IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 10, Issue 5 Ver. IV (Sep - Oct. 2015), PP 18-22
www.iosrjournals.org

In vitro Ovicidal Activity of Encapsulated Ethanolic Extract of *Prosopis juliflora* Against *Haemonchus contortus* Eggs

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Abstract: *Haemonchus contortus is one of the most prevalent and highly pathogenic nematode parasites of small ruminants. Development of resistance and degradation of active compounds of conventional anthelmintic drugs prompted evaluation of encapsulated medicinal plant extracts as alternative anthelmintics. The in vitro ovicidal activity of encapsulated extract of Prosopis juliflora was determined using Egg hatch inhibition assay on H.contortus eggs. The tests were evaluated at six concentrations (0.63, 0.13, 0.25, 0.5, 1.0 and 2.0 mg/ml) and in drug to polymer ratios of (1:2, 1:1 and 2:1). The results indicated that there was a significant difference in egg hatch inhibition at different concentrations and ratios ($P<0.05$). At 2.0 mg/ml, encapsulated leave ethanolic extract (ELEE) at drug to polymer ratio of (2:1), leaf ethanolic extract (LEE) and albendazole (positive control) all exhibited total inhibition of egg hatching. Of the plant extracts tested, ELEE showed higher ovicidal activity (IC_{50} =0.36 mg/ml) as compared to LEE, root ethanolic extract (REE) and encapsulated root ethanolic extract with IC_{50} values of 0.49, 0.65 and 0.74 mg/ml respectively. Generally, the mean inhibition rate of egg hatching increased with increase in extract concentration. However, there was no statistically significant difference in egg hatch inhibition between LEE and REE ($p>0.05$). In conclusion, the encapsulated P. juliflora extract have a potential anthelmintic activity and further in vivo evaluation and characterization of the plant extract is warranted to further ascertain its efficacy and the compounds responsible for the efficacy.*

Keywords: *Anthelmintic, Encapsulated, Ethanolic extract, Haemonchus contortus, Resistance*

I. Introduction

Livestock production covers up to 40 percent gross value of agricultural production worldwide (Bachaya *et al.,* 2006) and livestock especially small ruminants represent a major asset among resource-poor farmers (Perry *et al.,* 2002). Gastro-intestinal nematode parasites pose a major constraint in sheep industry and it cause production loss, increased cost of management, treatment and mortality in severe cases (Larsen *et al.,*1995; Barger and Cox, 1984). Control of these nematode parasites is essential for maximizing livestock productivity and feed efficiency. The main means of controlling this nematode infection is by use of anthelmintics (Roos, 1997).

High cost of modern anthelmintics and unavailability has limited the effective control of nematodes by rural farmers. In addition, widespread and intensive use of sometimes low quality anthelmintics has led to resistance (Mohammed *et al.,* 2005; Githiori, 2004; Ketzis, 2003). The earliest reports of anthelmintic resistance involved nematode parasites of sheep and horses. Now resistance has appeared in parasites that affect many animal species as well as humans (Sangster and Gill, 1999).

Chemical anthelmintics are reported to be toxic and pose side effects to the administrator and the animal (Kareru, 2008). Medicinal plants are economical, safe and generally have no problem of drug resistance. They can be matched as substitutes of synthetic anthelmintics (Jabbar *et al.,* 2005) and even considered as the best healing agents for the treatment of parasitic diseases (Kone *et al.,* 2012).

Prosopis juliflora (locally known as 'Mathenge') was introduced in Kenya in the early 1970s (Maghembe *et al.,* 1983; Ebenshade and Grainger 1980) and is generally considered a noxious weed locally. A study by Wamburu *et al* (2013) to investigate the toxicity and safety levels of *P. juliflora* using *Swiss albino* rats with an aim of exploiting its bioactive compounds revealed that toxicity symptoms were moderate and post mortem did not show any major gross effects on the internal organs. Saponins from its bark, roots and leaves have been reported to be effective against intestinal worms (Rechab *et al.,* 2014; Githiori, 2004).

The purpose of this study was to evaluate the *in vitro* ovicidal activity of encapsulated ethanolic extract of *P. juliflora* against *H.contortus* eggs. The main problem of using plant derived natural compounds is their degradation in gastrointestinal system before reaching the circulation system which limits the area of usage of

these compounds. Thus, there is need for encapsulation system to link this gap and maximize potential therapeutic benefits of natural compounds.

II. Materials And Methods

2.1 Sample collection and preparation

Prosopis juliflora leaves and roots were collected from Marigat in Baringo County, Kenya and authenticated by botanist at JKUAT botany laboratory. The plant samples were washed in water, chopped and shade dried for three weeks. Once completely dried, grinding into fine powder was done by a mechanical grinder and exhaustively extracted with ethanol. The extracts were concentrated using a rotary evaporator (BUCHI R-200) at 45ºc and stored at 4°C until required for phytochemical screening, encapsulation and bioassay.

2.2 Phytochemical screening

Qualitative phytochemical screening was performed as described by Harborne (1998). The extracts were tested for tannins, saponins, alkaloids, flavonoids and sterols. The screening tests were based on observation of color change and precipitin formation.

2.3 Preparation of *Prosopis juliflora* extracts microspheres

A determined quantity of ethyl cellulose was dissolved in 100 ml of ethanol to form a homogenous solution. Calculated quantity of *P. juliflora* extract was then added to the homogenous polymer solution and mixed thoroughly. The resulting mixture was added to 200 ml of aqueous mucilage of sodium carboxymethly cellulose (CMC) to emulsify the added dispersion as fine droplets. The ethanol solvent was then removed by evaporation during the continuous stirring at room temperature for three hours to produce spherical microspheres. The encapsulated microspheres from leaves and roots were prepared in drugs to polymer ratios of 1:2, 1:1 and 2:1.

Microspheres were subjected to drying and their weights determined. Percentage yield of the prepared microspheres was then calculated using the following formula.

$$\text{Percentage yield} = \frac{\text{Weight of microsphere x 100}}{\text{Weight of the polymer + Drug}}$$

2.4 *In vitro* drug release

Encapsulated *P. juliflora* extracts on concentrations ranging from 0.0625 mg/ml to a maximum of 2.0 mg/ml and in drug to polymer ratios of (1:2, 1:1 and 2:1) were redispersed on 2.0 ml of Phosphate Buffered Saline (PBS, pH 7.4) and kept in an incubator at 37^0C for 48 hours without agitation. The supernatant was collected and filtered after centrifugation at (35,000 rpm, 30 min). The released drug was kept at 4°C until required for egg hatch assay.

2.5 *In Vitro* Anthelmintic activity

2.5.1 Egg hatch assay

Egg hatch assay was conducted according to World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines (Coles *et al.,* 1992). Mature adult parasites of *H. contortus* were collected directly from abomasums of slaughtered sheep from a local slaughter house. The worms were transported to the laboratory in PBS (pH 7.4). Identification and separation of adult mature female were done according to the method by Hansen and Perry (1994) using a microscope. The worm was then washed and crushed to liberate eggs. The eggs used for this test were aged less than 2 hours. The encapsulated *P. juliflora* extracts of leaves and roots were used as the active treatment. The extracts were dissolved in PBS to a concentration ranging from 0.0625 to 2.0 mg/ml. Albendazole was used as positive control while untreated eggs in PBS were used as negative control. The test was conducted in 96-well flat-bottomed microtitre plate and eggs were incubated for 48 hours at 27ºC and 70 % relative humidity. After 48 hours of incubation, a drop of lugol's iodine was added to stop the eggs from hatching. There were three replicates for each concentration and control. Hatched larvae (live or dead and unhatched eggs) were counted using a compound microscope at X40 magnification with the help of a counter (Rechab *et al.,* 2014).

2.6 Determination of inhibitory concentration(IC_{50})

Mean percentage egg hatch rates from encapsulated (leaves and root) extracts, Albendazole (ALB) and ethanolic extracts of leaves (LEE) and roots (REE) were compared using one-way ANOVA test at $p<0.05$ significant levels. The concentration required to inhibit 50% (IC_{50}) was determined using the regression line of probit according to the $\log_{10}$ of the extract concentration

III. Results And Discussion

Table 1: Phytochemical screening of leaves and roots ethanolic extract of *P. juliflora*

Secondary metabolite screened		Saponins	Tannins	Flavonoids	Alkaloids	Sterol
Plant part	Leaves	+	+	+	+	+
	Roots	+	+	+	+	+

'+' Present

Phytochemical screening of *P. juliflora* leave and root ethanolic extract tested positive for saponins, tannins, flavonoids, alkaloids and sterols. The phytochemicals identified could be responsible for anthelmintic activity.

3.1 Yield of Encapsulated Extract

The percentage yield of the six preparations was ranging from 79.1% to 90.7% as shown in (Table 2). These higher percentage yields show that this method is very appropriate for adoption in the formulation of encapsulated *P. juliflora* extracts.

Table 2: Percentage encapsulation yield

Plant part	Ratio (Drug : Polymer)	Encapsulation yield in %
Roots	2:1	90.7 ± 1.7
	1:1	84.2 ± 5.2
	1:2	79.4 ± 1.7
Leaves	2:1	87.9 ± 1.5
	1:1	81.0 ± 3.6
	1:2	79.1 ± 3.2

The encapsulation technology applied in the current study is a well known process used to preserve active agents (Nedovic *et al.*, 2011). In addition, the usage of encapsulation technology on natural compounds has gained great interest in order to maximize its potential therapeutic benefits (Munin and Edwards-Lévy, 2011).

3.2 Egg-Hatch Inhibition (EHI) Assay

The results of EHI assay are as shown in Figure 1. The results obtained from the study indicated that there was a statistically significant difference in egg hatch inhibition at different concentrations and ratios of encapsulated roots and leaves ethanolic extract ($P<0.05$). This difference is due to variation in proportion of polymer with respect to amount of drug. Encapsulated Leave ethanolic extract at (2:1) drug: polymer ratio, leaf ethanolic extract and albendazole exhibited 100% inhibition of egg hatchability at 2.0 mg/ ml. The higher drug concentration resulted in higher egg hatch inhibition compared with lower concentration suggesting a concentration dependent response.

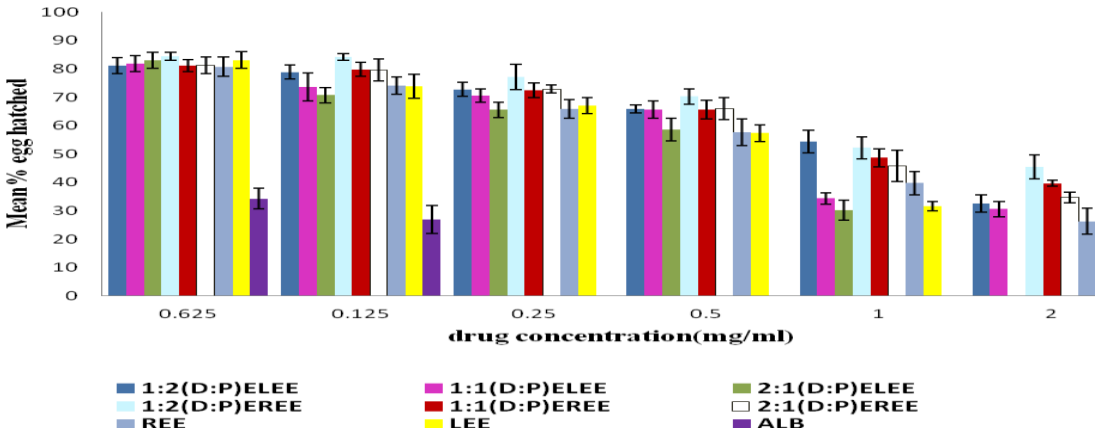

Figure 1: Showing *In vitro* egg-hatch inhibition assay (EHA) in various ratios of encapsulated (leaves and root) extracts, Albendazole (ALB) and ethanolic extracts of leaves (LEE) and roots (REE)

D: P: Drug: Polymer, **ELEE**: Encapsulated Leave Ethanolic Extract, **EREE**: Encapsulated Root Ethanolic Extract, **ALB**: Albendazole, **LEE**: Leave Ethanolic Extract; **REE**: Root Ethanolic Extract

Encapsulated leave extracts in different ratios showed (Table 3) a higher activity with IC_{50} values ranging from 0.355 to 0.949 mg/ml as compared with encapsulated root extract with IC_{50} values from 0.741 to 1.206 mg/ml. This could be attributed to the difference in proportions of the active components that are responsible for anthelmintic activity. However, the results showed no statistically significant difference in egg hatch inhibition on both LEE and REE ($p>0.05$) in comparison with ALB which showed a significant difference in activity ($p<0.05$). These results are in agreement with previous studies on *in vitro* ovicidal activity of ethanolic extract of *P.juliflora* (Rechab *et al.,* 2014).

Table 3: IC_{50} values for *in vitro* Egg Hatch Inhibition Assay (EHI)

	95% confidence limits for concentration (mg/ml)		
Sample type	**IC_{50} values (mg/ml)**	**Lower Boundary**	**Upper Boundary**
EREE(1:2)D:P	1.206	0.910	1.792
EREE(1:1)D:P	0.877	0.683	1.205
EREE(2:1)D:P	0.741	0.599	0.950
ELEE(1:2)D:P	0.949	0.725	1.358
ELEE(1:1)D:P	0.616	0.302	1.667
ELEE(2:1)D:P	0.355	0.024	1.378
REE	0.646	0.463	1.074
LEE	0.493	0.296	1.310
ALB	0.053	0.000	0.092

A number of medicinal plants have been used to treat parasitic infections in man and animals (Akhtar *et al.,* 2000). *Dregea volubilis* (family Asclepediaceae) is widely used as anthelmintic in traditional system of medicine in India (Hossain *et al.,* 2012). In Kenya plants that have been used as anthelmintic in livestock include *Vernonia lasiopus* (Njonge *et al.,* 2013) and *E. leptostachya* (Njonge *et al*., 2013; Kareru *et al.,* 2007). A study done by Kareru (2008) reported the presence of triterpenes, tannins, saponins and glycosides in *Entada leptostachya* while triterpenes, saponins, tannins and anthraquinones were found in *Albizia anthelmintica.* The anthelmintic activity of condensed tannins against *H. contortus* in sheep has been evidenced by inhibited egg hatching (Zafar *et al.,* 2007).

Saponins, tannins and cardiac glycosides are the phytochemicals responsible to have anthelmintic effect. They uncouple oxidative phosphorylation of the parasite thus hindering energy productions which cause parasite death (Danquah *et al.,* 2012).

IV. Conclusion

The findings of this study revealed that the encapsulated *P. juliflora* extract has a potential anthelmintic activity *in vitro*. However, *in vivo* evaluation of encapsulated *P. juliflora* extract is needed to make use of this plant for beneficial purpose and also to open up a new era of plant extract encapsulation technology for use in animal health care. Further studies should also be carried out in order to isolate, identify, characterize and purify the bioactive compounds and thus its activity increased.

Acknowledgements

The authors express their special thanks to Research, Production and Extension (RPE) division of Jomo Kenyatta University of Agriculture and Technology for funding the project.

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