. Phytochemical screening of the aqueous extract of *Stevia rebaudiana* leaves.

S. No.	Phytochemical parameter	Observation
1.	Alkaloid	+
2.	Carbohydrates	+
3.	Glycoside	++
4.	Protein	-
5.	Tannins and Phenolic compounds	++
6.	Flavonoide	+
7.	Steroid	+
8.	Amino acid	-
9.	Fats	+

TLC Analysis of the Leaf Extract

The Thin Layer Chromatographic analysis of the aqueous extract of *Stevia rebaudiana* leaves was carried out as explained. The chromatogram revealed 7 bands in visible light corresponding to various compounds present in the leaf extract. **Table 2** and **Figure 2**.

Table 2.TLC profile of aqueous leaves extracts.

No. of Spots	Solvent system	R_f value	Color of spot
7	(Ethyl Acetate: Ethanol: Water) (80: 20: 12)	0.26	Spot 1-Brownish red
		0.37	Spot 2- Brownish red
		0.42	Spot 3- Brownish red
		0.60	Spot 4 -Green
		0.75	Spot 5 -Greenish Brown
		0.82	Spot 6- Green
		0.91	Spot7- Green

Figure 1. TLC profile of aqueous extracts of *Stevia rebaudiana* leaves

Anti-inflammatory activity

In present investigation, absorbance of haemoglobin was determined in HRBC membrane stabilization method. The haemoglobin is released as a result of lyses of RBC membrane, due to less stabilization of membrane. The leaf extracts exhibited membrane stabilization activity by hypotonic induced lyses of erythrocyte membrane. The research is based on to evaluate for newer anti-inflammatory agents from herbal medicine with potent activity and lesser side effect substitutes for drugs. The effect of the aqueous extract of *Stevia rebaudiana* leaves on stabilization of RBC membrane is shown in **Table 3**. The maximum percentage of stabilization was showed in aqueous extract of *Stevia rebaudiana* leaf is 68.12±1.16% at 200µg/ml as compared with standard drug. The minimum protection 12.87±1.03% was observed in aqueous extract of *Stevia rebaudiana* leaf at the 100µg/ml. It possesses significant anti-inflammatory activity comparable with standard drug diclofenac sodium reference.

Table 3. *In vitro* anti-inflammatory activity of the aqueous extract of *Stevia rebaudiana* leaves.

Concentration of plant extract	% Anti-inflammatory activity	
	Aqueous extract of *Stevia rebaudiana* leaf	Diclofenac sodium(1mg/ml)
100µg	12.87±1.03	15. 49±1.63
120µg	15.37±1.14	17.53±1.54
140µg	30.62±0.92	30.58±1.72
160µg	40.12±1.67	42.62±1.91
180µg	55.37±1.66	57.70±1.30
200µg	68.12±1.16	70.79±1.78

Value are expressed in mean ± SEM of 3 replicates

DISCUSSION

Tissue protein denaturation might be the reason behind the generation of auto-antigens in specific arthritic aliments. Consequently it might be said that denaturation of tissue proteins is act as important marker for arthritic and inflammatory diseases. Causes that can avert the protein denaturation, consequently, would be promising contender for the development of anti-inflammatory medicaments. Considering this thought, the ***in vitro*** test was completed as a primary screening to ensure the occurrence of anti-inflammatory property. During the present investigation, the bioassay of protein denaturation was decided for ***in vitro*** evalution of anti-inflammatory activity of aqueous extract of *Stevia rebudina* leaves with a greater range of dose concentrations.

The current investigations showed a concentration dependent inhibition of protein (albumin) denaturation by the test extracts throughout the concentration range of 100-200 µg/ml. Diclofenac sodium (100-200 µg/ml) was utilized as the standard drug, which also showed concentration dependent inhibition of protein denaturation **[**Table 3**].** [18,19] This was chosen as a standard NSAID on the basis of previous reports of its use for the same purpose. The increased absorbance in the test extracts and the standard drug with respect to control indicates the protein stabilizing activity (denaturation is inhibited) with increased dose. The standard drug is approximately more potent than the crude leaf extract. Hence it is obvious, that if these crude extracts are purified, the pharmacological activity will increase significantly and might even match those of the standard drug.

During preliminary phytochemical screening of the crude extracts of the leaves having the important therapeutic principles such as alkaloids, saponins, flavonoids, tannins etc., were detected. Therefore, the current findings can be attributed to these groups of chemical compounds. Further study is need on these plant extracts to find the exact mechanism of action for its pharmological properties over its anti-inflammatory effects.

CONCLUSSION

Stevia rebaudiana is worldwide spread small plant. This plant have the number of pharmacological and therapeutic utility. During this investigation the phytochemical, thin layer chromatography and antinflammatorty investigation confirms the therapeutic potential of *Stevia rebaudiana*. The phytochemical screening and thin layer chromatography of the leaves of *Stevia rebaudiana* is revealed that, it is the greater resource of various phytochemicals with pharmaceutical potential. The leaf extract showed potent anti inflammatory activity, which indicates that the plant could "lead" for the isolation of novel agents with good efficacy to treat various diseases and disorders.

CONFLICT OF INTREST

The authors confirm that this article content has no conflict of interest.

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