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Research Article

Phyto Chemical Characterisation and *In-vivo* Antipyretic Activity of *Allophyllus cobbe* L. (Raeusch.).

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ABSTRACT

The present study was planned to evaluate and compare the in-vivo antipyretic activity of aqueous, hydroalcoholic, methanolic and petroleum benzene extracts of leaves of A*llophylus cobbe* (L.) Raeusch. in endotoxin (Lipopolysaccharide from *E. coli* serovar 0127: B_8) induced pyretic models in adult Wistar rats of either sex. Acute oral toxicity study was conducted as per OECD guidelines. Preliminary phytochemical analysis as per standard procedures and HPTLC fingerprinting of the various extracts was done using solvent hexane: ethyl acetate. *In vivo* antipyretic activity was determined by LPS induced pyrexia model and oral administration of 250 mg/kg of various extract of *A. cobbe* and lipopolysaccharide at dose rate of 50µg/kg intramuscularly. The reduction in temperature of aqueous extract treated group was comparable to the standard antipyretic drug paracetamol by the second hour of study. No acute toxic symptoms were observed on oral administration of any of these extracts at 2000 mg/kg body weight which indicated the safety of extracts. Phytochemical analysis revealed the presence of secondary plant metabolites like as phenolics, alkaloids, glycosides, tannins, terpenes and flavonoids. The HPTLC chromatogram and densitometry of different leaf extracts of A. cobbe at visible, short and long UV revealed maximum no of peak 13 in petroleum ether extract followed by 6 peaks in aqueous, 7 peaks in hydroalcoholic and methanolic extract respectively. The results of the study indicated that the antipyretic response observed could be due to the presence of one or more components of polar nature. Further fractionation and isolation study needs to be undertaken for identifying the active compound responsible for the antipyretic activity of the extract and development of potent safe herbal antipyretic drug from this plant.

Key words: Antipyretic, *A. cobbe*, HPTLC profiling, phytochemical screening.

INTRODUCTION

Pyrexia is a complex physiologic response triggered by infectious/aseptic stimuli, one of the sequelae of tissue damage, trauma, inflammation, graft rejection or pathological conditions due to virus, bacteria, fungus as well as protozoans[1,2]. Elevations of body temperature occur when concentrations of interleukins, interferons, cytokines and tumor necrosis factor alpha formed are in large quantity leading to prostaglandin E_2 (PGE_2) increase within certain areas of the brain[1,2] These elevations alter the firing rate of neurons that control thermoregulation in the hypothalamus. It is described as the body's natural defense mechanism to create an unconducive environment which infectious agents or damaged tissue cannot withstand. According to Ayurveda, pyrexia originates from a combination of indigestion, seasonal variation and significant alterations in daily routine[3]. Antipyretic drugs used for the management of fever are either NSAID's like ibuprofen, naproxen, ketoprofen or Aspirin and related salicylates such as choline salicylates, magnesium salicylates and sodium salicylates. Most antipyretic drugs function by inhibiting the expression of cyclooxygenase 2 (COX-2) to reduce the elevated body temperature by inhibiting the bio-synthesis of prostaglandin E2 (PGE2)[4,5,6] . However, these synthetic agents irreversibly inhibit COX-2 with high selectivity but are toxic to the hepatic cells, glomeruli, cortex of the brain and heart muscles[5,6]. The quest for a noble antipyretic drug is perpetual.

Allophylus is an important genus of the family Sapindaceae, found to grow on up line edges of hills in Western Ghats. The plant grows in mixed dipterocarp. It can also be found in coastal and sub-montane forests at the altitude of 1,700 m (5,600 ft.). Leaves of *A. cobbe* are used by many tribal healers against bone fractures, rashes, several gastrointestinal disorders including dyspepsia, anorexia, diarrhea, stomach ache and cuts and wounds. The plant is used in Ayurveda, to treat problems like inflammation, elephantiasis, oedma, and fracture of bones[7]. However, there are very scanty scientific information on the pharmacological activity of this plant. Hence in the present study, an attempt has been made for preliminary phytochemical screening and HPTLC profiling as well as evaluating the antipyretic potential of different extracts of leaves of *A. cobbe* using LPS induced pyrexia model.

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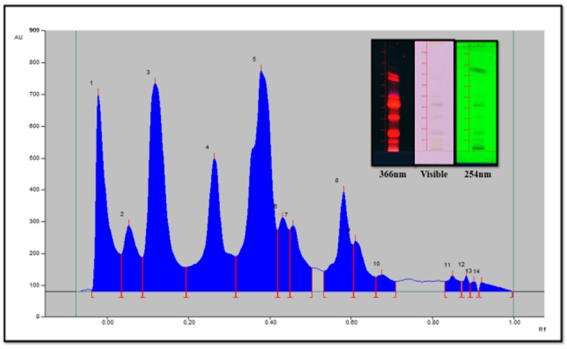
Table 1: Experimental design for oral administration of different extracts of leaves of *A. cobbe*. (n=6).

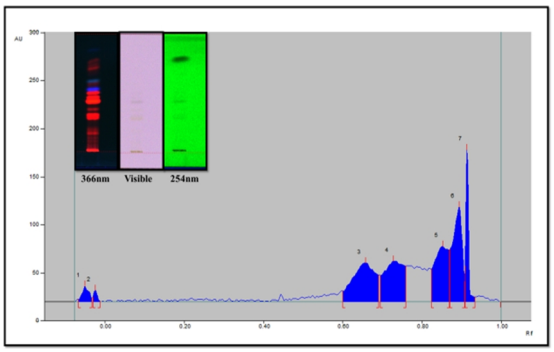
Group	Treatment
Group I (control)	0.1% Normal saline (Vehicle) p.o
Group II (standard)	Paracetamol @ 100 mg/kg body weight per os (p.o)
Group III	Aqueous extract of leaves of *A. cobbe* @ 250mg/kg body weight p.o
Group IV	Hydroalchoholic extract of leaves of *A. cobbe* @ 250mg/kg body weight p.o
Group V	Methanolic extract of *A. cobbe* @ 250mg/kg body weight p.o
Group VI	Petroleum benzene extract of leaves of *A. cobbe* @ 250mg/kg body weight p.o

Table 2: Qualitative determination of phytochemical constituents of various extracts of leaves of *A. cobbe*.

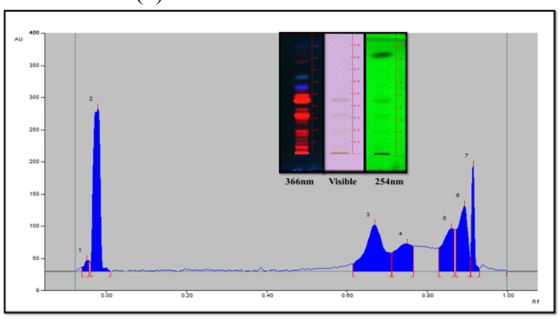
Phytoconstituents	Leaf extracts of *A. cobbe*			
	Aqueous	Hydroalchoholic	Methanolic	Petroleum benzene
Phenolics	+	+	-	+
Alkaloids	+	+	-	-
Steroids	-	-	+	-
Glycosides	-	-	+	-
Tannins	+	+	-	+
Terpenes	+	-	-	-
Saponins	+	-	-	-
Flavonoids	+	-	-	+



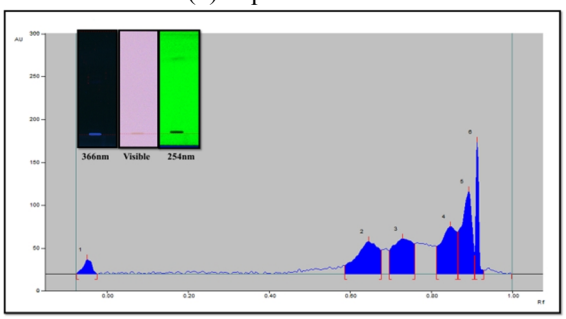
(a) Petroleum ether extract.



. (b) Aqueous extract.



(c) Methanolic extract



. (d) Hydroalcoholic extract.

Figure 1: HPTLC chromatogram of different leaf extracts of *A. cobbe* at long, visible and short UV rays.

MATERIALS AND METHODS

Plant material

The leaves of plant was collected from the Wayanad district, Kerala. The collected leaves were identified and authenticated by a botanist at M. S. Swaminathan Research Foundation, Kalpetta. The plant was cleaned, dried in shade and pulverized to coarse powder using temperature controlled plant sample grinder. The powdered leaves were extracted with different solvents such as aqueous, hydroalcoholic (1:1 methanol: water), methanol and petroleum benzene in a Soxhlet extraction apparatus. Solvents were evaporated by using a rotary vacuum evaporator (M/s Buchi, Switzerland) under reduced pressure 175 mbar at temperature ranging from 40^0C to 60^0 C.

Drugs and chemicals

All the solvents (synthetic /HPLC grade) and chemicals used in this study were purchased from M/S Merck India Ltd., and M/s Sigma-Aldrich India Ltd., Bangalore. The standard drug and Lipopolysaccharide (***E. coli***, serotype 0127, B_8) were obtained from M/s Sigma-Aldrich India Ltd., Bangalore.

Phytochemical analysis

The different extracts of *A. cobbe* were analyzed qualitatively for various phytochemical constituents as per the standard procedures[8].

Table 3: R_f values of different solvent extracts of leaves of *A. cobbe* @ 254 nm.

Extract	No. of peaks	Major peaks	R_f value	Area %
Petroleum ether	13	2	0.03	23.57
		9	0.61	28.56
Aqueous	6	2	0.59	20.77
		3	0.70	20.50
		4	0.81	21.33
		5	0.87	23.95
Hydroalcoholic	7	3	0.60	22.68
		4	0.70	20.64
		5	0.82	20.14
		6	0.87	22.61
Methanol	7	2	0.04	28.20
		3	0.62	22.33

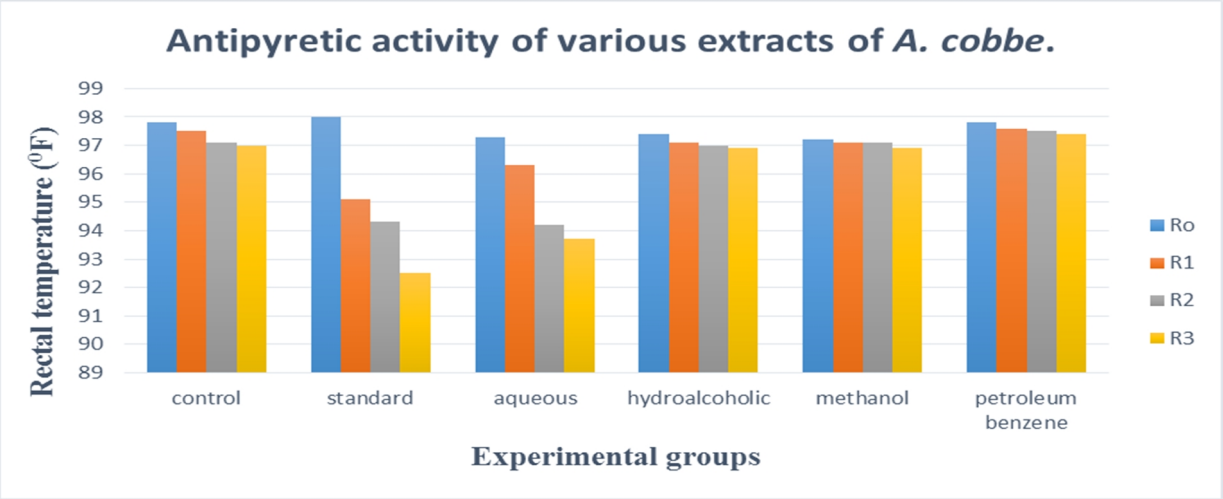


Figure 2: Antipyretic activity of various extracts of leaves of *A. cobbe*.
(R_0, R_1, R_2, R_3 = Rectal temperature at 0^{th}, 1^{st}, 2^{nd} and 3^{rd} hr. respectively)

HPTLC profiling of different extracts

High performance thin layer chromatography analysis was carried out (Camag, Switzerland) with different solvent extracts of *A. cobbe*. Chromatographic separation was performed on Merck TLC plates precoated with silica gel 60 F_{254} (20cm x 10 cm with 200 µm layer thicknesses). The different extract solution (5 µl) was applied onto the plates as a band with 6 mm width using Camag 100 µl sample syringe (Hamilton, Switzerland) using Camag Linomat 5 applicator (Camag, Switzerland). Linear ascending development was carried out in a twin trough glass chamber using hexane: ethyl acetate (8:2) as a mobile phase. Scanning was performed using Camag TLC scanner at 366 nm through fluorescence mode and operated by win CATS software (version 1.4.1, Camag). Plates are visualized under 254 nm, 366 nm and visible light[9].

Experimental animals

Thirty six albino Wistar rats of either sex were used for the experiment. Animals were procured from the Small Animal Breeding Station, College of Veterinary and Animal Sciences, Pookode and they were maintained and acclimatized under standard laboratory conditions. They were divided into six groups each comprising of six animals and given various treatment as depicted in Table 1.

Assessment of antipyretic activity

Initial rectal temperature of animals was recorded. Lipopolysaccharide (LPS from ***E. coli*** serovar O127, B_8) at the dose rate of 50µg/ml/kg i/m was given to all animals. After 18 hours, those animals showing a rise in body temperature of more than 1°F were selected and divided into six groups of 6 animals each. This record of increase in temperature was taken as 0^{th} hour (R-0) recording and extracts/drugs were administered as described above in Table 1. Rectal temperature of animals was recorded every hour after the treatment till 5 hrs.[10]. Experimental model and animal grouping for antipyretic study is shown in Table 1.

Assessment of the acute oral toxicity

The acute oral toxicity of all the extracts tested were done in rats at the dose of 2000 mg/kg as per OECD guidelines 420.

Statistical analysis

Statistical analysis was performed by following the student T test described by Snedecor and Cochran (1985)[11].

RESULTS

The results of phytochemical analysis of the aqueous, hydroalcoholic, methanolic and petroleum benzene extracts of *A. cobbe* are presented in Table 2.

Phytochemical analysis revealed the presence of phenolics and tannins in all extracts whereas steroids and glycosides were present only in the methanolic extract of *A. cobbe.*

The different solvent extracts of *A. cobbe* were subjected to HPTLC profiling for the conformation of active constituents. The extracts showed several polyvalent compounds with the solvent system hexane: ethyl acetate (8:2). The HPTLC chromatogram and densitometry of different leaf extracts of *A. cobbe* at visible, short and long UV are depicted in Figure 1 and tabulated in Table 3. Among the four extracts, the petroleum benzene extract showed maximum peaks followed by aqueous, hydroalcoholic and methanolic extract.

A maximum no. of 13 peaks with major peak at 2 and 9 corresponding R_f value 0.03 and 0.61, area % corresponding to 23.57 and 28.56 respectively are observed in petroleum ether extract followed by 7 peaks in hydroalcoholic and methanolic extract while 6 peaks in aqueous extract following significant antipyretic activity, R_f value 0.59, 0.70, 0.81, and 0.87, area % corresponding to 20.77, 20.50, 21.33 and 23.95 respectively was observed. The antipyretic activity of the treated as well as the control groups are presented in Figure 2.

Acute oral toxicity test

No mortality was observed in animals treated with the extracts and no untoward clinical signs were noticed in any of the animals treated with the extracts during the entire period of study.

DISCUSSION

The aqueous extract of *A. cobbe* alone significantly reduced the elevated body temperature of rats treated with LPS when compared with hydroalcoholic, methanolic and petroleum benzene extracts. The significant antipyretic activity in rats with ample safety profile can be attributed for the presence of secondary plant metabolites like as phenolics, alkaloids, glycosides, tannins, terpenes and flavonoids [12, 13].

The variation in the HPTLC peaks of aqueous and hydroalcoholic, methanol and petroleum benzene can be correlated to their different activity and phytoconstituents eluted according to polarity. But detailed research has to be undertaken to further substantiate the chromatographic findings.

The antipyretic and anti-inflammatory activity of alkaloids is attributed to their inhibition of a number of inflammatory molecules including lipooxygenase, cyclooxygenase, leukotrienes and prostaglandins[14,15,16]. Tannins are reported to possess anti-inflammatory property evident by the inhibition of prostaglandin synthesis[17]. Flavonoids have potent inhibitory activity against liopoxygenases, cyclooxygenases and related enzymes. They are also reported to have anti-oxidant property and are used as UV-light protective compounds by the plants[18,14]. Tannins along with flavonoids exhibit anti-inflammatory and analgesic effect attributing to their free radical scavenging activity[18,10]. Phenolics are a class of antioxidant agents which acts as free radical terminators which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxidase[19]. The antioxidant activity of phenolics is reported to be mainly due to their redox properties[19,17]. Saponins is reported to possess hyperglycemic component, anti-oxidant and anti-cancerous activity[20,21].

Since there was no potent toxicities evidenced, it may be concluded that more extensive study needs to be conducted to determine exact mechanism of action for antipyretic activity using different experimental models. Further fractionation and isolation study of aqueous extract of *A. cobbe* needs to be undertaken for the development of potent safe herbal antipyretic remedy from this plant.

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CONFLICT OF INTEREST

None declared.

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