International Journal of
Medicinal Chemistry & Analysis
e ISSN 2249 – 7587
Print ISSN 2249 - 7595
www.ijmca.com
Research Article

PHYTOCHEMICAL AND ANTIDIABETIC ACTIVITY OF ETHANOLIC EXTRACT OF ACTINODAPHNE TADULINGAMII

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ABSTRACT

This research aimed to determine the phytochemical and anti-diabetic activity of ethanolic extract of *Actinodaphne tadulingamii*. The extraction of leaves of *Actinodaphne tadulingamii* was done by cold maceration with ethanol. The ethanolic extract of *Actinodaphne tadulingamii* were subjected to qualitative analysis to identify the presence of various phytoconstituents and anti-diabetic activity was carried out by in-vitro method using α-amylase inhibition assay. Preliminary phytochemical analysis of ethanolic extract of leaves of *Actinodaphne tadulingamii* showed that the plant have a wealthy possession of phytochemicals like carbohydrate, phenols, flavonoids, glycosides, terpenoids, steroids, sterols, saponins. In the in-vitro study the ethanolic extract of *Actinodaphne tadulingamii* shows maximum inhibition of 68.34% on α-amylase at 400µg/ml. From this study, the ethanolic extract of *Actinodaphne tadulingamii* leaf has a affluent amount of phytochemicals like flavonoids, steroids, terpenoids. Based on the obtained results and observations, the leaves of *Actinodaphne tadulingamii* could be used for the supportive treatment of diabetes mellitus. Further studies are essential to determine the anti-hyperglycemic activity of *Actinodaphne tadulingamii* in terms of molecular mechanism(s) concerned within the activity.

Keywords: Ethanol Extract, *Actinodaphne Tadulingamii*, Phytochemical, In-Vitro, Anti-Diabetic.

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INTRODUCTION

In drug discovery and development, medicinal herbs have consistently been considering the leading source of pharmaceuticals employed in the treatment of various human diseases due to their high chemical diversity and broad biological functionality[1]. According to WHO herbal medicines are defined as finished, labeled medicinal products that contain active ingredients , aerial or underground part of plants or other plant material or their combinations. The annual herbal sales have skyrocketed and the global traditional market is growing at a rate of 7-15% annually.

In the present scenario, herbal drugs are claimed for almost every disorder ranging from diabetes to rejuvenators[2]. Diabetes mellitus (DM) is that the commonest endocrine disorder. It affects over one hundred million individuals worldwide and its incidence is increasing steady with changes in life designs[3]. Diabetes mellitus may be a chronic disorder characterized by deregulating in sugar, protein and fat metabolisms caused by the whole or relative insufficiency of hormone (insulin) secretion or hormone (insulin) action.

Approximately 50% of diabetic cases can be adequately controlled by diet alone, 20-30% will need an oral antidiabetic medication and 20-30% will require

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Received:28.04.21 | **Revised**:10.05.21 | **Accepted**:25.06.21

insulin [4]. Insulin has proved to be effective to some extent in increasing the life expectancy of diabetic patients, but not a permanent solution since there are many draw backs of this therapy. Also the therapy with oral hypoglycemic agents is not satisfactory. Thus, the search for a new therapeutically agent devoid of adverse effects originating from plants used in traditional medicines would be of interest[5]. Plants have been used in traditional medicine since ancient times for the treatment of various diseases of man and animals. Traditional medicines and herbs would probably open new therapeutic venues for multifactorial disease, such as diabetes mellitus, since their complex complications often provide versatile bioactivity and varied mechanism of action. In accordance with the recommendations of the WHO expert committee on diabetes mellitus, an investigation of antihyperglycaemic agents of plant origin used in traditional medicines seems important [6].

Recent years have witnessed a renewed interest in plants as pharmaceuticals because they synthesize a variety of secondary metabolites with antioxidant potential which can play a major role in protection against molecular damage induced by reactive oxygen species (ROS)[7]. Several anti-inflammatory, digestive, antinecrotic, antidiabetic, neuroprotective and hepatoprotective drugs have recently been shown to have antioxidant or radical scavenging mechanism as part of their activity[8]. Keeping this in view, one such plant *Actinodaphne tadulingamii* which possesses good antioxidant property was chosen for study and evaluated for its antidiabetic activity.

MATERIALS AND METHODS:

Collection and authentication of plant material:

The leaves of the *Actinodaphne tadulingamii'* used in this study were collected from talakona forest near tirupati (Chitoor District, Andhra Pradesh). The plant was authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara, Tirupati, Andhra Pradesh.

Preparation of plant extract:

The leaves of '*Actinodaphne tadulingamii*' were dried under shade for 15 days and powder using mechanical grinder. The powder was sieved through sieve no.22 to get uniform particle size. About 500g of coarse powder was cold macerated with ethanol (1 L) for 72 hours with occasional shaking, after completion of extraction, the ethanolic extract was filtered using No.1 Whatmann filter paper and it was concentrated at 55oC on water bath, till it acquires 3/4th volume. The extract shows dark green viscous residue.

Preliminary phytochemical analysis:

The preliminary phytochemical analysis of the ethanol extract of leaves of '*Actinodaphne tadulingamii'* were tested for carbohydrate with Molish and Fehling's test, phenol with $FeCl_3$, flavonoids with shinoda's test, glyscosides with keller killani test, terpenoids with salkowski test, steroids test with acetic anhydride test,sterols with 5% KOH, saponins with foam test[9].

In-vitro antidiabetic activity of ethanol extract:

α-amylase inhibition assay: The α-amylase inhibition assay was carried out according to the method developed by Ramachandran et al with slight modification[10]. 1.7 ml of the various concentrations of the ethanol extract and standard agarbose (100-400 µg/ml) were separately mixed with 1.5 ml of the α-amylase enzyme (1%) and 1.7 ml of 0.1 M of sodium acetate buffer (pH-7.2). After incubation at room temperature for 30 minutes, 2 ml of 1% starch solution was added. The above mixture was incubated for 30 minutes at 37°C. Then 2.0 ml of 3,5-dinitrosalicylic acid reagent was added to the mixture. They were kept in a boiling-water bath for 5 minutes. The absorbance was recorded at 540 nm by UV-Visible spectrophotometer. Each experiment was carried out in triplicate and the average was taken.

Calculation of percentage inhibition:

The percentage inhibition of different concentration of acetone, ethylacetate and ethanol extract of leaves of *Actinodaphne tadulingamii* and standard drug for α-amylase inhibition assay were calculated by using the following formula

$$\% \text{ inhibition} = (A0 - A1 / A0) \times 100$$

Where A0 is the absorbance of control and A1 is the absorbance of test or standard.

DISCUSSION:

Many species have been reported to present antidiabetic activity. Hence, *Actinodaphne tadulingamii* was selected for phytochemical studies and anti-diabetic activity.

Preliminary phytochemical analysis of the *Actinodaphne tadulingamii* leaf acetone, ethylacetate and ethanol extracts showed that the plant have a wealthy possession of phytochemicals like alkaloids, carbohydrates, steroids, phenols, tannins, glycosides, flavonoids and sterols.

α-Amylase is the that catalyzes the hydrolysis of 1,4-glucosidic linkages in the starch into glucose. Degradation of dietary starch leads to increase the postprandial hyperglycemia. Inhibition of α-amylase in the digestive tract reduces the rate of degradation of starch in the stomach that leads to decrease the postprandial hyperglycemia. α-amylase inhibitors such as acarbose and miglitol lowering the postprandial glucose level by decreasing glucose release from the starch and delaying carbohydrate absorption by inhibiting the activity of α-amylase in the small intestine.

RESULT:

Table 1: Phytochemical screening of Ethanolic extract of leaf of *Actinodaphne tadulingamii*

S. No.	Constituents	Test	Observation	Inference
1.	Alkaloids	a) Mayer's reagent b) Dragendorff's reagent c) Hagner's reagent d) Wagner's reagent	No Cream colour was developed No Reddish brown colour precipitate was produced No Yellow colour was developed No Brown colour precipitate was produced	Absent Absent Absent Absent
2.	Carbohydrates	a) Molisch's reagent b) Fehling's solution A and B c) Benedict's reagent d) Barfoed's reagent	Violet ring was formed between the two layer Reddish brown colour was developed Reddish orange colour precipitate was produced Reddish orange colour precipitate was produced	Present present present present
3.	Protein	a) Biuret test b) Millon's reagent	No violet colour was developed No pink colour was developed	Absent Absent
4.	Steroids	a) Acetic anhydride test	Violet ring was formed between the organic and aqueous layers and green colour was formed in aqueous layer	Present
5.	Phenols	a) Ferric chloride	Pink colour was developed	Present
6.	Tannins	a) 10% Lead acetate solution b) Aqueous bromine solution	No white colour precipitate was produced No White colour precipitate was formed	Absent Absent
7.	Flavanoids	a)Shinoda's test b)Alkaline reagent test	Magenta or pink colour was formed Yellow colour was formed	Present Present
8.	Gums and Mucilage	Swelling test	No swelling characteristics was observed	Absent
9.	Glycosides	Keller-Killani test	Reddish brown colour ring was formed between organic and aqueous layers	Present
10.	Sterols	5 % Potassium Hydroxide	Pink colour was formed	Present
11.	Saponins	Foam test	Foam was liberated on the upper part	Present
12.	Terpenoids	Salkowski test	Reddish brown colouration	Present

Table 2: Percentage inhibition of α-amylase by ethanol extract of leaves of Actinodaphne tadulingamii

S. No	Concentration (µg/mL)	% of activity (±SEM)	
		Ethanol extract	Acarbose
1	100	19.16±0.427	22.14±0.852
2	150	27.62±0.712	34.46±0.673
3	200	32.56±0.545	39.66±0.707
4	250	39.69±0.478	50.34±0.652
5	300	49.34±0.492	62.84±0.643
6	350	58.75±0.546	71.49±0.511
7	400	68.34±0.467	79.19±0.509
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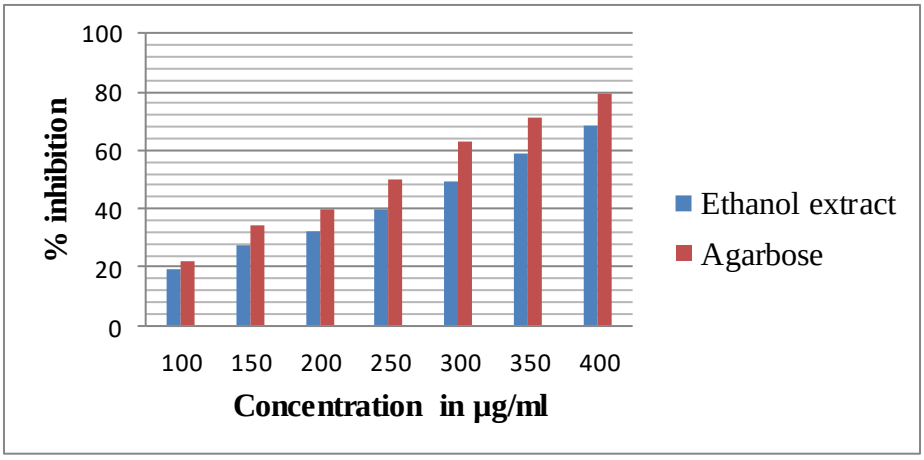
*All values are expressed as mean ± SEM for three determinations

Figure 1: Percentage inhibition of α-amylase by ethanol extract of leaves of *Actinodaphne tadulingamii*



The inhibitory effect was increased with the increasing the concentration of acetone, ethylacetate and ethanol extract and acarbose from 100 to 400 µg/mL. The experimental data showed that percentage inhibition values of standard acarbose were high when compared to ethanol extract.

CONCLUSION

Preliminary phytochemical analysis of the *Actinodaphne tadulingamii* leaf extract showed that the plant has a affluent amount of phytochemicals like flavonoids and steroids, terpinoids.

Based on the obtained results and observations, the leaves of *Actinodaphne tadulingamii* could be used for the supportive treatment of diabetes mellitus. From this study, the ethanolic extract of *Actinodaphne tadulingamii* leaf has a affluent amount of phytochemicals like flavonoids, steroids, terpenoids. Based on the obtained results and observations, the leaves of *Actinodaphne tadulingamii* could be used for the supportive treatment of diabetes mellitus. Further studies are essential to determine the anti-hyperglycemic activity of *Actinodaphne tadulingamii* in terms of molecular mechanism(s) concerned within the activity.

ACKNOWLEDGEMENT:

The author would like to thank all the staff of JKKMMRF'S for the support.

CONFLICTS OF INTEREST:

Nil

SOURCE OF FUNDING:

None

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