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Research Article

COMPARING THE EFFECT OF ANTIDIABETIC ACTIVITY OF ANDROGRAPHIS PANICULATA, SALACIA RETICULATA AND OCIMUM SANCTUM BY INVITRO SCREENING

N. PREM KUMAR[A],SOUMYA.K.VIJAYAN*, J.N.DHARSANA[B], SEENA K.X[C], ANJANA A.K[D]

[a]Department of Pharmacology, Academy of Medical science, Kannur, Kerala,*Department of Pharmaceutics, Academy of Pharmaceutical sciences, Kerala,[b]Department of Pharmaceutical chemistry, Academy of Pharmaceutical sciences, Kerala.[c]Department of Pharmacognosy,Manipal College of Pharmaceutical Sciences,Karnataka.[D]Department of Pharmacology,Jamia Salafiya Pharmacy College,Kerala. **Email:** prem.n1977@gmail.com

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ABSTRACT

Objective: The primary objective of this study was to determine comparing the effect of antidiabetic activity of andrographis paniculata, salacia reticulata and Ocimum sanctum by invitro screening. Study design: Cells were cultured on 6 well plates and incubated for 48 hrs at 37°C in CO_2 incubator. When semi confluent monolayer was formed, the culture were renewed with serum free DMEM containing 0.2 % BSA and incubated for 18 hrs at 37°C in CO_2 incubator. The cells are treated with Insulin, Standard drug and plant extract and added glucose (1M) and incubated for half an hour. The supernatant was collected for glucose estimation and glucose uptake was terminated by washing the cells three times with 1 ml ice-cold KRP buffer. Cells were subsequently lysed by freezing and thawing three times. Glucose uptake was calculated as the difference between the initial and final glucose content in the incubated medium by GOD-POD method. Results: In vitro study on glucose utilization in L-6 cells showed that the effects of both the extract were found to be mild over control. Andrographis paniculata enhanced the glucose uptake by 16.11 ± 2.76% over control salacia reticulata stimulated the uptake of glucose only by 12.44 ± 2.35% over control Ocimum sanctum enhanced the glucose uptake by 11.75 ± 2.06 over control. Conclusion The drugs discussed in these studies have exhibited hypoglycemic activity and stimulates glucose uptake in L-6 skeletal muscle cells .Antidiabetic activity of *Andrographis paniculata* was found to be prominent over *salacia reticulate,* which has shown better activity than *Ocimum sanctum.* This study can bring a promising role for these plants in the management of Diabetes mellitus.

Keywords:

INTRODUCTION

The medicinal plants are widely used by the traditional medical practitioners for curing various diseases in their day to day practice. The medicinal plants are rich in secondary metabolites (which are potential sources of drugs) and essential oils of therapeutic importance. The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability [1, 2]. Because of these advantages the medicinal plants have been widely used by the traditional medical practitioners in their day to day practice.

According to a survey (1993) of World Health Organization (WHO), the practitioners of traditional system of medicine treat about 80% of patients in India, 85% in Burma and 90% in Bangladesh [2, 3]. In traditional systems of medicine the Indian medicinal plants have been used in successful management of various disease conditions like bronchial asthma, chronic fever, cold, cough, malaria, dysentery, convulsions, diabetes, diarrhea, arthritis, emetic syndrome, skin diseases, insect bite etc. and in treatment of gastric, hepatic, cardiovascular & immunological disorders [1, 4–8]. The *Andrographis paniculata* , *Salacia reticulata* and Ocimum sanctum L. has also been suggested to possess antifertility, anticancer, antidiabetic, antifungal, antimicrobial, hepatoprotective, cardio protective, antiemetic, antispasmodic, analgesic, adaptogenic and diaphoretic actions.

Andrographis paniculata is an erect annual herb to a height of 30–110 cm in moist, shady places, extremely bitter in taste in all parts of the plant body. The lance-shaped leaves have hairless blades measuring up to 8 centimeters long by 2.5 wide. The slender stem is dark green, squared in cross-section with longitudinal furrows and wings along the angles

Uses: The plant extracts exhibits antityphoid and antifungal activities. Kalmegh is also reported to possess antihepatotoxic,antidiabetic, antibiotic, antimalarial, antihepatitic, antithrombogenic, anti-inflammatory,[4] anti-snake venom, and antipyretic properties to mention a few, besides its general use as an immunostimulant agent.

Chemical constituents

Bitter diterpenoid lactones, especially deoxy-andrographolide, andrographolide and neoandrographolide have been isolated from the whole plant and leaves. Diterpene dimmers, Flavonoids, available in the roots. a Panicoline, Paniculide-A , Paniculide-B,Paniculide-

Ocimum sanctum, among the plants known for medicinal value, the plants of genus Ocimum belonging to family Labiatae are very important for their therapeutic potentials. Ocimum sanctum L. is an example of known important species of genus Ocimum, which grow in different parts of the world and are known to have medicinal properties. Ocimum sanctum L is an erect softy hairy aromatic herb or under shrub found throughout India.

Uses: recommended for the treatment of bronchitis, bronchial asthma, malaria, diarrhea, dysentery, skin diseases, arthritis, painful eye diseases, chronic fever, insect bite etc. The Ocimum sanctum L. has also been suggested to possess antifertility, anticancer, antidiabetic, antifungal, antimicrobial, hepatoprotective, cardioprotective, fever, bronchitis, arthritis, convulsion, antiemetic, antispasmodic, analgesic, adaptogenic and diaphoretic actions

Chemical constituents Ocimum sanctum leaves are abundant in tannins like gallic acid, chlorogenic acid, alkaloids, glycosides, saponins, volatile oil oleanolic acid**,** ursolic acid, rosmarinic acid, eugenol, carvacrol, linalool, β-caryophyllene (about 8%),[21] β-elemene (c.11.0%), and germacrene D (about 2%).

Salacia Reticulata: A woody climber grows on bushes and hedges. Leaves simple, opposite, ovate oblong, acuminate, coriaceous, crenate, glabrous and shining**.** It belongs to the family Hippocrateacaea. Roots are mainly used for therapeutic purpose.

Uses: They are useful in conditions of vata, diabetes, leprosy, skin diseases, antirheumatic, hepatoprotective and colic. It was attributed to its intestinal α-glucosidase inhibitory activity. α-Glycosidase inhibitors retard the digestion and hence absorption of carbohydrates in the small intestine that prevents the increase in blood glucose concentration after a carbohydrate load.

Chemical constituents: xanthone-e-glucoside, mangiferin , salacinol and kotalanol. polyphenols viz,. epicatechin, (epigallocatechin, methylepigallocatechin etc;) triterpenoids viz.,

kotalagenin 16-acetate, 26-hydroxy-1,3-fridelanedione, maytenfolic acid, dihydroxyolean-12-en-29-oie acid etc,.

Literature Review

Andrographis paniculata contain Andrographolide, the major constituent of the extract, is implicated in its pharmacological activity. A study has been conducted on the cellular processes and Andrographolide treatment inhibited the *in vitro* proliferation of different tumor cell lines, representing various types of cancers. The compound exerts direct anticancer activity on cancer cells by cell cycle arrest at G0/G1 phase through induction of cell cycle inhibitory protein p27 and decreased expression of cyclin dependent kinase 4 (CDK4)Immunostimulatory activity of andrographolide is evidenced by increased proliferation of lymphocytes and production of interleukin 2. Recent (2011) randomized, double-blind, multicentre, study found *Andrographis paniculata* as effective as mesalazine (mesalamine) in ulcerative colitis.[9] In one Chilean study, the herb had a significant drying effect on the nasal secretions of cold sufferers who took 1,200 milligrams of andrographis extract daily for five days.[8] Andrographis has been shown to be a safe traditional botanical for supporting upper respiratory tract health; the herb has been shown to inhibit RANTES secretion in inflamed bronchial cells.[10] Andrographolide, an active ingredient in Andrographis, has been shown to be responsible for the herb's inflammatory modulating actions, including the reduction of cytokine and peritoneal deposition of neutrophils, and modulation of lung inflammation in vivo.[16] Extracts of Andrographis exhibit potent inflammatory modulating and antioxidant actions in mouse models.[11]Andrographis paniculata extracts are mosquito repellent and can also be adulticidal to mosquitoes, viz., Culex quinquefasciatus and Aedes aegypti[12].

Recent studies suggest *tulsi* may be a COX-2 inhibitor, like many modern painkillers, due to its high concentration of eugenol (1-hydroxy-2-methoxy-4-allylbenzene).[13] One small study showed it to reduce blood glucose levels in type 2 diabetics when combined with hypoglycemic drugs.[14] The same study showed significant reduction in total cholesterol levels with *tulsi*. Another study showed its beneficial effect on blood glucose levels is due to its antioxidant properties.[14] Some of the main chemical constituents of *tulsi* are: oleanolic acid, ursolic acid,rosmarinic acid, eugenol, carvacrol, linalool, β-caryophyllene (about 8%),[12] β-elemene (c.11.0%), and germacrene D (about 2%).[13] β-Elemene has been studied for its potential anticancer properties,[14] but human clinical trials have yet to confirm its effectiveness.

Traditionally used in Ayurvedic medicine to treat diabetes, more recently it has been used as a supplementary food in Japan to prevent diabetes and obesity[22]. Salacia also has potent antioxidant properties, and triglyceride and LDL cholesterol-lowering effects that aid in weight loss. It contains mangiferin, a polyphenol, which enhances the body's sensitivity to insulin, and contains inhibitors of sugar digestion and absorption. A study at the Kyoto Pharmaceutical University, Japan, investigating the antiobesity effects of Salacia on laboratory animals found that its polyphenols inhibit fat metabolizing enzymes and enhance lipolysis (the breakdown of fat stored in fat cells), *suppresses glucose absorption,* Alpha-glucosidase inhibitors decrease the absorption of carbohydrates from the intestine, resulting in a slower and lower rise in blood glucose throughout the day, especially right after meals. They are important constituents of the traditionally used anti-diabetic medicines, and are also therapeutic for other carbohydrate-metabolic disorders, such as Syndrome X. Salacia contains two potent alpha-glucosidase inhibitors: salicinol[23] and kotalanol[27].

In a recent study, Salacia extract exerted an inhibitory effect on the increase of serum glucose levels in rats that had been give high doses of sucrose (table sugar) and maltose (a sugar molecule consisting of two linked glucose molecules). In fact, the scientists concluded that Salacia is a more potent glucose inhibitor than acarbose, a commercial alpha-glucosidase inhibitor found in diabetic medication[28]. A study published in April 2004 by the Department of Food Science and Nutrition, School of Human Environmental Sciences, Mukogawa Women's University, Nishinomiya, Japan, discusses the effects that various teas had on slowing down the digestion and absorption of sugar in laboratory animals[24]. Out of the ten teas tested (including mulberry and guava), Salacia's inhibitory effect lasted 110 minutes, which was significantly longer than any of the other teas .Salacia has also been found to inhibit aldose reductase[25], an enzyme that is normally present in the eye and in other parts of the body that helps change glucose into a sugar alcohol called sorbitol. Too much sorbitol trapped in eye and nerve cells can damage. A recent research report revealed that Salaretin (*Salacia reticulate* extract) effectively inhibits a-amylase, the enzyme that catalyzes the breakdown of dietary starch to simple sugars, thereby potentially inhibiting starch digestion[26].

MATERIALS AND METHODS

Cell Lines (Ian Freshney, 2005) L6The cell line was obtained from National Centre for Cell Sciences, Pune, India.

Plant material and extract preparation (raaman2006)

The leaves of *Andrographia paniculata,Oscimum sanctum* and *roots of Salacia raticulata* were collected from Kannur district (Kerala), The collected leaves and roots of above plants were dried and coarsely powdered. The powdered leaves and roots then subjected to extraction by hot continuous extraction and Cold maceration.

Phytochemical studies (Raaman, 2006; Kokate , 1993; Singh, 1995)The concentrated extracts of all plants were subjected to chemical test as per the standard methods for the identification of the various constituents.

Dose calculation

Dose calculation for the plant extract was carried out by cytotoxicity assays

***In vitro* Anti Diabetic Screening**

Glucose uptake assay

Cells were cultured on 6 well plates and incubated for 48 hrs at 37°C in CO_2 incubator. When semi confluent monolayer was formed the culture were renewed with serum free DMEM containing 0.2 % BSA and incubated for 18 hrs at 37°C in CO_2 incubator. After 18 hrs discarded the media and cells were washed with KRP buffer once. The cells are treated with Insulin, Std. drug and plant extract and added glucose (1M) and incubated for half an hour. The supernatant was collected for glucose estimation and glucose uptake was terminated by washing the cells three times with 1 ml ice-cold KRP buffer. Cells were subsequently lysed by freezing and thawing three times. Cell lysate was collected for glucose estimation.

Glucose uptake was calculated as the difference between the initial and final glucose content in the incubated medium by GOD-POD method.

RESULTS

Phytochemical studies: Qualitative Phytochemical analysis of Andrographis paniculata, salacia reticulata and ocimum sanctum extract showed the presence of majority of the compounds including carbohydrates, proteins, amino acids, tannins, alkaloids, flavonoids and saponins. The results from chemical tests are shown in Table 1, 2 and 3.

Table 1: Phytochemical Studies of Andrographis paniculata

Andrographis paniculata	Soxhelet extraction						Cold maceration	
Test	**Pet.eth**	**CHCl3**	**E.A**	**MeOH**	**Wtr**	**50%HA**	**50%HA**	**100%Wtr**
Alkaloids	-	+	+	+	+	+	-	-
Carbohydrates	-	-	+	+	+	+	+	+
Phytosterols	+	-	+	+	-	-	-	-
Fixed oils and Fats	-	-	-	-	-	-	-	-
Saponins	-	-	-	+	+	+	+	+

Phenolics andTannins	-	-	+	+	+	+	+	+
Proteins andAmino acids	-	-	-	+	+	+	+	+
Glycosides	-	-	-	+	+	+	-	-
Flavonoids	-	-	-	+	+	+	+	+
Volatile oil	-	-	-	+	+	+	-	+

Table 2:Phytochemical Studies of Salacia reticulata

Salacia reticulata	**Soxhelet Extraction**						**Cold Maceration**	
Tests	**Pet.eth**	**CHCl3**	**E.A**	**MeOH**	**Wtr**	**50%HA**	**50%HA**	**100%Wtr**
Alkaloids	-	+	+	+	+	+	+	+
Carbohydrates	-	-	+	+	+	+	+	+
Phytosterols	+	-	+	+	-	-	-	-
Fixed oils and Fats	-	-	-	-	-	-	-	-
Saponins	-	-	-	+	-	+	-	+
Phenolics and Tannins	-	-	-	+	+	+	+	+
Proteins andAmino acids	-	-	-	+	+	+	+	+
Glycosides	-	-	-	+	+	-	-	-
Flavonoids	-	-	-	+	-	+	-	-
Volatile oils	-	-	-	-	-	-	-	-

Table. No: 3. Phytochemical Studies of Ocimum sanctum

Ocimum sanctum	**Soxhelet Extraction**						**Cold Maceration**	
Tests	**Pet.eth**	**CHCl3**	**E.A**	**MeOH**	**Wtr**	**50%HA**	**50%HA**	**100%Wtr**
Alkaloids	-	+	+	+	+	+	+	+
Carbohydrates	-	-	+	+	+	+	+	+
Phytosterols	+	-	+	+	-	-	-	-
Fixed oils andFats	-	-	-	-	-	-	-	-
Saponins	-	-	-	+	-	+	-	+
Phenolics and Tannins	-	-	-	+	+	+	+	+
Proteins andAmino acids	-	-	-	+	+	+	+	+
Glycosides	-	-	-	+	+	-	-	-
Flavonoids	-	-	-	+	-	+	-	-
Volatile oils	-	-	-	-	-	-	-	-

In vitro cytotoxic screening

Determination of CTC $_{50}$ by using MTT and SRB assay in L6 cell cultures

Extracts	**Concentration in µg/ml**	**CTC $_{50}$ in (µg/ml)** MTT	SRB	**Mean CTC $_{50}$ in (µg/ml)**
Andrographis paniculata	500 250 125 62.5	122	120.5	121.25
salacia reticulata	500 250 125 62.5	370	344	357
Ocimum sanctum	500 250 125 62.5	385	388	387

The extracts of Andrographis paniculata, salacia reticulata and Ocimum sanctum were screened for their cytotoxicity activity by MTT and SRB assay methods on L6 cell lines. Andrographis paniculata *extract* showed higher cytotoxicity against L-6 cell line; whereas salacia reticulata extracts were moderately cytotoxic to cell lines. On the basis of cytotoxicity assay we decided the dose for Glucose uptake assay using L-6 cell line.

Results for *In vitro* antidiabetic screening

***In vitro* glucose uptake activity by L-6 cell line**

The effect of Andrographis paniculata, salacia reticulata and ocimum sanctum extracts on glucose uptake in L-6 skeletal muscle cells was determined. The values showed that the effect of both the extract on glucose uptake were not significant compared to control. Results indicate that aqueous extract of leaves of Andrographis paniculata, salacia *reticulata* and Ocimum sanctum stimulates glucose uptake in in L-6 skeletal muscle cells. In the above plant extracts, antidiabetic activity of *Andrographis paniculata* was found to be prominent over *salacia reticulata,*

Effect of Andrographis paniculata, salacia reticulata and Ocimum sanctum extracts on glucose uptake in L-6 cells.

Sl.no.	**Incubation Medium**	**% Glucose uptake over control**
GroupI	Insulin (1 IU/ml)	153.11 ± 2.08
GroupII	Metformin (100 µg/ml)	61.76 ± 2.45
GroupIII	Insulin (1 IU/ml) + Metformin (100 µg/ml)	158.51 ± 1.68
GroupIV	Andrographis paniculata	16.11 ± 2.76
Group V	Andrographis paniculata + Insulin (1 IU/ml)	156.93 ± 1.40
GroupVI	salacia reticulata	12.44 ± 2.35
GroupVII	salacia reticulata + Insulin (1 IU/ml)	155.58 ± 2.03
GroupVIII	Ocimum sanctum	11.75 ± 2.06
GroupIX	Ocimum sanctum+ Insulin (1 IU/ml)	151.87±2.11

SUMMARY

In vitro Antidiabetic studies

Glucose uptake activity by L-6 cell line

In vitro study on glucose utilization in L-6 cells showed that the effects of both the extract were found to be mild over control.

- Andrographis paniculata enhanced the glucose uptake by 16.11 ± 2.76% over control
- salacia reticulata stimulated the uptake of glucose only by 12.44 ± 2.35% over control

- Ocimum sanctum enhanced the glucose uptake by 11.75 ± 2.06 over control

CONCLUSION

Aqueous extract of leaves of Andrographis paniculata, salacia *reticulata* and Ocimum sanctum stimulates glucose uptake in L-6 skeletal muscle cells. In the above plant extracts, antidiabetic activity of *Andrographis paniculata* was found to be prominent over *salacia reticulata,* which has shown better activity than *Ocimum sanctum*.

All the drugs discussed in these studies have exhibited hypoglycemic activity. Since herbal drugs are proved to have high potency and negligible side effects, there is increasing demand by patients to use the natural products with antidiabetic activity. Isolation & identification of active constituents from these plants, preparation of standardized dose &dosage regimen can bring a promising role for these plants in the management of Diabetes mellitus.

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