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Original Article

SCREENING OF PHYTOCHEMICAL CONSTITUENTS OF THE LEAVES OF *CLINACANTHUS SIAMENSIS* BREMEK AND *CISSAMPELOS PAREIRA* L USED AS ANTIDOTE FOR SNAKE BITE IN INDIGENOUS MEDICINE

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ABSTRACT

Objective: The study is aimed to screen the phytochemical constituents of the medicinal plants *Clinacanthus siamensis* Bremek and *Cissampelos pareira* L used as anti snake venom in tribal medicine.

Methods: The phytochemical compounds such as proteins, carbohydrates, lipids, phenols, tannins, flavonoids, saponins, steroids, terpenoids, coumarins, anthocyanin, glycosides, reducing sugars, lignins, anthoquinone and alkaloids were extracted by standard methods and compared the components for its antivenom activity.

Results: Qualitative analysis of methanolic and aqueous extracts of leaves confirmed the presence of primary metabolites like proteins, carbohydrates, lipids and reducing sugars and secondary metabolites like phenols, tannins, flavonoids, glycosides, saponins, steroids, terpenoids, coumarins, lignins, anthocyanin, anthoquinone and alkaloids.

Quantitative estimation of primary and secondary metabolites showed that the presence of proteins, flavonoids, tannins, alkaloids and lipids are more in *Cissampelos pareira* L when compared to *Clinacanthus siamensis* B. whereas the phenols and carbohydrates were more in *Clinacanthus siamensis* B.

Conclusion: The study helped in the successful screening of phytochemical constituents which supports the traditional knowledge of the use of the plants as important medicine, as an antidote for poisonous snake bites and in curing various ailments.

Keywords: Traditional medicinal plants, Phytochemical constituents, Secondary metabolites, Antidote, Snake bite.

INTRODUCTION

Clinacanthus siamensis Bremek, a medium sized perennial shrub endemic to western ghats grows up to 2 meters in height[1]. Leaves simple, opposite, acute or acuminate and slightly serrated, crowded at the end. Stem round, green coloured, and turns grey when old. Plant pacifies vitiated kapha, pitta, poison bites, inflammation, traumatic oedema and swelling due to poison stings [2]. Used as anti snake venom, in the treatment of diabetes, herpes infection, effective against influenza, gives immune response [3, 4]. It also has anti-inflammatory and antiarthritic properties [5]

Cissampelos pariera L is a medicinal climber endemic to Tropical regions. The plant has a great demand in tribal and ayurvedic system of medicine. The plant is antispasmodic, antihemorrhagic, muscle relaxant, uterine relaxant, hypotensive etc: The leaves of the plant are light green in colour. Stem is flexible and slender and twines for support. The root system consists of light brown lateral roots with abundant fine roots. Flowers are bisexual, with yellow to white in colour. Fruits are juicy red or yellow, hairy drupes [6]. The plant is also used as antidote for poisonous snake bite by the tribes of Amazon region [7]. Studies showed that a number of alkaloids and flavanoids are present in the plant. Ethanomedical and traditional uses of the plant is antidote for poisonous snake bites, analgesic, antiseptic, aphrodisiac, cardiotonic, diaphoretic, hepatoprotective, stimulant, tonic etc [8].

The phytochemicals are naturally occurring and biologically significant chemical compounds in plants. They may be primary or secondary. Primary constituents like carbohydrates, lipids, aminoacids and its compounds are directly involved in the plant metabolism whereas secondary metabolites like phenolic compounds may directly or indirectly help in the metabolism in plants [9]. Leaf juices of both the plants under study are used as antidote for sanke venom in traditional and tribal medicine [10]. The phytochemicals mostly responsible for this are polyphenols, ellagic acid, alkaloids, steroids, saponins, glycosides and tannins. In places where poisonous snakes live, several plant species were reported to be used for treatment of ophidian envenomation, by the local people [11,12,13]. A number of plants were screened for secondary metabolites for their medicinal values like *Svensonia hyderobadensis* [14], *Boswellia ovalifoliolata* [15], *Allamanda catharitica* [16] and *Cochlospermum religiosum* [17] *Shorea thunbergia* [18], *Thespesia populnea* [19], *Catheranthus roseus* [20] etc.

This study is aimed at the extraction and comparison of various phytochemical constituents responsible for its use as anti snake venom from the leaves of the two plants.

MATERIALS AND METHODS

Clinacanthus siamensis Bremek was collected from the natural habitats of Western Ghats (Kerala) and *Cissampelos pariera* L was collected from the locally available habitats of Thiruvananthapuram. Both the plants were compared with the preserved species in the Botanical Garden of Panchakarma Ayurvedic Research Centre, Thiruvananthapuram. The plants were planted in S.V.U. Botanical Garden. Further Taxonomic identification of the plants was studied with the help of Gamble [21].

Fresh and healthy leaves of these plants species were collected and cleaned with distilled water and wiped dry with blotting paper. The leaves were shade dried for a week and then ground to make a fine powder. Phytochemical screening was carried out with these dry powders by using the methods of Peach and Tracey [22], Gibbs [23], Trease and Evans [24] and Harborne [25] and also quantitative analysis of proteins [26], carbohydrates [27], lipids [28], phenols [29], flavonoids [30], alkaloids [31] and tannins [32] etc. were done.

RESULTS AND DISCUSSION

The phytochemical analysis and qualitative studies showed that the leaves of both the plants contain sufficient amount of carbohydrates,

proteins, lipids, phenols, flavanoids, alkaloids and tannins. The results are summarized in Table-1. It was noted that the methanolic extract of the leaf juice of both the plants showed maximum number of phytochemical constituents when compared with the aqueous extract of the same. The analysis shows that the amount of proteins, lipids, flavanoids, tannins and alkaloids were more in *C.par*eira when compared to that of *C.siamensis*. The carbohydrate and phenolic compounds were more in *C.siamensis*.

Table 1: Phytochemical Screening of leaves of *Cissampelos pariera* L and *Clinacanthus siamensis* Bremek

S. No.	Phyto chemicals	*C.par*eira		*C.siamensis*	
		Methanolic extract	Aqueous extract	Methanolic extract	Aqueous extract
1	Carbohydrates	+	+	+	+
2	Proteins	+	+	+	+
3	Lipids	+	-	+	-
4	Flavanoids	++	-	+	-
5	Saponins	-	-	-	-
6	Phenols	+	-	++	-
7	Steroids	+	-	++	-
8	Tannins	++	-	++	-
9	Terpenoids	-	-	+	-
10	Coumarins	+	-	+	-
11	Anthocyanin	-	-	+	-
12	Glycosides	+	-	++	-
13	Alkaloids	+	-	+	-
14	Reducing sugars	+	+	+	+
15	Lignins	+	-	+	-
16	Anthoquinone	-	-	-	-

Note: '+' – indicates presence: '-' – indicates absence

Studies showed that glycoproteins isolated from *Withania somnifera* were able to inhibit the cobra venom [33]. Sufficient amounts of proteins were screened in both *C.par*eira and *C.siamensis* to explore the possibility of proteins being used as antivenom. Flavanoids have the ability to bind to biological polymers and can inhibit the effect of toxins.

Flavanoid content in *C.par*eira leaf is more than that of *C.siamensis* (Table- 2). Effectiveness of flavanoids in inhibiting the venom was reported in *Primula denticulate* [34]. Flavonoids also have many useful properties like anti-inflammatory, antimicrobial, enzyme inhibition, oestrogenic, antiallergic, antioxidant and anti- tumour activity [35,36].

Table 2: Quantitative Analysis of phytochemical constituents of leaves of *Cissampelos pariera* and *Clinacanthus siamensis*

S. No.	Biochemical Constituents	*C.par*eira	*C.siamensis*
1	Proteins	0.9 ± 0.05	0.13 ± 0.03
2	Carbohydrates	0.06 ± 0.05	0.09 ± 0.02
3	Lipids	0.27 ± 0.09	0.19 ± 0.03
4	Flavanoids	0.37 ± 0.16	0.25 ± 0.02
5	Phenols	0.33 ± 0.08	0.42 ± 0.06
6	Tannins	0.44 ± 0.07	0.42 ± 0.06
7	Alkaloids	0.08 ± 0.06	0.06 ± 0.01

'±'Values are the average of triplicates standard error.

Effect of alkaloids as antidotes for snake venom was reported and used from various plants [37]. Presence of alkaloids like seeperine, bebeerines, cissampellin etc. in the leaves of *C.par*eira are effective against snake bite [38]. Almost equal amount of alkaloids were found to be present in both the plants and the use of alkaloids in *C.siamensis* as potential snake venom is explorable. Phenolic compounds and tannins can bind to proteins and can directly act on venom constituents [39]. Presence of phenols was more in C.siamensis and tannins were present almost in the same amount in both the species of *C.par*eira and C.siamensis. The phenols have been shown to contain higher levels of antioxidant activities [40]. Saponins and steroids from different plants have demonstrated anti snake venom activity in lab tests [41]. Qualitative analysis has shown the presence of steroids in both the plants. Tests for saponins were negative with the aqueous and methanolic extracts of leaves in these plants.

CONCLUSION

In reviewing the above analysis it can be concluded that the secondary metabolites in the plants can be used as potential antivenoms in snake bite. Exploration of this pharmacological property needs further investigation of active ingredients by implementation of techniques like extraction, purification, separation, crystallization and identification. Studies on *C.par*eira plant extract for its anti-hemorrhagic and antiproteolytic activities against *Bothrops asper* venom have been reported [42]. The use *C.siamensis* as potential antidote needs to be further studied.

CONFLICT OF INTERESTS

Declared None

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