Int J Pharm Bio Sci 2014 April ; 5 (2) : (B) 580 - 587

Research Article **Allied Sciences**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

PRELIMINARY PHYTOCHEMICAL ANALYSIS AND ANTHELMINTIC ACTIVITY OF THE AQUEOUS EXTRACT OF *OCIMUM SANCTUM* (LINNAEUS, 1767) LEAVES (GREEN AND BLACK) AGAINST *COTYLOPHORON COTYLOPHORUM* (FISCHOEDER, 1901)

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ABSTRACT

One of the major sources of wool and meat production is sheep. But sheep are affected mostly by helminthiasis, the most important animal disease which leads to heavy production loss. *Cotylophoron cotylophorum* is one of the trematode parasites that cause helminthiasis in sheep. Since chemotherapeutic anthelmintics create resistance and side effects, there is a need for an alternative plant based anthelmintics, which could be effective in expelling these worms. *Ocimum sanctum*, which is the most sacred herb of India, is of two varieties Rama Tulsi (green) and Krishna Tulsi (black). Apart from religious significance it has got amazing number of health benefits. The present investigation deals with the comparison of phytochemical constituents and anthelmintic activity in the aqueous extract of the two leaf varieties (green and black) of *O. sanctum* against *Cotylophoron cotylophorum*. The results of the preliminary phytochemical constituents revealed that the secondary metabolites like triterpenoid, flavonoid, phenol, tannin, alkaloid, saponin and acid were present and steroid was absent in both leaf varieties (green and black) of *O. sanctum*. Though, dose dependent anthelmintic activity was observed in both the leaf varieties, black leaves are with more efficacy than green.

KEYWORDS: ***Ocimum sanctum*, *Cotylophoron cotylophorum*, anthelmintic, helminthiasis and phytochemicals.**

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INTRODUCTION

In today's world, more than half of the human population suffers from various types of infection while majority of cattle suffer from worm infections [1]. Sheep are primarily useful for wool and meat production. But they are affected by various parasitic diseases. Helminthiasis is the most important animal disease inflicting heavy production loss. This disease is highly prevalent, particularly in third world countries [2], due to poor management. Chemical control of helminthes coupled with improved management has been the worm control strategy throughout the world. Because of increasing anthelmintic resistance with conventional anthelmintic compounds it is important to look for an alternate strategy against gastrointestinal helminthics. India is one of the countries in the world which is rich in large varieties of medicinal plants. Medicinal plants have served through the ages, as a constant source of medicaments, for the treatment of variety of diseases [3].Herbs are still, widely used as a remedy for many diseases in this advanced technological world. Since plant based drugs are easily available at low cost and are comparatively safe, people have good faith in such remedies and hence, WHO encourages such herbal remedies in National health care programmes.

The medicinal plants are rich in phytochemicals (secondary metabolites) and essential oils of therapeutic importance. The most important of these phytochemicals are alkaloids, tannins, flavonoids and phenolic compounds [4 and 5]. A number of medicinal plants have been used to treat parasitic infections in man and animals [3, 6, 7 and 8]. *Ocimum sanctum* (Tulsi) which is considered as "Queen of herbs" is held sacred by Hindus and is used as medicinal plant in day to day practice in Indian homes for various ailments [9]. Among the plants known for medicinal value, *O. sanctum* leaves have also been used as anthelmintic agent[10]. Rama Tulsi, with green leaves and Krishna Tulsi with deep magenta or purple leaves which is also called as black Tulsi [11], are the two varieties of *O. sanctum. Cotylophoron cotylophorum* is a digenetic trematode that parasitizes the rumen and reticulum of livestock. Adults destroy part of rumen and cause inflammation of the intestine [12]. Since, chemotherapeutic anthelmintics create resistance, relative toxicity and side effects, an alternative plant based anthelmintics are used to destroy and expel parasitic worms from the gastrointestinal tract [13 and 14]. Since, there is no previous report on the anthelmintic activity of *O. sanctum* against *C.cotylophorum,* in the present investigation it is attempted with the aqueous extract of two *O.sanctum* leaf varieties (green and black). Phytochemical screening of the secondary metabolites was also carried out to substantiate the anthelmintic activity of *O. sanctum* varieties.

MATERIALS AND METHODS

(i) Collection and authentication of O. sanctum

Green and black *O. sanctum* leaf varieties used for various purposes in day to day life of Indians were collected from local areas of Chennai and Thiruvallur, Tamil Nadu, India. After identifying the leaf varieties by its aromatic odour and morphological features, they were authenticated by Dr. A. Manoharan, Head of the Department of plant biology and biotechnology, Presidency College, Chennai, India.

(ii) Preparation of extracts

The fresh leaves of both varieties (green and black) of *O. sanctum* were shade dried in normal environmental condition and coarsely powdered. In 1.5 litres of water, 100 grams of this powder was soaked for 24 hours with frequent mixing. The preparation was filtered through a Whatman filter paper and the collected filtrate was concentrated at 100º C in a water bath.

(iii) Preliminary phytochemical analysis

Organic phytochemicals like steroid, triterpenoid, flavonoid, phenol, tannin, alkaloid, saponin and acid were identified according to the standard methods [15 and 16] in the aqueous extract of *O. sanctum.*

(iv) Collection of C. cotylophorum and selection of survival medium

C. cotylophorum was collected from the rumen of the sheeps autopsied at the Corporation slaughter house, Pulianthope, Perambur, Chennai. The species identification was done as per the diagnostic keys [17 and 18]. Live worms were collected and kept in normal saline till they are transferred to the laboratory. Hedon-Fleig solution [19] was selected as the survival medium for this study, as it was reported to be a suitable medium for *C. cotylophorum* in this laboratory [20].

(v) Evaluation of anthelmintic activity in the aqueous extract of green and black leaves of O. sanctum against C. cotylophorum for 24 and 48 hours

Adult specimens of *C. cotylophorum* irrespective of sex with uniform size were selected from the collection and acclimatized in Hedon-Fleig solution (pH 7) for 6 hours. After acclimatization a group of ten trematodes were transferred to 25 ml of freshly prepared Hedon-Fleig solution containing different concentrations of the aqueous extract of *O. sanctum* ranging from 10 to 80 mg at an interval of 10 mg for green and black *O. sanctum* leaves. For the higher concentration the extract was taken in the multiples of hundred from 100 to 600 mg. Percentage survival of *C. cotylophorum* at different concentrations was noted at an interval of every 2 hours. The lethal, median and sub lethal concentrations of *O. sanctum* aqueous extract were noted for an exposure period of 24 and 48 hours separately and were tabulated. Hedon-Fleig solution was replaced freshly after 24 hour.

RESULTS

Triterpenoid, flavonoid, phenol, tannin, alkaloid, saponin and acid are the organic secondary metabolites reported to be present in the aqueous extract of both the leaf varieties (green and black) of *O. sanctum,* while steroid is absent (Table 1).

Table 1
Preliminary phytochemical analysis of organic substances in the aqueous extracts of O. sanctum leaves (green and black)

S.No.	Organic substances	Aqueous extract of *O.sanctum*	
		GL	BL
1	Steroid	-	-
2	Triterpenoid	+	+
3	Flavonoid	+	+
4	Phenol	+	+
5	Tannin	+	+
6	Alkaloid	+	+
7	Saponin	+	+
8	Acid	+	+

(+) – Present ***GL – Green Leaves***
(-) – Absent ***BL – Black Leaves***

In 24 hours survival study with *C. cotylophorum*, 100% mortality occurred in 600mg extract /10 parasites/ 25ml Hedon-Fleig solution; 50% mortality occurred in 400mg extract/10 parasites/ 25ml Hedon-Fleig solution and no mortality occurred in 100mg extract /10 parasites/ 25ml Hedon-Fleig solution. These concentrations are considered respectively as lethal, median and sub lethal concentrations of green *O. sanctum* leaf extract (Table 2 and Figure 1).

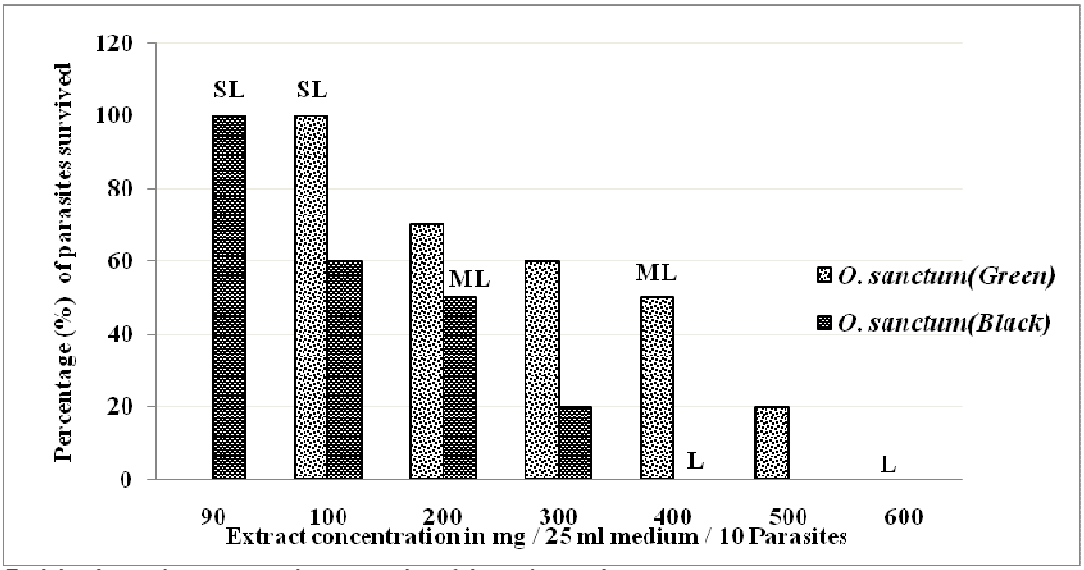
Table 2
Evaluation of anthelmintic activity of O. sanctum leaf varieties (green and black) against C. cotylophorum for 24 hours

S. No.	Extract concentration in mg / 25 ml medium / 10 Parasites	Percentage (%) of parasites survived		
		GL	BL	
1	90	-	100	**SL (BL)**
2	100	100	60	**SL (GL)**
3	200	70	50	**ML (BL)**
4	300	60	20	
5	400	50	0	**ML (GL),L(BL)**
6	500	20	-	
7	600	0	-	**L (GL)**

GL – Green Leaves, BL – Black Leaves, SL – Sub lethal, ML – Median lethal, L - Lethal

When the survival test period is increased to 48 hours 80, 50 and 10mg extract /10 parasites/ 25ml Hedon-Fleig solution are recorded as lethal, median and sub lethal concentrations for green leaves respectively (Table 3 and Figure 2). Whereas, for black variety *O. sanctum,* 24 hours lethal, median and sub lethal concentrations are 400, 200 and 90mg extract /10 parasites/ 25ml Hedon-Fleig solution respectively (Table 2 and Figure 1).

Figure 1
Anthelmintic activity of O. sanctum leaf varieties (green and black) against C.cotylophorum for 24 hours



Each bar in graph represents the mean value of three observations
SL – Sub lethal, ML – Median lethal, L - Lethal

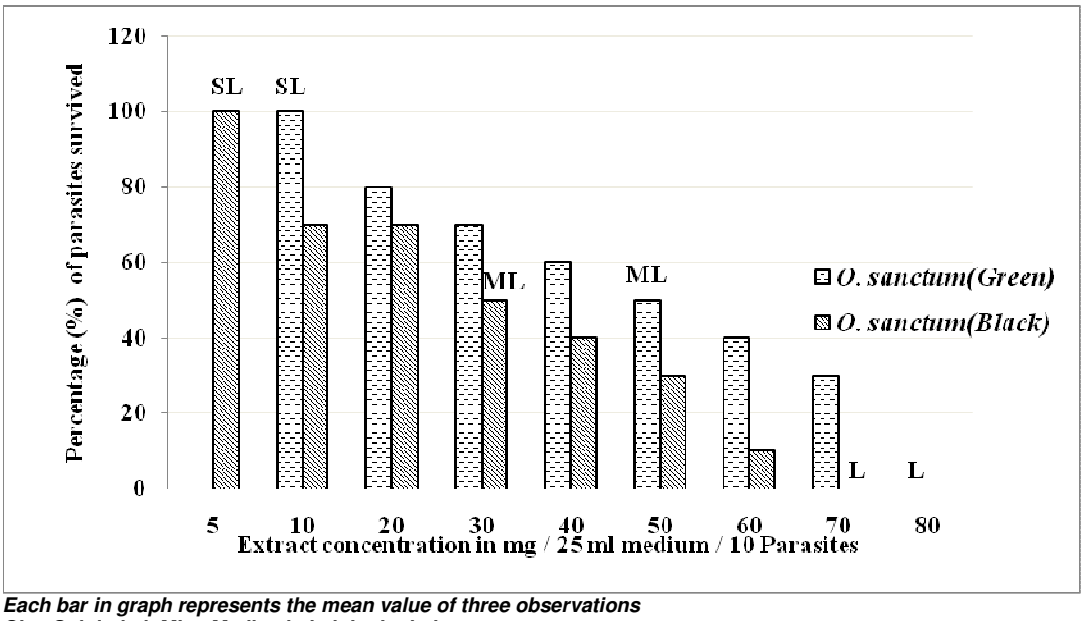
By extending the study period to 48 hours, the black leaf extract recorded lethal, median and sub lethal concentrations at 70, 30 and 5mg extract/10 parasites/ 25ml Hedon-Fleig solution respectively (Table 3 and Figure 2).

Table 3
Evaluation of anthelmintic activity of O. sanctum leaf varieties (green and black) against C. cotylophorum for 48 hours

S. No.	Extract concentration in mg / 25 ml medium / 10 Parasites	Percentage (%) of parasites survived		
		GL	BL	
1	5	-	100	SL (BL)
2	10	100	70	SL (GL)
3	20	80	70	
4	30	70	50	ML (BL)
5	40	60	40	
6	50	50	30	ML (GL)
7	60	40	10	
8	70	30	0	L (BL)
9	80	0	-	L (GL)

GL – Green Leaves, BL – Black Leaves, SL – Sub lethal, ML – Median lethal, L – Lethal

Figure 2
Anthelmintic activity of O. sanctum leaf varieties (green and black) against C.cotylophorum for 48 hours



Each bar in graph represents the mean value of three observations
SL – Sub lethal, ML – Median lethal, L - Lethal

Anthelmintic activity of the two varieties (green and black) of *O. sanctum* leaf aqueous extracts is dose dependent. It is also reported from the present result that the aqueous extract of black *O. sanctum* leaf is more effective against trematodes than the green *O. sanctum*.

DISCUSSION

Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas[21]. According to circumstances, depending on their efficacy, plant anthelmintics offer sustainable and environmentally acceptable alternate that can overcome problems of parasitic infections. Earlier works [22, 23 and 24] also reports dose dependent activity of their plant extract against the worms selected. Anthelmintic activity of *O. gratissimum* essential oil and eugenol has been reported against *Haemonchus contortus*, which is a gastrointestinal nematode of small ruminents[25]. Anthelmintic activity of the essential oil of *O. sanctum* and eugenol was

evaluated against *Caenorhabditis elegans* [26]. However, regarding the anthelmintic activity of *O. sanctum* leaf varieties (green and black) against *C. cotylophorum*, this work is first of its kind. The secondary metabolites reported in the present investigation may be responsible for the anthelmintic activity as reported earlier [27 and 28].The different degree of anthelmintic activity of the aqueous extract of *O. sanctum* (green and black) might be due to the level of tannins present [29]. The varying levels of effectiveness of the two varieties of *O. sanctum* aqueous extract could also be due to the varying contents of total alkaloids [30]. It is also possible that phenolic content in the extracts of *O. sanctum* leaf may contribute to the anthelmintic activity.

Several anthelmintic agents, including certain plant extracts, have been reported to cause considerable structural alteration against paramphistomes [31 and 32] during deworming. Similar effect could also be expected from *O. sanctum* leaf extracts. Further histochemical reports may substantiate this assumption. In the present investigation, though both the leaf varieties (green and black) of *O. sanctum* has got similar phytochemical constituents, the anthelmintic activity of black *O. sanctum* leaf variety was found to prove more effective. This might be due to the variation in the quantity of the phytochemicals responsible for anthelmintic activity [33]. Thus the present investigation suggests that *O. sanctum* leaf extract could be an effective helminthicide against parasitic infection, in particular the black leaf variety.

CONCLUSION

O. sanctum (Tulsi) which is regarded as a kind of “Elixir of life” in Ayurveda has got various therapeutic potentials. Recognising the importance, the World Health Organisation has recommended that traditional health and folk medicine system to be integrated with modern medicinal therapies, which could effectively overcome the health problems worldwide. In future it is necessary to identify and isolate the active phyto-constituents responsible for anthelmintic activity of black *O. sanctum* leaves and study its pharmacological action which will be useful to human beings.

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