ORIGINAL RESEARCH PAPER

Ayurveda

PHARMACOGNOSTICAL, MEDICINAL, PHYTOCHEMICAL AND INVITRO *ANTIDIABETIC STUDIES* ON *ANDROGRAPHIS PANICULATA* (BRUM.F.) WALL. EX NEES

KEY WORDS:
Pharmacognostical, in vitro antidiabetic, *Andrographis paniculata*

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ABSTRACT

Background: *Andrographis paniculata* (brum.f.) wall. ex nees., of family Acanthacae is a bitter herb commonly used in siddha, Ayurveda and homeopathy medicines as well as tribal medicines in India and some other countries. It is commonly called as king of bitters. The various secondary metabolites present in this palnt have considerably enchanced its importance in the arena of medicinal plants. The present study was to perform pharmacognostic, Medicinal importance, preliminary phytochemical study physichochemical parameters and in-vitro antidiabetic activity of *Andrographis paniculata.* The measures taken for pharmacognostic evaluation are organoleptic, macroscopy, powder microscopy, preliminary phytochemical screening, physichocemical parameters and invitroantidiabetic activity done by haemoglobin glycosylation method and glucose uptake by yeast cells method. The powder microscopy of aerial parts of *Andrographis paniculata* showed the prescence of cork cells, xylem fibers, Prismatic calcium oxalate crystals and fibers. More number of secondary metabolites were in this plant. Phytochemical studies revealed the prescence of flavonoids, tannins, alkaloids, phenols, carbohydrates, glycosides. Physical parameters revealed resulted a valuble data to establish standards for the plant. Invitro antidiabetic activity revealed that hydroalcoholic extract of plant *Andrographis paniculata* exhibited higher inhibition of glycosylation when compared with the standard drug metformin . Over the period of 72 hour the plant extracts decreases haemoglobin glycosylation by decreasing the formation of the glucose-haemoglobin complex and amount of free haemoglobin increases. Glucose transport takes place through facilitated diffusion in yeast. Type 2 Diabetes is characterized by the deficiency of insulin causing increased amount of glucose in blood. After the treating yeast cells by these plant extract, the glucose uptake was found to increase as per dose. The results shows the % increase in glucose uptake by yeast cells at different glucose concentrations, the hydro alcohol extract of 4 mg/ml has showed significant activity when compared to the standard drug.

1. INTRODUCTION

Medicinal plants have been used by human beings since time immemorial for curing health *Andrographi spaniculata* belongs to the family *Acanthacae* is an annual herbaceous plant. It is annual herbaceous plant extensively cultivated in southern Asia, China and some parts of Europe[1]. According to Indian ayurveda, *A.paniculata* cools and relives internal heat, inflammation and pain. It is also known as Nelavembu meaning "neem of the ground"[1]. It has a strong bitter taste as that of Neem tree. The plant is widely cultivated for its multiple uses. The plant has been showed various potential therapeutic actions like liver disorders, cold and cough in humans[2]. The *A.paniculata* aerial parts, roots and whole plant has been used for centuries in Asia as traditional medicine for the treatment of various ailments. It is traditionally useful for stomachaches, inflammation, pyrexia, and intermittent fevers [3] . The whole plant has been used for several applications such as anti-dote for snake-bite and poisonous stings of some insects and to treat dyspepsia, influenza, dysentery, malaria and respiratory infections[3],[4] . The leaf extract is a traditional remedy for the treatment of infectious disease, fever-causing diseases, colic pain, loss of appetite, irregular stools and diarrhea[5]. A decoction of the aerial parts is used to treat common cold, malaria, diabetes, hypertension, cancer, and snakebite[6]. The plant has been reported to exhibit various biological activities such as in vivo as well as in-vitro viz.., anti-viral, anti-bacterial, anti-inflammatory, anti-cancer, anti-HIV and Immunomodulating/immunostimulatory. Considering this in the present study the powder microscopy, preliminary phytochemical analysis , physicochemical parameters and in vitro antidiabetic activity were recorded.

1.1 Taxonomical classification:

Kingdom	: *Plantae*
Subkingdom	: *Viridiplantae*
Infrakingdom	: *Sterophyta*
Superdivision	: *Embryophyta*
Division	: *Tracheophyta*
Subdivision	: *Spermatophyta*
Class	: *Magnoliopsida*
Superorder	: *Asteranae*
Order	: *Lamiales*
Family	: *Acanthaceae*
Genus	: *Andrographis*
Species	: *paniculata*

1.2 Synonyms

Andrographi subspathulata C.B.Cl.
Justica laterbrosa Russ. Ex Wall.
Justica paniculata Burm. fil.
Justica stricta Lam. Ex Steud.

1.3 Vernacular names

English	: *Kalamegh, green chiretta, Andrographis*
Hindi	: *Kiryat, kalpanath.*
Telugu	: *Nelavembu*
Tamil	: *Nela vaembu*
Kannada	: *Nelaberu*
Malayalam	: *Nelavepu, kiriyattu.*+

A.paniculata is native to India, Taiwan, Mainland, China, Java, Malaysia, Indonesia, West Indies and America.

1.4 Medicinal Importance(7): The whole plat is mainly useful for dyspepsia, influenza, malaria and respiratory infection. The aerial parts are mainly useful for common cold, urinary tract infections, tuberculosis, mouth ulcers, bronchitis gastro-intestinal disorder and sores [9,10,11] . And leaf parts are useful for loss of appetide, irregular stools, cough, fever, hepatitis, mouth ulcer, bronchitis gastro-ntertional disorders

and sores.

1.5. PHYTOCHEMISTRY[1,14,15,16]

The plant contains bitter glucosides such as andrographolide, paniculoside, flavonoids, andrographonin, panicalin, neoandrographolide, apigeninin 7-4-dimethyl ether. The plant contains diterpenoids like 14 deoxy-11-oxoandrographolide, 14- deoxy-11,12-didehydroandrographolide, neoandrographolide and 14 deoxyandrographolide. It consists of flavones like 5-hydroxy-7,8,2`3`-tetra methoxyflavone, andrographonin, flavonesapigenin-7,4`di-o-methyl ether, panicolin and α-sitosterol. The leaves contain andrographosterol, homoandrographolide and andrographone. Whole plant, leaves and root contains a furonoid diterpine Andrographolide; 2',5-dihydroxy-7,8-dimethoxyflavone-2'-o-β-(D)-Glucoside, 3β-hydroxy5-stigmasta-9(11),22(23)-diene, panicolin, diterpene glucoside-neoandrographolide, flavones-5-hydroxy-7,8,2',3'tetramethoxy flavones, andrographin,5-hydroxy-7,8-flavone, apigenin, 7,4-dioxymethyl ester, mono-oxymethylwigthin, deoxyandrographolide-19β-D-glucoside, flavones glucoside A, B, C, D, E and F(root), 5-hydroxy-3,7,8,2'-tetramethoxyflavone, 7-o-methylwogonin, α-sitosterol, apigenin-7,4'-di-omethylether, β-sitosterol glucoside, bitter substances, carcrol, neoandrographolide, eugenol, caffeic, hentriacontane, chlorogenic, pan icolide, eugenol, caffeic, hentriacontane, dicaffeoylquinic acids, tritricontane, 3,14-dideoxyandrographolide, andro-graphoside, en-14 β-hydroxy-8(17),12-laabadein-16,15-olide-3 β199-oxide(aerial part); oroxyliinA, homoandrographolide, wogonin, andropanoside, 14-deoxy-12-methoxyandrographolide, andrograpanin, 14-deoxy-11-oxoandrographolide, 5-hydroxy-2',7,8-trimethoxy flavones, andrographoside, 14-deoxy-11,12-didehydroandrographolide, 2',5-dihydroxy 7,8-dimethoxy flavones, 14 deoxyandrographoside(plant).

Andrographolide is colourless or light yellow crystal compound with a very bitter taste. There are four lactones in Andrographais paniculata viz..,(1) 14-deoxyandrographolide, which was also identified. Andrographolide, neo andrographolide (a non bitter, C3 O glucoside derivative of the major constituent andrographolide) and 14-deoxy-11,12-di-dehydro-andrographolide which were also identified. The other medicinal chemical principles are diterpenoids viz. 14-deoxyandrographolide, -19β-D-glucoide which has been isolated from leaves. Andrographolide and neoandrographolide were seperated from leaves of *Andrographis paniculata*.

2. MATERIALS AND METHODS:

2.1 Collection and authentification of plant:

The plant material of *Andrographis paniculata*was collected in Narsipatnam, vishakapatnam (dist) Andhra Pradesh, India in January 2017.The plant species was authenticated by Prof. BodaihPadal ,taxonomist, department of botany, Andhra university, Visakhapatnam. The voucher specimen no: 22279 were deposited in the herbarium, college of pharmaceutical sciences, Andhra University.

2.2 chemicals:

Phluroglucinol, HCL, glycerine, methanol and other analytical grade chemicals.

2.3 Macroscopic analysis:

Macroscopic features of the leaf, stem and fruit were analysed by the standard methods.

2.4 Powder microscopy: The dried aerial parts were powdered and studied under microscope. Different staining reagents (such as iodine for detection of starch grains and phloroglucinol for detection of lignified components) were used. To a little quantity of aerial parts powder taken over a microscopic slide, 1-2drops of 0.1%w/v phloroglucinol solution and a drop of concentrated hydrochloric acid were added and covered with a cover slip. The slide preparation was mounted in glycerol and examined under microscope. The characteristic structures and cell components were observed.

2.5 Preliminary phytochemical screening:

The hydroalcoholic extract of *Andrographispaniculata* phytochemical analysis[17,18,19]. A series of identification tests were performed to detect the presence of alkaloids, flavonoids, saponins, proteins and aminoacids, fixed oils and fats, glycosides, tannins and steroids.

2.6 In-vitro anti-diabetic activity[20,21]:

It was done by 2 methods - Non enzymatic haemoglobin glycosylation method and glucose uptake by yeast cells.

I. Non enzymatic glycosylation method: Antidiabetic activity of *Andrographis paniculata* aerial parts was investigated by estimating the degree of nonenzymatichemoglobin glycosylation, measured colorimetrically at 520 nm Glucose (2%), hemoglobin (0.06%) and gentamycin (0.02%) solution were prepared in phosphate buffer 0.01M, pH 7.4. 1 ml each of above solution was mixed. 2mg/ml 4mg/ml, 8mg/ml, 10mg/ml extract was added to above mixture. Mixture was kept in dark at room temperature for incubation for 72 hrs. At 520nm hemoglobin glycosylation was measured colorimetrically .The standard drug used for assay was Metformin. %inhibition was calculated.

II. Glucose uptake by yeast cells: The commercial baker's yeast was washed by repeated centrifugation (3,000×g; 5 min) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of both plant extracts (1–5 mg) were added to 1ml of glucose solution (5, 10 and 25 mM) and incubated together for 10 min at 37 °C. Reaction was started by adding 100µl of yeast suspension, vortex and further incubated at 37°C for 60 min. After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and glucose was estimated in the supernatant. Metformin was taken as standard drug.

3. RESULTS AND DISCUSSION

The powder microscopy of aerial parts of *Andrographis paniculata* showed the prescence of cork cells, xylem fibers, Prismatic calcium oxalate crystals and fibers etc.

Its height is 40 to 80 cm, length 2 to 4 cm, apex acuminate, base cuneate,margin shallow unduneate.

The stem is dark green 2 to 6 mm in diameter, quadrangular with longitudinal furrows and wings at angles of the younger parts, slightly enlarged at the nodes. It can be broken easily due to its fragile nature.

Leaves are lanceolate measuring up to 2 to 12 cm long by 1 to 3 cm wide, simple, opposite, acute, glabrous, slightly undulated, pale beneath with tapering base.

The total ash values of aerial parts of *Andrographis paniculata* was found to be 10.12%w/w which indicates the presence of earthy matter. The water soluble ash was found to be 5.10 %and acid insoluble ash was found to be 2.26%. The loss on drying values of aerial parts was found to be 8.40% which indicates the presence of moisture content. The swelling index of *Andrographis paniculata* was found to be <100. The foaming index of *Andrographis paniculata* was found to be absent. The extractive values of aerial parts was found to be more hydro- alcohol solvent. The preliminary phtochemical screening of *Andrographis paniculata* revealed the presence of alkaloids, carbohydrates, tannins, glycosides, flavanoids, steroids, saponins. The microscopic study revealed the

presence of xylem, sclerides, corkcells, curved fibres, calcium oxalate crystals. High glucose levels in body leads to its binding to haemoglobin which may result in the production of reactive oxygen species. End products of glycosylation can be inhibited by plant extracts. Upon incubation of haemoglobin with different concentration of glucose over a period of 72 hour will increase glycosylation. However, upon increasing the concentration of haemoglobin the plant extracts inhibited haemoglobin glycosylation. *Andrographis paniculata* exhibited higher inhibition of glycosylation when compared with the standard drug. Over the period of 72 hour the plant extracts decreases haemoglobin glycosylation by decreasing the formation of the glucose-haemoglobin complex and amount of free haemoglobin increases. Glucose transport takes place through facilitated diffusion in yeast. Type 2 Diabetes is characterized by the deficiency of insulin causing increased amount of glucose in blood. After the treating yeast cells by these plant extract, the glucose uptake was found to increase as per dose. The results shows the % increase in glucose uptake by yeast cells at different glucose concentrations, the hydro alcohol extract of 4 mg/ml has showed significant activity when compared to the standard drug.

CONCLUSION

Present report on pharmacognostic characterization and in vitro antidiabetic activity of *Andrographis paniculata* provides a vital diagnostic tool for identification, authentification and development of quality parameters of the species. The physicochemical parameters like total ash value , acid insoluble ash, water soluble ash, loss on drying, foaming index and extractive values using solvent (Methanol, distilled water, chloroform, acetone, ethyl acetate, hexane, hydro alcohol). The extractive values determine the active constituents present in the extract. The extractive values of aerial parts of *Andrographis paniculata* was found to be more hydro alcohol solvent. The preliminary phytochemical screening of hydro-alcoholic bark extract of *Andrographis paniculata* revealed the presence of different phytochemical like tannins, phenols ,flavonoids, alkaloids, carbohydrates and steroids.

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Innovations & breakthroughs

The present research work represents a pharmacognostical, medicinal , phytochemical number of structures of the compounds isolated from this palnt species. Furthermore it gives better idea about the specific compound or the extract for a specific biological activity. And it gives good knowledge about the traditional therapeutic use of the plant all around the world.

Conflicts of interest

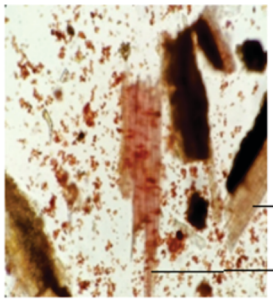
There are no conflicts of interest.

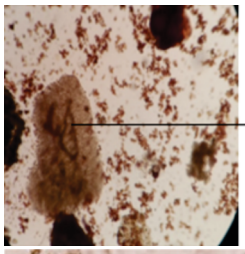
Related reports:

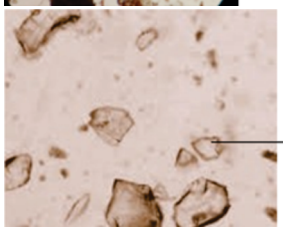
This work is based on literature study on scientific journals, books, electronic sources from 1973 to till2018

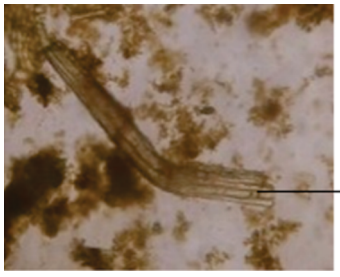
Fig:1 *Andrographis paniculata* plant

Fig:2 *Andrographis paniculata* stem









The powder microscopy of aerial parts of Andrographis paniculata showed the prescence of cork cells, xylem fibers, Prismatic calcium oxalate crystals and fibers etc.

Table: 1 Phytochemical Analysis:

Constituents	Chloroform	Ethyl acetate	Petroleum ether	Methanol	Water
Alkaloids	_	+	_	++	+
Steroids	_	+	++	++	_
Tannins	_	+	+	+++	+
Phenols	_	+	_	+++	++
Flavonoids	+	_	+++	+++	+++
Saponins	+	_	_	+	_
Carohydrates	_	_	_	++	+
Glycosides	+	_	_	++	_
Terpenoids	+	+	_	+	_
Gums and mucilage	_	+	_	+	+

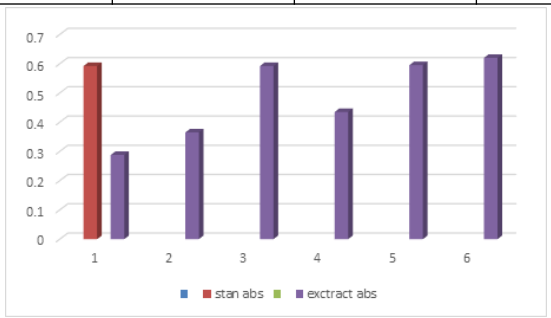
*Weak (+), moderate(++), strong (+++), very strong(++++), absent (-).

Table-2 Physicochemical parameters:

Parameters	Values obtained on dry weight basis
Total ash	10.12
Acid insoluble ash	2.26
Water isoluble ash	5.10
Loss on drying	0.26
Swelling index	_
Foaming index	_

Table-3 Results of in-vitro anti-diabetic method:

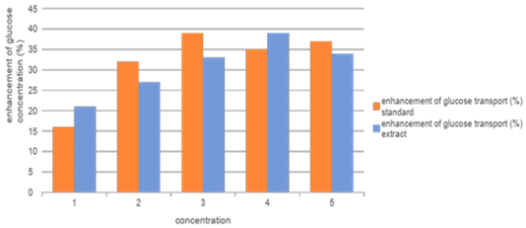
Blank	Standard			Hydro alcoholic extract		
Absorbance	Concentration	Absorbance	%inhibition	Concentration	Absorbance	%inhibition
0.129	5	0.592	77%	2	0.288	55%
				4	0.365	64%
				6	0.435	70%
				8	0.595	78%
				10	0.620	79%



Graph: 1 Non enzymatic glycosylation of haemoglobin assay

Table: 5 Results of glucose uptake by yeast cells:

Concentration	blank	Standard		Extract	
		Absorbance	% inhibition	Absorbance	% inhibition
1.0	0.102	0.121	16%	0.130	21%
2.0		0.152	32%	0.140	37%
3.0		0.169	39%	0.171	40%
4.0		0.157	35%	0.151	32%
5.0		0.162	37%	0.155	34%



Graph:2 Glucose uptake by yeast cells

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