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In Vitro Anthelmintic Activity of *Allium sativum, Zingiber officinale, Curcurbita mexicana and Ficus religiosa*

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ABSTRACT

Methanol extracts of some commonly used plant materials of ethnoveterinary importance in Pakistan were screened for their *in vitro* anthelmintic activity. Results revealed that *Zingiber officinale* killed all the test worms (*Haemonchus contortus*) within two hours post exposure being 100% effective. *Allium sativum* and *Cucurbita mexicana* extracts were equally effective at 2 and 4 h post exposure; by 6 h post exposure, however, the earlier was 100% effective; whereas, *C. mexicana* could not kill all the worms and was 83.4% effective. *Ficus religiosa* was 100% effective by 4 h post exposure, and was as good as *A. sativum* and *Z. officinale* by 6 h post exposure. Majority of the worms exposed to control (normal saline) remained alive till 4 h post exposure, and thereafter, 50% of them died by 6 h post exposure. It was concluded that all the studied plants had some anthelmintic activity, therefore, *in vivo* trials may be conducted for further evidence for their use in animals on scientific basis.

Key Words: Anthelmintic activity; *Haemonchus contortus*; *Allium sativum; Zingiber officinale; Ficus religiosa; Cucurbita mexicana*

INTRODUCTION

Development of anthelmintic resistance in helminths reported in a number of countries (Jackson, 1993; Sanyal, 1996; Rolfe, 1997; van Wyk *et al*., 1997; Waller, 1997) gives a clear indication that control programs based exclusively on their use are not sustainable. The development of integrated programs to control helminths is vital, but such control programs require viable alternatives to the use of anthelmintics (Waller, 1999). Medicinal plants have served through ages, as a constant source of medicaments for the exposure of a variety of diseases. The history of herbal medicine is almost as old as human civilization. The plants are known to provide a rich source of botanical anthelmintics, antibacterials and insecticides (Satyavati *et al.,* 1976; Lewis & Elvin-Lewis, 1977). A number of medicinal plants have been used to treat parasitic infections in man and animals (Nadkarni, 1954; Chopra *et al.,* 1956, 1958; Said, 1969; Akhtar, 2000). The present study was conducted to evaluate the *in vitro* anthelmintic activity of some palnt materials commonly used in ethnoveterinary medicine in Pakistan.

MATERIALS AND METHODS

Preparation of extracts. Each material (Table I) was thoroughly cleaned with water and dried under shade for five to seven days until it became grindable. The dried materials were ground with the help of electric grinder and separately preserved for further use. About 200 g of each plant material was used for extraction. Each plant material was boiled for 24 h in 500 mL methanol in Soxhlet's apparatus. The extracts thus obtained were placed in small test tubes separately and stored in a refrigerator for further use.

Table I. Plant materials evaluated for their anthelmintic activity

Botanical name	Family	Parts used	Common Names
Allium sativum (a bulbous herb)	Liliaceae	Bulb	Garlick, Lehsan, Lassan, Lasun, Lashan
Zingiber officinale (a rhizomatous herb)	Zingiberaceae	Rhizomes	Ginger, Adrak, Sonth (dried), Ada
Curcurbita mexicana (a trailing herb)	Cucurbitaceae	Whole fruit	Kuddu
Ficus religiosa (a large tree)	Urticaceae	Bark	Pippal, Pipla, Pipur, Ashatwa, Aswat, Asvatha

Evaluation of *in vitro* anthelmintic activity. *In vitro* anthelmintic activity of the plant materials was evaluated by exposing the adult *Haemonchus contortus* to undiluted extracts of plants. For each extract, five petridishes were used i.e. four for extract to be tested and one for normal saline as control. Adult motile *H. contortus* were collected from the gastrointestinal tract of sheep slaughtered at Faisalabad slaughterhouse and immediately transferred to the petridishes containing plant extracts and normal saline. Observations were made on the motility/survival of worms at 0, 2, 4 and 6 h post-exposure (PE).

The number of worms dead at 6 h PE to various plant extracts was compared statistically with those exposed to

control group by z-test using Microsoft Excel 2000 program.

RESULTS AND DISCUSSION

It is evident from Table II that *Zingiber officinale* killed all the worms within two hours PE being 100% effective. *Allium sativum* and *Cucurbita mexicana* extracts were equally effective at 2 and 4 h PE; however, by 6 h PE, the earlier was 100% effective; whereas, *C. mexicana* could not kill all the worms and was found 83.4% effective, which was not different ($P < 0.05$) from control. *Ficus religiosa* was 100% effective by 4 h PE, and was as good as *A. sativum* and *Z. officinale* by 6 h PE. Majority of the worms exposed to control (normal saline) remained alive till 4 h PE; therefater, three out of six worms were found dead. The justification as to why the aforesaid plant materials exerted lethal effect on *H. contortus* is not known by now. However, following information on the use, chemical composition and available evidence suggest detailed studies to generate more precise knowledge about these materials.

Allium sativum. Garlic is mentioned in the Bible and the Talmud. Its historical or traditional use (may or may not be supported by scientific studies) has been mentioned by Hippocrates, Galen, Pliny the Elder, and Dioscorides for many conditions, including parasites, respiratory problems, poor digestion, and low energy. Its use in China was first mentioned in A.D. 510. Louis Pasteur confirmed the antibacterial action of garlic in 1858. It is mildly antihypertensive (Silagy & Neil, 1994) and has antioxidant activity (Kleijnen, 1989). Garlic has antibacterial, antiviral, and anti-fungal activity (Hughes & Lawson, 1991). The fresh bulbs are also cooked and eaten to treat asthma, colds, coughs, and to control worms. The alcoholic extract of bulb of *A. sativum* has also shown moderate *in vitro* anthelmintic activity against human *Ascaris lumbricoldes* (Kalesaraj, 1975). *A. sativum* has been reported to be effective in the exposure of dysentery and also act as vermifuge (Nadkarni, 1976; Schavenberg & Paris, 1977). Oil of *A. sativum* has also been reported to possess anthelmintic activity (Steenis-Kruseman, 1953; Hoppe, 1975; Nadkarni, 1976; Perry, 1980; Kirtikar & Basu, 1981) and discards all injurious parasites in the intestine (Nadkarni, 1976). Garlic is the best known source of selenium. The sulfur compound allicin, produced by crushing or chewing fresh garlic, in turn produces other sulfur compounds: ajoene, allyl sulfides, and vinyldithiins (Koch & Lawson, 1996).

Zingiber officinale. Ginger is a perennial plant and is considered to be the universal medicine in Ayurveda. Traditional Chinese medicine has recommended ginger for over 2,500 years. It is used for abdominal bloating, coughing, vomiting, diarrhea and rheumatism. Classified as an aromatic bitter, it stimulates digestion. It also keeps the intestinal muscles toned (Bradley, 1992).

The anthelmintic activity of alcoholic extracts of rhizomes of *Z. officinale* against human *Ascaris lumbricoldes* is appreciable (Kalesaraj, 1974; 1975). Goto *et al.* (1990) reported lethal effect of *Z. officinale* on Anisakis larvae *in vitro*. The antifilarial effect of *Z. officinale* against *Dirofilaria immitis* has been reported by Datta and Sukul (1987). Adeewunmi *et al.* (1990) have reported molluscicidal and antischistosomal activities of *Z. officinale*. The dried rhizome of ginger contains approximately 1–4% volatile oils. These are the medically active constituents of ginger, and they are also responsible for ginger's characteristic odor and taste. The aromatic principles include zingiberene and bisabolene, while the pungent principles are known as gingerols and shogaols (Tyler, 1994). There is speculation that the mechanism of action of ginger may be both central and peripheral i.e. anticholinergic and antihistaminic (Quian & Liu, 1992).

Cucurbita mexicana. The aqueous, etheral and alcoholic extracts of *C. mexicana* seeds have exhibited good

Table II. Effect of methanol extracts of different plants on the motility/survival of *Haemonchus contortus*

Botanical name of plant (Common name)	Time post exposure (hours)				Efficacy (at 6 hours post exposure)
	0	2	4	6	
Allium sativum (Garlick)	Alive=6	Alive=4	Alive=2	Alive=0	100%*
	Dead=0	Dead=2	Dead=4	Dead=6	
Zingiber officinale (Ginger)	Alive=6	Alive=0	Alive=0	Alive=0	100%*
	Dead=0	Dead=6	Dead=6	Dead=6	
Cucurbita mexicana (Kaddu)	Alive=6	Alive=4	Alive=2	Alive=1	83.4% NS
	Dead=0	Dead=2	Dead=4	Dead=5	
Ficus religiosa (Pipal)	Alive=6	Alive=6	Alive=0	Alive=0	100%*
	Dead=0	Dead=0	Dead=6	Dead=6	
Normal saline (control)	Alive=6	Alive=6	Alive=4	Alive=3	50%
	Dead=0	Dead=0	Dead=2	Dead=3	

* indicates significant ($P < 0.05$) difference compared with control independently; NS = Non-significant

anthelmintic activity against *Moniezia expansa, Fasciolopsis buski, Ascaris lumbricoides* and *Hymenolepis diminuta*. The order of decreasing potency of the extracts *in vitro* was aqueous, alcoholic and etheral in decreasing order (Shrivastava & Singh, 1967). Sharma *et al*. (1971) have reported significant *in vitro* effect of extracts of *Cucurbita pepo* on the motility of mature *H. contortus* of goat origin.

Ficus relegiosa. Like *A. sativum* and *Z. officnale*, *F. relegiosa* bark methanolic extract was also 100% lethal for *H. contortus* worms. Kaushik *et al.* (1981) evaluated stem and bark extracts of *F. relegiosa*, which proved lethal to *Ascaridia galli in vitro.* The latex of some species of *Ficus* (Moraceae) i.e. *Ficus inspida, F. carica* was also reported to have anthelmintic activity against *Syphacia obvelata, Aspiculuris tetraptera* and *Vampirolepis nana* (de Amorin, 1999). Pharmacological studies on *Ficus glabrata* latex with live *Ascaris* demonstrated a lethal effect at concentrations down to 0.05% latex in physiological saline solution (Hansson *et al.*, 1986). It has been accepted that anthelmintic activity is due to a proteolytic fraction called ficin. It is evident from above that methanolic extracts of *F. relegiosa* possibly exerted anthelmintic effect because of ficin.

CONCLUSION

It is concluded based on the findings of the present study that *Allium sativum*, *Zingiber officinale*, *Cucurbita mexicana* and *Ficus relegiosa* all possess varying degree of anthelmintic activity. However, dose and the form in which they be used require standardization. Moreover, phytochemical studies are also needed to lay down recommendation on scientific grounds.

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