

Determination of Anthelmintic Activity of the Root extract of *Orthosiphon thymiflorus* (Roth)

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Abstract

Anthelmintic activity for the *Orthosiphon thymiflorus* (Roth) root was studied with the aim of drawing the pharmacological standards for this species. The study also deals with the proximate chemical investigation of the roots with various extracts such as petroleum ether to water. The anthelmintic property of the methanolic extract of plant carried out using *Pheretima posthuma* as a test worm. Major observation in present bioassay was determination of time of paralysis and time of death. The proximate chemical investigation results showed that the presence of carbohydrates, protein, glycosides- flavonoid, phenols, tannins, terpenoids and keto-steroids. The glycosides, tannins and terpenoids may be responsible for anthelmintic activity. The presence study includes anthelmintic activity of *Orthosiphon thymiflorus* whole plant first time.

Key words *Orthosiphon thymiflorus*, flavonoids, terpenoids, anthelmintic.

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Introduction

Orthosiphon is a genus of plants in the Labiatae family native to Africa, Southern Asia and Queensland, with one species (*O. americanus*) in Colombia. It is an herbaceous shrub which grows to a height of 1.5 m (5 ft). *Orthosiphon* is a popular garden plant because of its unique flower, which is white and bluish with filaments resembling a cat's whiskers. In the wild, the plant can be seen growing in the forests and along roadsides. *Orthosiphon thymiflorus* is a medicinal plant, slightly aromatic sub-shrub commonly seen in India, It is grown in Hills above 600m on the slopes, in crevices of rocks; more numerous by arable lands, etc. It is common plant found at western ghat, Maharashtra, India (Anonymous,

2001). It have a number of pharmacological uses as, aqueous extract of leaves is reported to have diuretic activity in rats and acetylcholine antagonistic activity in frog skeletal muscle contraction, also have antioxidant activity (Sundarammal S *et al.*2012). It is antidiarrheal, alexiteric, vulnerary and febrifuge. Even though it has been widely used in medicinal preparations and in folk medicines (Bashpa P, *et al.*, 2017; Rastogi RN, 1986). There is no anthelmintic activity reported on *Orthosiphon thymiflorus* and proximate chemical profile together.

Materials and Methods**Procurement of Plant Material**

Roots of plant *Orthosiphon thymiflorus* was collected from 'Jejuri' region, Maharashtra, India, in the month of Aug-Sep 2019. Botanical

identification was carried out and voucher specimen of the plant material has been deposited at Institute level.

Preparation of Plant Material

Fresh roots of plant *Orthosiphon thymiflorus* are shade dried and powdered was prepared by passing through sieve # 40, and kept in air tight polythene bags for further study.

Collection of Worm

The Indian earthworms were collected from water logged area of soil. The collected earthworm authenticated as a *Pheretima posthuma* (Indian earthworm), are long cylindrical worms of brownish colour.

Chemicals and Instruments

Solvents and reagents were procured from Pallav Chemicals and Solvents Pvt. Ltd., Mumbai, India. Soxhlet's apparatus and other common glassware and instruments used for the study.

Preparation of Extract

The dried plant material of *Orthosiphon thymiflorus* extracted using Soxhlet's apparatus and methanol as a solvent. The extract concentrated and dried at room temperature accordingly to remove traces of solvent, finally stored in desiccators for further study (Khandelwal KR, 2005).

Phytochemical Investigation

The successive extractive values carry out as per the procedure of C. K. Kokate, 1994).

Anthelmintic Assay

The anthelmintic assay carried out as per procedure of Gururaja M.P. et al., 2009. The

different concentration of alcoholic extract of *Orthosiphon thymiflorus* (10, 20, 40 mg/ml) and piperazine citrate (10mg/ml) as a standard were prepared. Then formulations were prepared of all concentration of sample and Standard by triturating them with 50 ml 15% tween 80. After triturating the formulation mix well for 30 minute using a mechanical stirrer. The earthworms kept in petridish the sample and standard tested separately, 15% tween 80 with distilled water is a negative control. Observation was made for time of paralysis and time for death required for earthworm. When there is no movement after vigorous shaking of petridish with earthworm consider as a time of paralysis. Time for death also noted after ascertaining that worm neither moved when shaken vigorously nor when dipped in warm water (50°C).

Results and Discussion

Phytochemical Investigation the presence of primary and secondary metabolites as carbohydrates, protein, glycosides- flavonoid, phenols, tannins, terpenoids and keto-steroids (Table 1). In dose dependant manner the anthelmintic activity of methanolic extract of *Orthosiphon thymiflorus* observed. At dose 40mg/ml the *Orthosiphon thymiflorus* showed paralysis in 23.13 minute and death in 41.11 minutes compared to the reference standard Piperazine citrate (10mg/ml) showed the paralysis at 20.33 minutes and death at 45.25 minutes (Table 2).

Conclusion

These data and parameters have been investigated for *Orthosiphon thymiflorus* to set pharmacological and phytochemical standards which could be useful to find the authenticity of this traditional medicinal system plant. In conclusion the use of *Orthosiphon thymiflorus* roots as an anthelmintic has been confirmed and further studies are suggested to isolate the active principle responsible for the anthelmintic activity.

Table 1: Phytochemical Investigation of *Plectranthus mollis*

Phytoconstituents	Extract		
	Pet. ether	Ethanol	Water
Glycosides	-	+	-
Flavonoid	-	+	-
Steroids	+	-	-
Tannins	-	+	+
Phenolic Substances	-	+	+
Terpenoids	+	-	-
Carbohydrates	-	-	+
Protein	-	-	+

+ present; - absent

Table 2: Anthelmintic Activity of *Hyptis suaveolens*

Substance	Conc. mg/ml)	Paralyzing time (min)	Death time (min)
Alcoholic Extract	10	41.17	63.20
	20	32.33	54.33
	40	23.33	41.11
Piperazine citrate	10	20.33	45.25
15% Tween	--	--	--

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