



In-vitro hemolytic activity of *Allium stracheyi* Baker

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ABSTRACT

The present study reveals the phytochemical profile and *in-vitro* hemolytic property of *Allium stracheyi* Baker, an ethno botanically important medicinal and aromatic plant endemic to western Himalaya. The leaves were sequentially extracted by using different solvents according to their increasing polarity. The qualitative phytochemical screening of the extracts revealed the presence of alkaloids, saponins, fixed oils, phytosterols, phenolics and flavonoids. The extracts were tested for their hemolytic property with three different concentrations. The butanol extract has shown maximum amount of hemolysis where as the water extract has shown least amount of hemolysis. All the extracts have shown dose dependent activity. Thus the present study indicates the hemolytic potential of this plant.

Key words: *Allium stracheyi*, hemolytic activity, soxhlet extraction, phytochemical profile

INTRODUCTION

India is distinguished as a global biodiversity hotspot where ecology and evolutionary factors favoured huge species diversity with over 1740 Medicinal and Aromatic Plants with various traditional and modern medicinal uses. Phytochemicals from medicinal plants serve as lead compounds in drug discovery and design^{[1], [2]}. It is seen from recent epidemiological studies that various phytochemicals e.g., polyphenols, flavonoids, vitamin c are correlated with reduced risks of fatal and chronic diseases^[3]. *Allium stracheyi*, locally known as jambu or pharan, is a multipurpose ethno botanically important aromatic and medicinal herb endemic to western Himalaya. It was originally brought from Tibet decades ago. The plant has long been known for its uses as spices, pickles and medicines for jaundice, cold, cough, wound healing and other stomach problems^{[4], [5], [6]}. The total production and consequently the total monetary gain from this plant have been reported maximum due to the larger area under their cultivation, thus making it an economically important plant^[7]. But till date no scientific investigations have been done on the plant except some studies on vegetative propagation and chromosomal behaviour.

In vitro hemolytic activities are becoming a new area of research in drug lead discovery. Researchers are exploring ethno botanically important plants to find out potential natural products with antiaggregant action. These studies are important because some patients have become resistant to the already existing drug e.g. aspirin^[8] and/or conventional medication (warfarin, aspirin) in association with medicinal plant formulations. This is posing a serious problem to the society. Moreover the constant use of synthetic drugs is leading the society to face great danger^[9]. In recent years, many antiplatelet aggregating agents have been isolated from plants and have demonstrated potent activity^[10].

Though *Allium stracheyi* is an ethno medicinally important plant, no scientific work has been done on its bioactivities. As per our knowledge this will

be first investigation focused on the phytochemical profiling and hemolytic potential of this plant.

2. MATERIAL AND METHODS

2.1 Plant materials

The plant was collected from Tolma village, Nanda Devi Biosphere Reserve Region, Chamoli District, Uttarakhand. It was authenticated and the voucher specimen was deposited at Dept. of Botany, HNB Garhwal University, Srinagar, India and the voucher specimen number is GUH 7251.

2.2 Extraction of phytochemicals

The leaves were washed under running water and then dried by using blotting paper. The leaves were then kept for air drying for about a week. It was then grinded to semi granulated powder by a grinder. Then the dried powder was placed inside the soxhlet extraction apparatus. The phyto compounds were then extracted according to their polarities using six different solvents (petroleum ether, benzene, n-butanol, ethyl acetate, 85% ethanol and water). The extraction was carried out separately for 36 hours for each solvent. The extracts were then dried by rotary vacuum evaporator and then kept in the refrigerator at 4°C for future uses.

2.3 Qualitative phytochemical test

The six dried extracts were subjected to various tests for the detection of phenolics, flavonoids, alkaloids, fixed oil, phytosterols, saponins.^[11]

2.4 Hemolytic assay

Hemolytic assay was carried out by adopting the method of Bulmus et al.^[12]. Freshly collected human red blood cells were taken and washed three times by 150 mM NaCl (2500 rpm for 10 minutes). The serum was removed and the cells were suspended in 100 mM sodium phosphate buffer. Three different concentrations (50 µg, 250 µg and 500 µg) of extracts were mixed with 200 µL of RBC solutions and the final reaction mixture volume was made up to 1 ml by adding sodium phosphate buffer. The reaction mixture was then placed in water bath for 1 hour at 37°C. After the incubation time the reaction

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Table 1. Qualitative analysis of crude extracts obtained from the leaves of *Allium stracheyi*

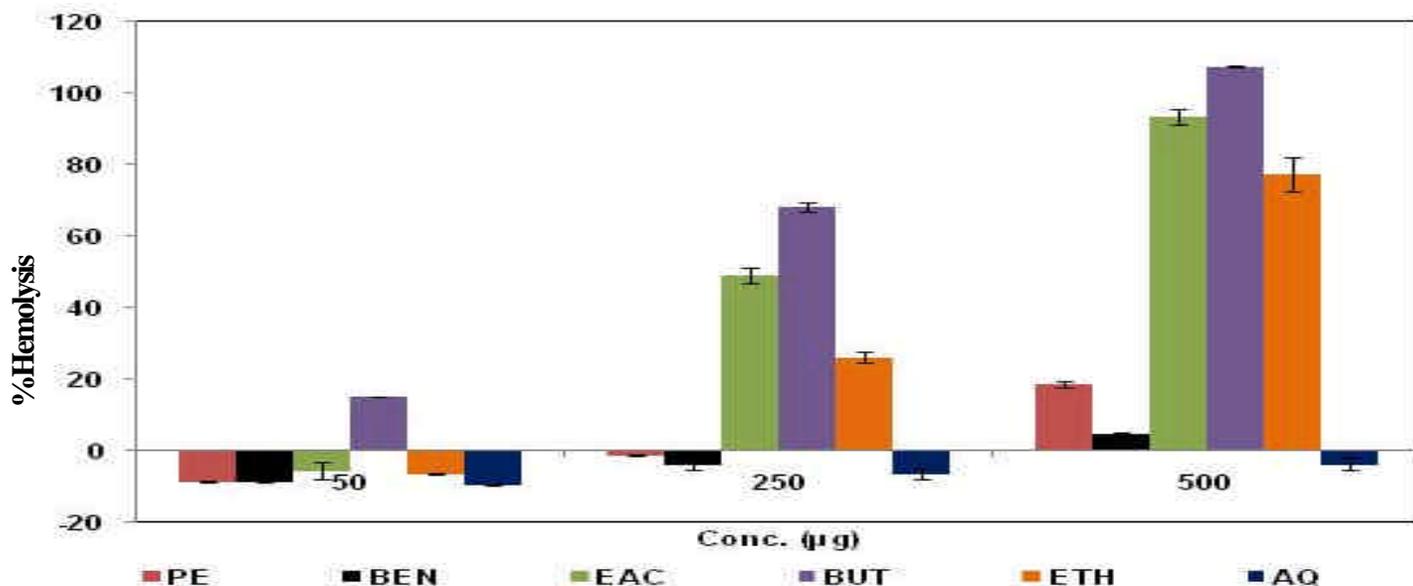
Class of compounds	PE	BEN	BUT	EAC	ETH	AQ
Alkaloid	-	-	+	+	-	-
Phytosterols	+	++	+	++	-	-
Phenolics	-	-	+	+	+++	++
Flavonoids	-	-	+	++	+++	+
Saponins	-	-	++	++	+	+++
Fixed oil	+	++	++	+	-	+++

- = not present; + = present; ++ = moderately present; +++ = highly present

and flavonoids were found in n-butanol, ethyl acetate, ethanol and water extract. Phytosterols were found in petroleum ether, benzene, butanol and ethyl acetate extracts where as fixed oil was found in all the extracts except ethanol extract. Saponins were found in all the extracts except petroleum ether and benzene.

The results for hemolytic test have shown that butanol extract possesses the maximum amount of hemolytic activity followed by ethyl acetate. The water extract was seen not to possess any hemolytic activity. The hemolytic activities of the extracts were in the following order : butanol > ethyl acetate > ethanol > petroleum ether > benzene > water (Figure 1). The hemolytic effect of the extracts is seen to increase with the increasing concentrations of the extracts. Thus the extracts have shown dose dependent hemolytic activity.

Figure 1. Hemolytic activity of different extracts obtained from leaves of *Allium stracheyi*



mixture was centrifuged again at 2500 rpm for 15 minutes. The supernatant was collected and the optical density was measured at 541 nm keeping sodium phosphate buffer as blank. Deionised water was used as a positive control. The experiment was done in triplicate and mean ± S.D. was calculated.

$$\text{Percentage hemolysis} = \frac{(\text{Absorbance of sample} - \text{Absorbance of blank})}{\text{Absorbance of positive control}} \times 100$$

3. RESULTS AND DISCUSSION

Natural products have been shown to be a tremendous and consistent resource for the development of new drugs^[13]. It has been suggested that phytochemical extracts from plants holds promises to be used in allopathic medicine as they are potential sources of antiviral, antitumoral and antimicrobial agents^[14]. Sometimes plant derived natural compounds have gained attention because of their potential to act as cytotoxic and chemopreventive activity^[15]. Various plants have already been proved to possess high antioxidant property containing high amounts of phenolics and flavonoids^[16].

The phytochemical screening of the extracts have shown the presence of alkaloids, flavonoids, phenolics, phytosterols, saponins and fixed oil (Table 1). Alkaloids were found in n-butanol and ethyl acetate extract where as phenolics

Various non specific mechanisms can cause hemolysis. Hemolytic activity can be a result of pore formation in the cell membranes thus changing membrane permeability or it can be due to the alteration of sodium-potassium and calcium-magnesium ATPase activities^[17]. Sometime some reduced compounds like phenols can cause oxidation of hemoglobin producing methaemoglobin thus finally causing hemolysis^[18]. Measuring hemolytic activity is important as it is an indicator for cytotoxicities. The *in vitro* hemolysis test has also been employed by many different groups for the toxicological evaluation of different plants^[19]. Mechanical stability of erythrocytes membrane is good indicator of various *in-vitro* cytotoxicities^[20]. Performing hemolytic assay is important to determine whether a drug possessing antioxidant and other bioactivities can be used in pharmacological applications^[15].

In recent years many plants and their active components have been isolated which are proved to have antiplatelet aggregating potency^[10]. Some of the important studied plants with antiplatelet aggregating potency are *Panax ginseng* C.A. Mey., *Allium sativum* L., *Ginkgo biloba* L., *Matricaria chamomilla* L., *Angelica sinensis* (Oliv.) Diels, *Camellia sinensis* L., *Salvia miltiorrhiza* Bunge, *Zingiber officinale* Roscoe^[21]. Among natural compounds quercetin, myricetin, chrysin, naringin, naringenin, hesperidin and apigenin are noted for their hemolytic activities^[22,23]. Catechins from green tea (*Camellia sinensis*) can inhibit *in vitro* platelet aggregation induced by COL, AA and U46619 (9,11-dideoxy-9a,11a-methanoepoxy-prostaglandin F2a) and *ex vivo* induced by

AA.^[14] The whole fruit from *Paullinia cupana* has antiaggregant properties *in-vitro* and *in-vivo*. Active principles vicine and covicine from aqueous extracts of broad bean (*Vicia faba*) were correlated with whole blood glutathione levels for their hemolytic activity^[24]. Mono, di, tri and tetra sulphides derived from various allium plants e.g. *Allium cepa* and *Allium sativum* have been recorded with hemolytic activities^[25]. Thus the hemolytic potency of the leave extracts from this plant can be due to the presence of various phytochemicals whose presence has been shown qualitatively.

This is the first time where the plant has been proved for its hemolytic activities. Thus *Allium stracheyi* Baker can be a potent natural source for antiplatelet aggregating agent.

Abbreviations:

PE: Petroleum ether extract

BEN: Benzene extract

BUT: Butanol extract

EAC: Ethyl acetate extract

ETH: Ethanol extract

AQ: Water extract

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