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In vitro Adulticidal Efficacy of Albendazole, *Capparis decidua* stems and *Moringa oleifera* leaves against *Fasciola gigantica*

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ABSTRACT

The *in vitro* adulticidal effect of aqueous and methanolic extracts of *Capparis decidua* stems, *Moringa oleifera* leaves and albendazole was investigated against *Fasciola gigantica* worms. Three different concentrations of the plant extracts 1200, 600, 300 µg/ml and 1%, 0.5%, 0.25% of albendazole were used. Albendazole drug exhibited highest mortality ($P < 0.05$) after 11 hours. *C. decidua* stems methanolic extract showed significant ($P < 0.05$) toxic effect on *F. gigantica* adult worms after 13 h at 1200 µg/ml compared with the low concentration of albendazole (0.25%). Moreover, methanolic extract of *M.oleifera* leaves did not show any significant ($P > 0.05$) fasciolicide activity against adult worms at the concentrations used in this study compared with albendazole and the negative control. These results demonstrate that *C. decidua* stems may have anthelmintic activity especially at higher doses.

INTRODUCTION:

The use of herbal remedies for the prevention and treatment of a variety of illnesses in small animals has increased tremendously in recent years (Wynn and Fougere, 2007). Fasciolosis is an economically important liver disease of domestic livestock, in particular cattle and sheep and occasionally man. *Fasciola gigantica* is found in many countries primarily in tropical regions (Andrews, 1999). Fasciolosis especially the chronic form is one of the major anaemia causing helminths infections in the tropics. The disease causes considerable economic loss through mortality, organ condemnation at meat

inspection, reduced productivity and lowered resistance to other infections (Mathewos *et al.,* 2001).

Chemotherapy with drugs remains the most cost-effective way of treating parasitic diseases, and is usually at the heart of any major control campaign (Gaasenbeek *et al.,* 2001). Several drugs are used in Sudan for the treatment of fasciolosis, which have activities against adult flukes, and the parenchymal stages. These include rafoxanide, nitroxynil, closantel, albendazole. oxclozanide and triclbendazole. Albendazole is widely used for treating roundworms and fluke. It affects energy production in worms (Brander *et al.,* 1991).

However, anthelmintic resistance has become a major practical problem in many countries (Varady and Corba, 1999). This disadvantage has stimulated a search for alternative control methods, such as the use of traditional medicinal plants based on their wide use in natives' therapy (Donald, 1994).

Capparis decidua (Altundoub), belongs to the family Capparaceae, is widely distributed in northern and central Sudan especially on sandy soils and in low rainfall savanna on clays (El-Amin, 1990). Different parts of the plant were claimed to have various medicinal uses among the local population. The bark extract is used in asthma and cough and as diuretic, antigout, anti-inflammatory, astringent, stomachic, laxative, antidote and used for skin diseases (Al-Yahya, 1986; Rahman *et al*., 2004). The decoction of fresh twigs is taken against jaundice, the fumigation of the stems are used as anti-rheumatic (El Ghazali *et al*., 1997). Furthermore, Chahlia, (2009) reported that the alcoholic extracts of every tested part of *C. decidua*, manifests significant hypoglycemic activity. The extract of *C. decidua* has also been found to have hepatoprotective (Ali *et al*., 2009), antioxidant properties (Yadav *et al*., 1997) and anthelmintic (Mali and Mehta, 2008). Moreover, some members of the family Capparaceae e.g. *Capparis spinosa*, *Cleome icosandra* and *Gynandropsis gynandra* are claimed to possess anthelmintic property against *Fasciola gigantica* (liverfluke), *Taenia solium* (Tapeworm) and *Pheretima posthuma* (earthworm) (Mali and Mehta, 2008).

Moringa oleifera (Family *Moringaceae*), commonly called drumstick and horseradish tree and locally known as al-rawag. *M. oleifera* a multipurpose tree found almost all over the Asian and African countries and its fruit and leaves are consumed as food by the people (El-Amin, 1990). Studies have reported that the plant extract has a hepatoprotective action against antitubercular drugs such as isoniazid and rifampicin-induced liver injury (Pari and Kumar, 2002), antihypertensive (Faizi *et al*., 1995), anthelmintic (Rastogi *et al.,* 2009) antihyperglycemic (Kar *et al*., 2003), antioxidant (Siddhuraju and Becker 2003) a highly potent anti-inflammatory (Ezeamuzie *et al*., 1996), and also as antitumor (Guevara *et al*., 1999). The plant seed powder may be useful in chelation therapy (Kumari *et al*., 2006).

The preliminary phytochemical screening of the powdered materials of *C. decidua* stems and *M. oleifera* leaves revealed the presence of flavonoids, tannins, alkaloids, saponins, glycosides, terpenoids and cumarins (Ali *et al*., 2009; Ali *et al*., 2010). Several compounds are known to be active against a large range of organisms and could be responsible of anthelmintic activity such as saponins, trepenoids and

tannins (Garg, 1997; Deore and Khadabadi, 2010).

This study was carried out to evaluate *in vitro* efficacy of albendazole as fasciolicide drug and to investigate the fasciolicide activity of the aqueous and methanolic extracts of *Capparis decidua* stems and *Moringa oleifera* leaves which are known to have therapeutic effectiveness as anthelmintics in natives' therapy or according to the scientific researches (Mali and Mehta, 2008; Rastogi *et al.*, 2009).

MATERIALS AND METHODS:

Plants material

C. decidua stems and *M. oleifera* leaves were collected from Khartoum state, authentication was done at Medicinal and Aromatic Plants Research Institute (MAPRI) National Centre of Research (NCR), Khartoum, Sudan.

Plant extract

500 g of the material of plants were extracted by soxhlet apparatus using chloroform, petroleum ether, methanol (60 – 68 °C). The filtrates were then collected and evaporated using rotavaper. Aqueous extract was prepared by infusion method, the filtrate was collected and freeze dried to obtain a powdered extract. The residues percentages were calculated, the extracts were stored sheltered from light and humidity (Harborne 1984).

Collection of liver flukes

Adult worms of *F. gigantica* were collected in RPMI 1640 media (pH 7.2 – 7.8) at 37°C from the livers of infected cattle slaughtered for consumption at the local slaughter houses. After washing the flukes several times with RPMI 1640 media, the healthy ones with good motility were selected.

Adult worm motility assay

Fasciolicide activity was studied by *in vitro* petri dish method as described by Jiraungkoorskul *et al*., (2005) and Githiori *et al*., (2006). 0.5 g of crude extracts were first dissolved in 100 ml of normal saline (0.9%) stock solution. The mobile worms were put into plastic plates, 6 worms per plate in media containing RPMI only (negative control), other groups of RPMI media included dissolved different concentration of albendazole (Alb 1%, Alb 0.5%, Alb 0.25%) or different plant extracts; *M. oleifera* methanolic extract (MOM), *C. decidua* methanolic extract (CDM) and *C. decidua* aqueous extract (CDA) at the concentrations 1200, 600 and 300 µg/ml. All dilutions were performed in RPMI media plus penicillin (50 iu/ml) and streptomycin (50µg/ml). Adult worm motility was evaluated by observation under magnifying glass or after being removed from the experimental medium and dipped in slightly warm water and on gentle stimulation, the paralyzed worms confirmed motility. The numbers of dead worms were counted every hour and the percentage of mortality was reported from the average of three replicates. The numbers of dead worms were evaluated as the ratio: number of dead worms/ total number of worms per plate.

Phytochemical screening

General phytochemical screening for the active constituents was carried out for plant extracts according to the standard methods described by Wall *et al*., (1952); Harborne, (1984) and Sofowora, (1993) with minor modifications. The procedure based on the addition of specific reagents to the methanolic and aqueous extracts of *C. decidua* stems

and *M.oleifera* leaves, and observing changes of the solution colour or precipitate formation. The presence of the several chemicals compounds was evaluated.

Statistical analysis

The results are expressed as mean mortality percentage of worms ± standard deviation. The analysis was performed using ANOVA and compared by multiple comparisons (Bonferroni method) using SPSS. Results with $P < 0.05$ were considered to be statistically significant.

RESULTS:

Adult worm motility assay

Fasciolicidal activity of extracts of *M. oleifera* leaves, *C. decidua* stems and albendazole drug are presented in Table 1 and 2. Albendazole (1%) exhibited higher mortality (77.8%) than MOM 300, CDM 600, CDM 300, CDA 1200, CDA 600 and CDA 300 after 11h ($P < 0.05$). The mean percentage of mortality after 12h for albendazole 0.5% and 0.25% was 91.7% and 100% for albendazole 1% there was a significant difference between negative control (RPMI) and albendazole groups ($P < 0.05$). The methanolic extract of *C. decidua* stems (CDM 1200) showed significant efficacy at 12h when compared with different concentration of albendazole. After 13h there were no differences between RPMI control and other treatments, but there was a significant difference between MOM 300, CDA 600, CDA 300, CDM 600 and CDM 300 and albendazole groups ($P < 0.05$).

Phytochemistry

The phytochemical screening indicated that the *M. oleifera* leaves methanolic extract contained alkaloids, triterpenes, flavonoids, saponins, cumarins and tannins. Similar result was obtained in the screening of *C.decidua* stems methanolic extract in addition of the presence of sterol compounds. No anthraquinone compounds were detected in both extracts. The phytochemical screening of the methanolic extracts of *M. oleifera* leaves and *C.decidua* stems is presented in Table 3.

Table 1: Fasciolicidal activity of albendazole and methanolic and aqueous extracts of *M. oleifera* leaves and *C.decidua* stems.

	Mortality • %±SD						
	9h	**10h**	**11h**	**12h**	**13h**	**14h**	**15h**
Treatments							
RPMI	0.00± 0.00	5.6±0.58	11.1±1.16	11.1±1.16°	22.2±1.16	50.0±2.65	83.3±1.73
Alb 1%	38.9±3.22	44.4±3.06	77.8±1.53	100±0.00*	100±0.00	100±0.00	100±0.00
Alb 0.5%	33.3±3.46	44.4±3.22	61.1±3.06	91.7±3.2 *	100±0.00	100±0.00	100±0.00
Alb 0.25	33.3±3.46	38.9±3.22	61.1±2.08	91.7±0.58*	100±0.00	100±0.00	100±0.00
MOM1200	11.1±1.16	11.1±1.16	11.1±1.16	16.7±1.73 °	27.8±2.08	50.0±1.73	77.8±2.31
MOM 600	16.7±1.73	16.7±1.73	16.7±1.73	16.7±1.73 °	27.8±2.08	44.4±2.31	66.7±3.47
MOM 300	0.00± 0.00	0.00± 0.00	0.00± 0.00°	5.6±0.58 °	5.6±0.58 °	33.3±2.00	66.7±3.47
CDM1200	0.00± 0.00	0.00± 0.00	22.2±1.53	44.4±1.16	77.8±2.31	83.3±1.73	100±0.00
CDM 600	0.00± 0.00	0.00± 0.00	0.00± 0.00°	0.00± 0.00°	11.1±0.58 °	22.2±0.58	72.2±0.58
CDM 300	0.00± 0.00	0.00± 0.00	0.00± 0.00°	0.00± 0.00°	11.1±0.58 °	11.1±0.58 °	44.2±1.53
CDA1200	0.00± 0.00	0.00± 0.00	0.00± 0.00°	0.00± 0.00°	38.9±2.52	77.8±1.16	100±0.00
CDA 600	0.00± 0.00	0.00± 0.00	0.00± 0.00°	0.00± 0.00°	11.1±1.16 °	72.2±2.08	100±0.00
CDA 300	0.00± 0.00	0.00± 0.00	0.00± 0.00°	0.00± 0.00°	22.2±1.00 °	77.8±1.16	100±0.00

• Mean value of three replicates. * $P < 0.05$ when compared with RPMI control, ° $P < 0.05$ when compared with albendazole, Alb: albendazole, MOM: *M. oleifera* methanolic extract, CDM: *C.decidua* methanolic extract, CDA: *C.decidua* aqueous extract.

Table 2: Effect of albendazole and methanolic and aqueous extracts of *M. oleifera* leaves and *C. decidua* stems on *Fasciola gigantica* adult worms.

Treatments	**Death time (min)**
RPMI	948±1.88
Alb 1%	576±2.22
Alb 0.5%	656±1.75
Alb 0.25	681±1.55
MOM1200	779±3.57
MOM 600	816±4.42
MOM 300	978±1.50
CDM1200	816±1.25
CDM 600	916±0.87
CDM 300	924±0.89
CDA1200	857±0.77
CDA 600	869±0.62
CDA 300	853±0.79

Alb: albendazole, MOM: *M. oleifera* methanolic extract, CDM: *C.decidua* methanolic extract, CDA: *C.decidua* aqueous extract.

Table 3: The phytochemical screening of *M. oleifera* leaves and *C.decidua* stems

Compounds	Score	
	***M. oleifera* leaves**	***C.decidua* stems**
Alkaloids	+++	++
Sterols	-	++
Triterpenes	++	+++
Flavonoids	+++	+++
Saponins	+++	++
Cumarins	+++	+++
Tannins	+++	++
Anthraquenones	-	-

- Negative; +: Weak colouration; ++: Moderate colouration; +++: strong colouration

DISCUSSION:

In the present study, the fasciolicidal activity of albendazole drug (1%, 0.5%, 0.25 %,), *M. oleifera* leaves and *C. decidua* stems extracts were evaluated on *F. gigantica* adult worms. Three different concentrations were used to investigate the efficacy of the plant extracts at three different doses *viz*: 1200, 600 and 300 µg/ml. Albendazole exhibited strong *in vitro* efficacy on adult worms of *F. gigantica*, the mean percentages of mortality for the higher concentration 1% after 11h was 77.8% ($P < 0.05$) and 100% ($P < 0.05$) after 12h. The present results indicate that albendazole has a potent *in vitro* activity especially at higher concentration. The activity of different concentrations of albendazole was similar at 13h. The *in vitro* effect of albendazole on fluke motility is essentially long term in nature and required concentrations far higher than those which are effective *in vivo*, so they probably have little relevance to their mode of action. Albendazole produces a prolonged stimulation of motility before movement finally declines, while albendazole sulphoxide induces a gradual suppression of activity (Dalton, 1999). Phytochemical screening of the methanolic extracts of *M. oleifera* leaves and *C. decidua* stems indicated that the presence of several compounds which are known to have anthelmintic activity such as saponins, trepenoids and tannins (Garg, 1997; Deore and Khadabadi, 2010). *C. decidua* stems methanolic extract showed significant efficacy at higher concentration 1200 µg/ml after 12h when compared with albendazole. The ethanolic extract of root bark of *C. decidua* was evaluated against adult Indian earthworm. The activity was found to be dose dependant at higher concentration of 100 mg/ml of the extract (Mali *et al.,* 2004). A large number of medicinal plants have been claimed to have anthelmintic activities in traditional system of medicine and are also utilized by ethnic groups worldwide such as *Capparis decidua* (Altoundoub), *Alibbizzia anthelmintica* (Geref el-dood), *Boscia senegalensis* (Mukheit) and *Moringa oleifera* (Al Rawag) (Elghazali *et al*., 1994; Koko *et al.,* 2000; Satyanarayana *et al.,* 2008; Rastogi *et al.,* 2009). In Sudan, Koko *et al.,* (2000)

investigated the fasciolicidal efficacy of water extracts of *Albizia anthelmintica* and *Balanites aegyptiaca* in goats. They reported that the water extracts of *A. anthelmintica* and *B. aegyptiaca* showed strong fasciolicidal effect against *Fasciola gigantica*, based on the percentage reduction in fluke counts from the liver post mortum 2 weeks after treatment, compared with 20 mg/kg bw of albendazole.

No anthelmintic effect of extracts of *M. oleifera* leaves was detected against adult stages between the hours of incubation times in the concentrations in which they were tested, compared with negative control ($P > 0.05$) and albendazole drug ($p < 0.001$). Rastogi *et al.*, (2009) investigated the anthalmintc activity of ethanolic extract of *M. oleifera* against Indian earthworm. They reported that the extract showed strong anthelmintic activity at 25, 50 and 100 mg/ml.

In conclusion methanolic extract of *C. decidua* stems may have *in vitro* anthemintic activity on adult worm of *F. gigantica* especially at higher concentrations, while the aqueous extract did not show any similar activity in concentrations used during this study. This may be due to the presence of more polar compounds in methanolic extract. In contrast the methanolic extract of *M. oleifera* leaves used according to the present protocol did not prove to be effective in controlling adult worms of *F. gigantica*.

Further investigations should be done to examine other parts of both plant extracts as fasciolicide at higher concentrations through *in vitro* and *in vivo* models.

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