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PHYTOCHEMICAL ANALYSIS AND ORAL HYPOGLYCEMIC ACTIVITY OF LEAF EXTRACT OF LEAVES OF *ANDROGRAPHIS STENOPHYLLA* C.B. CLARKE (ACANTHACEAE)

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ABSTRACT: *Andrographis stenophylla* C.B. Cclarke (Acanthaceae) leaves are commonly used as folk medicine in south India. The present study is planned to determine the oral hypoglycemic activity of dichloroethane (DCE) extract of *Andrographis stenophylla* leaves. The *Andrographis stenophylla* leaves showed high yield extract values in DCE, DCM (dichloromethane) and carbinol. The DCE extract showed clear compound separation in thin layer chromatography, hence that extract was used for phytochemical analysis and pharmacological study. The DCE extract showed significant oral hypoglycemic activity in rats. The present study concludes, the DCE extract of *Andrographis stenophylla* leaves are suitable for isolate the terpene and it's showed significant oral hypoglycaemic activity.

Key words: Phytochemicals, Leaf extract, *Antrographis stenophylla*

INTRODUCTION

Andrographis stenophylla C.B. Clarke (Acanthaceae) leaves were commonly used as food substance and folk medicine by tribes in South India. The plant was identified as an erect glabrous perennial under shrub, with very narrow leaves and stems from a stout root stock. The leaves are simple lanceolak, acute at both the ends, 2.0-3.0 cm long and 0.5- 1.5 cm width green to dark green in colour, odourless and bitter in taste (Nair and Henry, 1987). Traditionally this plant leaves are used as anti-inflammatory, anti-diabetic agent. India is one of the 12 leading bio-diversity centres with presence of over 45,000 plants used in ayurveda, 600 in sidha, 700 in unani and 30 in modern medicines (Gamble and Dunn, 1915). But the medicinal values and phytochemistry of the plants were not well documented. So present study is planned to determine the phytochemical analysis and oral hypoglycemic activity of DCE extract of *Andrographis stenophylla* leaves.

Materials and Methods:

The taxonomically identified fresh plant leaves of *Andrographis stenophylla* C.B. clarke (Acanthaceae) were identified and collected in the region of Marudhamalai, Western catchments, Coimbatore districts with the help of tribes in Marudhamalai village from Mar-Jul 2003. This plant was authenticated by Botanical survey of India, Southern Circle, Coimbatore (BSI/SC/5/21/2000-358 dated on May 23, 2000). The voucher specimen of the plant was preserved and kept in the Department of Pharmacognosy, R.V.S college of Pharmaceutical sciences, Coimbatore.

Determination of physical constant values: The fresh plant leaves were washed with running water to remove adhering earth material and leaves were dried under the shadow in room temperature. The dried leaves were ground to coarse powder and passed through 40 mesh sieve, stored in air tight container. The ash values, extractive values and loss on drying were performed according to the official methods prescribed in Indian Pharmacopeia (Tests on herbal product. IP 1985). The dried leaves powder were extracted with various solvents like carbinol, dichloromethane (DCM), dichloroethane (DCE), ethanol 90% v/v, chloroform, chloroform: carbinol (1:1), dichloroethane: carbinol (1:1), dichloroethane: chloroform (1:1) and distilled water (Evens and Trease, 1989)

Behavioural aspects of the leaf power with different chemical reagents: The dried powder sample was treated with various chemical reagents like H_2SO_4, HCl, HNO_3, carbinol, chloroform, glacial acetic acid etc. and the mixture was mixed well, allowed to stand for few minutes and filtered. The filtrate was examined under both U.V. and visible light (Wagner and Bladt, 1996; Liang et al., 2004; Edeoga et al., 2005)

Qualitative analysis: The chemical test for alkaloids, carbohydrates, glycosides, saponins, phenolic compound/ tannins, flavanoids/ flavones, proteins, steroids, terpene/ diterpenoid, gum/ mucilage and fixed oils were performed to know the quality of the *Andrographis stenophylla* leaves (Edeoga et al., 2005; Rasul et al., 1989; Ukani et al.,1998)

Thin layer chromatography (TLC) profile of the *Antrographis stenophylla*: The 10 × 5 cm TLC plats were (stationary phase) prepared with homogenous suspension of silica gel G and silica gel GF254. The TLC plates were activated by heating at 110°C for at least an hour and protected from moisture. The different solvent extracts of leaves of Andrographis stenophylla were run with various solvent systems like chloroform, carbinol (95:05); chloroform, carbinol (98:02); chloroform, ethanol (95:05); ethylacetate, ethanol, water (75:15:10); toluene, ethylacetate (95:05) and hexane, ethylacetate (4;1). The different spots developed in various mobile phase system and the spots were identified by vanillin sulphuric acid reagent (vs reagent) (Liang et al., 2004; Edeoga et al., 2005; Rasul et al., 1989; Ukani et al.,1998).

Oral hypoglcemic activity: Healthy, adult, male albino rats of Wistar strain, weighing 160 – 180 g were obtained from Department of Pharmacology, R.V.S. college of Pharmaceutical sciences, Coimbatore. The rats were housed under 24 ± 2° C temperature, 40-60 % humidity and 12-12 ± 1 h light dark cycle. The animals were fed with water and rat pellets *ad libitum*. The rat pellets were supplied by M/s. Hindustan Lever Ltd., Bangalore, India. All animal experiments were carried out as per CPCSEA guidelines.

Twenty five male Wistar rats were divided into five groups containing five animals and grouped as follows.

Group I - Normal control (no treatment)
Group II - Control (0.5% w/v CMC)
Group III - Standard drug treatment (metformin- 250 mg/kg)
Group IV - DCE extract 100 mg/kg
Group V - DCE extract 200 mg/kg

Prior to the study blood samples were collected from the all the group of the animals to check random glucose levels. The animals which had a glucose level >130 gm/dL were excluded from the study.

All the test substances (DCE extract) and standard drugs were administered as an oral suspension in non-fasted animals. The test and standard substances were suspended in 0.5% w/v carboxymethyl cellulose (CMC). Group II to group V animals were received 5 g/kg glucose additionally after 30 min of standard drug/ test substance administration. The animals were restrained in the rat restrainer and blood samples were collected from tail vein (without anaesthesia) for glucose estimation at various time points like pre dose, 1.0, 2.0, 3.0 and 4.0 h after test substance/ standard drug treatment. At each time point 0.2 – 0.25 ml of the blood sample was collected from the tail vein and fluid loses were replaced with equivalent volume of Lactated ringer's solution (LRS). Glucose was estimated by using Microlab auto-analyzer (Vital Scientific N.V., The Netherland) with the help of Lab kit enzymatic kit (Vogel 2002, Nyunai 2006).

Statistical analysis: The values were expressed as mean ± SEM (n=5) for each group. Significant difference between groups was determined using one-way ANOVA followed by Tukey's multiple comparison test. A P value less than 0.05 was considered significant.

RESULTS

The dried *Andrographis stenophylla* leaves were extracted with various solvents. Dichloroethane solvent gave high yield extractive value than other solvents. The extract value of *Andrographis stenophylla* leaves were 28% in DCE, 25 % in DCM: carbinol (1; 1), 24 % in carbinol, 24 % in chloroform: carbinol (1:1), 22% DCM, 18% in DCE: carbinol (1:1), 17 % in 90% ethanol, 12 % in chloroform and 09% in distilled water. The physical constant values are mainly used to determine the purity and quality of the drug. Ash value of the powder will give inference about inorganic compound, impurities present in the leaves. The result (mean ± SEM) suggests that the powdered leaves having high acid insoluble ash (7.98 ± 0.36 %; n=6) and 3.82 ± 0.20 % (n=6) of water soluble ash, 10.80 ± 0.47 % (n=5) of sulphated ash and 6.67 ± 0.54 % (n=5) of loss on drying. The loss on drying infers about percentage of moisture present in the test substance.

The dried leaves powder was treated with H_2SO_4, HCl, HNO_3, carbinol, chloroform, glacial acetic acid etc. and characteristics were presented in the table 1. The DCM and DCE solvents gave high yield extractive value, so DCM and DCE extracts were subjected to chemical qualitative analysis to determine the nature of constitutes present in the extract. The *Andrographis stenophylla* DCM and DCE leaves extract were tested for presence of alkaloids, carbohydrates, glycosides, saponins, phenolic compound/ tannins, flavanoids/ flavones, proteins, steroids, terpene/ diterpenoid, gum/ mucilage and fixed oils. The test results showed positive for presence of carbohydrate, proteins, terpene and diterpenoid.

Table 1: Fluorescence analysis of various extract leaves powder of *Andrographis stenophylla*

S.No	Chemical reagent	day light	U.V. light
1	Nitric acid (specific gravity 1.42)	Pale green	Brown
2	HCl (specific gravity 1.16)	Yellowish green	Yellow with brown spot
3	H_2SO_4	Deep blackish green	Brown with black spot
4	CH_3COOH (glacial)	Yellowish green	Dark brown
5	Carbinol	Green	Brown
6	Petroleum ether	Yellowish green	Green
7	Dichloromethane	Yellowish green	Brown
8	Dichloroethane	Green	Yellowish green
9	90% ethanol	Green	Reddish brown

The DCM and DCE extracts were showed clear compound separation in TLC and other solvent extracts [carbinol, ethanol 90% v/v, chloroform, chloroform: carbinol (1:1), dichloroethane: carbinol (1:1), dichloroethane: chloroform (1:1) and distilled water] not showed clear compound separation in TLC, so DCM and DCE extract were used for the further chromatographic study. The TLC system of DCM and DCE extracts showed presence of terpene and diterpene. The TLC profile of DCM and DCE extracts of *Andrographis stenophylla* leaves were presented in the table 2. The DCE extract showed good separation of the terpene with wide range of solvent system, so DCE extract was used for further pharmacological study.

Table 2: TLC profile of DCM and DCE extracts of *Andrographis stenophylla* leaves

Extract	Solvent system	Visualizing agent	Detection colour	Rf value	Component
DCM extract dissolved in CH_3OH	$CHCl_3$: CH_3OH (95:5)	vs reagent	Violet	0.42	Terpene
DCE extract dissolved in CH_3OH	$CHCl_3$: CH_3OH (95:5)	vs reagent	Violet	0.24	Terpene
DCE extract dissolved in DCE	$CH_3COOC_2H_5$: C_2H_5OH: H_2O (75:15:10)	vs reagent	Violet	0.79	Terpene
DCE extract dissolved in DCE	$CHCl_3$: C_2H_5OH (95:5)	vs reagent	Violet	S1-0.65 S2-0.5 S3-0.357	Terpene
DCM extract dissolved in Toluene	$CH_3COOC_2H_5$: $C_6H_5CH_3$ (95:5)	vs reagent	Violet	0.85	Terpene
DCM extract dissolved in Toluene	$CH_3COOC_2H_5$: $C_6H_5CH_3$ (95:5)	Iodine chamber	Dark yellow	0.85	Terpene
DCM extract dissolved in Toluene	C_6H_{14}: $CH_3COOC_2H_5$ (4;1)	$KMnO_4$ in H_2SO_4	Rose pink	0.39	Diterpene

The oral hypoglycaemic effect of DCE extract of *Andrographis stenophylla* leaves was studied in non-fasted Wistar rats. The DCE extract of *Andrographis stenophylla* leaves significantly reduced ($P<0.05$- 0.001) hyperglycemia at the dose levels of 100, 200 mg/kg, compared with glucose treated group (group-2). The glucose control group showed significant ($P<0.001$) increase in glucose levels after 30 min of glucose administration in all the animals when compared with normal group animals. The hypoglycemic effect of DCE extracts of *Andrographis stenophylla* leaves comparably equivalent to the standard metformin 250 mg/kg treated group. Table 3 summarized the oral hypoglycemic effect of DEC extract of *Andrographis stenophylla* leaves.

Table 3: Effect of DCE extracts of *Andrographis stenophylla* leaves on oral hypoglycaemia

Groups	Blood glucose levels (mg/dL)					
	Pre-dose	1 h	2 h	3 h	4 h	5 h
Normal control	93.8± 2.85	97.6± 2.32	92.8± 3.62	96.8± 2.13	97.8± 2.08	97.6± 2.25
Glucose Control	101.4± 1.57	395.8± 2.31***	331.2± 3.51***	254.8± 2.01***	189± 2.28***	104.4± 4.47
Metformin 250 mg/kg	99. 6± 3.28	173± 3.63$^{\&\&\&}$	157.4± 4.44$^{\&\&\&}$	112.8± 3.21$^{\&\&\&}$	92.6± 2.09$^{\&\&\&}$	63.4± 1.25$^{\&\&\&}$
DCE extract 100	102.2± 3.61	192.6± 4.63$^{\&\&\&}$	150.2± 3.43$^{\&\&\&}$	105.4± 2.20$^{\&\&\&}$	90.0± 5.98$^{\&\&\&}$	91.2± 3.24
DCE extract 200	97.6± 2.66	149.0± 3.81$^{\&\&\&}$	116.8± 3.65$^{\&\&\&}$	94.0± 4.47$^{\&\&\&}$	87.6± 3.75$^{\&\&\&}$	89.6± 4.12$^{\&}$

Values are mean ± SEM of five animals; ****P<0.001 as compared with normal control group; $^{\&\&\&}$ P<0.001, $^{\&}$P<0.05 as compared with glucose control group; One way ANOVA by Tukey's multiple comparison test.

Discussion: DCE, DCM and carbinol are shown high percentage of extract value, but DCE shown clear compound separation in thin layer chromatography, hence the DCE extract were used for pharmacological study. The phytochemical analysis of the DCE extract of the *Andrographis stenophylla* leaves showed presence of terpene and diterpenoid. The DCE extract showed significantly equivalent hypoglycemic activity as compared to the metformin. So, further study is required to confirm the hypoglycemic activity in diabetic induced model.

Conclusion:

The present study concludes, the DCE extract of *Andrographis stenophylla* leaves are suitable for isolate the terpene and its showed significant oral hypoglycemic activity in male Wistar rats.

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