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In vitro anthelmintic activity of *Syzygium aromaticum* and *Melia dubia* against *Haemonchus contortus* of sheep

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Prolonged and indiscriminate exploitation of anthelmintics in small ruminants led to development of resistance (Chaudhry *et al.* 2015, Amulya *et al.* 2016) in strongyle nematodes. The speed of development of resistance by parasites across the world forced the scientific communities to search for alternative means like exploitation of ethno pharmacology. Medicinal plants with anthelmintic activity have been reported for use in rural India (Dhanraj and Veerakumari 2015, Swargiary *et al.* 2016). In the current study, attempts were made to know the anthelminthic activity of two plants (*Syzygium aromaticum* and *Melia dubia)* with medicinal properties. *Syzygium aromaticum (L)* (Family Myrtaceae), popularly known as clove is a well known spice of southern India. Its oil is traditionally applied as a remedy for bronchitis, common cold, cough, dyspepsia, flatulence, stomach distension and gastrointestinal spasms (Banker *et al.* 2011). *Melia dubia Cav.* (Family Meliaceae) known as malabar neem, is a large deciduous and native tree species of India. Every part of the plant is being used as traditional herbal medicine, such as anthelmintics, treatment of leprosy, eczema, asthma, malaria, fevers and cholelithiasis (Vennila and Mariyal 2015).

Dried unopened flower buds of *Syzygium aromaticum* were procured from local markets and the *Melia dubia* leaves were collected directly from fields during the months of June and July 2016. The plant materials were authenticated from a Botanist of Sri Venkateswara University, Tirupati. Collected plant parts were shade dried and ground into fine powder, and acetone extracts (AE) were prepared by cold maceration method as described by Sindhu *et al.* (2012a). Prepared AEs were refrigerated till further use.

Egg hatch test: The egg hatch test (EHT) was performed as described by Coles *et al.* (1992). Fresh ova were collected from fecal sample of sheep infected with *H. contortus.* Eggs were concentrated (approx. 200 eggs/ml) by flotation technique. Acetone extracts of two plant materials at varying concentrations (10, 5, 2.5, 1.25, 0.62, 0.31 and 0.15 mg/ml) were studied. Albendazole (Intas Private Ltd.) purchased from commercial sources was dissolved in DMSO (2.5 mg/ml) which acted as referral compound, while DMSO along with egg solution served as negative control. The plates were incubated at 28°C for 48 h and unhatched eggs were counted using stereomicroscope (20×). The hatching inhibition percentage from egg hatch assay was subjected to probit analysis to calculate inhibitory concentration (IC_{50} and IC_{90}) for respective compounds using Statistical Package for Social Sciences (SPSSv 19.0 IBM, Illinois, Chicago).

Adult motility assay: Mature *H. contortus* worms were collected from abomasums (four) of slaughtered sheep. Adult motility assay (AMA) was conducted as per the method described by Hounzangbe-Adote *et al.* (2005). The worms were exposed in triplicate to acetone extracts at seven different concentrations (10, 5, 2.5, 1.25, 0.62, 0.31 and 0.15 mg/ml) prepared in Phosphate buffer saline (pH 7.4). As positive control, Piperazine adepate @ 12 mg/ml was taken and PBS alone with worms served as negative control. Complete cessation of motility was taken as indication of worm mortality. Time for death of worms was determined after ascertaining that the worms neither moved when pricked with needle nor when dipped in warm water (50°C). In the same manner, observations were taken at regular time intervals until worms in the negative control completely lost their motility. The results from AMA were analyzed with ANOVA using statistical version.

In EHT, based on IC_{50} values (mg/ml), the most effective plant in its activity was *S. aromaticum* (1.612) followed by *M. dubia* (7.752). The concentration that inhibited (IC_{50}) the hatch of 50% of the ova was considered as the minimum ovicidal concentration. IC_{90} of *S. aromaticum* (5.445) was more than that calculated for albendazole (2.413) and it was statistically significant ($P<0.05$). The inhibitory concentration estimates of acetone extracts of two plants and albendazole are given in Table 1. The results of AMA showed that the acetone extracts of two plants showed dose and time dependent anthelminthic activity. The onset of

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Table 1. Egg hatchability inhibitions by two plant compounds

Compound	IC_{30}	IC_{50}	IC_{80}	IC_{90}	Slope (SE)	Intercept (SE)	χ^2 (df)
Syzygium aromaticum	0.980 (0.833-1.131)	1.612 (1.407-1.849)	3.586 (3.046-4.363)	5.445 (4.465-6.981)	2.425 (0.063)	-0.503 (0.029)	128.574 (19)
Melia dubia	4.297 (3.561-5.208)	7.752 (6.295-10.221)	19.988 (14.277-33.176)	32.792 (21.593-62.279)	2.046 (0.081)	-1.820 (0.057)	142.372 (19)
Albendazole	0.736 (0.570-0.899)	1.039 (0.849-1.298)	1.807 (1.426-2.638)	2.413 (1.814-3.939)	3.503 (0.117)	-0.059 (0.032)	179.592 (10)

Values are inhibitory concentrations with 95% Fiducial Confidence Intervals in parenthesis. IC, inhibitory concentration; Se, standard error; df, degrees of freedom.

activity with *S. aromaticum* (10 mg/ml) was very rapid (1 min) than *M. dubia* (5.33 min) and Piperazine adepate (5.0 min). The time taken to kill all the worms at the lowest concentration (0.16 mg/ml) of *S. aromaticum* and *M. dubia* was 36.67 min and 79.33 min respectively. However, in all treatment groups as the concentration of the compounds decreased the degree of immobilization got delayed (Table 2). While in PBS, worms stayed alive for 8 h.

AMA and EHT of clove bud oil revealed significant ($P<0.05$) anthelmintic property against *H. contortus* worms. Clove bud extracts at high concentrations were very effective in killing the worms where 100% mortality was attained within minutes of exposure. IC_{50} concentration of *S. aromaticum* (1.612 mg/ml) and standard compound (1.039 mg/ml) were very close, indicating similar range of activity. Kumar and Singh (2014) recorded *in vitro* toxicity of eugenol against adult *Fasciola gigantic* whose LC_{50} was 0.31 mg/ml concentartion after 8 h of exposure. Likewise, Dhanraj and Veerakumari (2014) tested ethanolic extract of *S. aromaticum* on the motility of *Cotylophoron cotylophorum* and reported that maximum level of inhibition of the motility was observed at 0.5 mg/ml after 8h of exposure. Bioactive constituent of clove oil is eugenol, but its exact mechanism of action on nematodes is unknown (Yang *et al.* 2003). Wormicidal activity of clove extracts could be attributed to its strong corrosive action on cuticle and tegument of helminthes which needs further evaluation.

Table 2. Wormicidal activity of acetone extract of *Syzygium aromaticum*

Concentration (mg/ml)	Time (min) for paralysis	Time (min) for mortality
10.00	1.00±0.00	2.00±0.00
5.00	7.00±0.58	9.67±0.33
2.50	10.67±0.33	14.00±0.58
1.25	15.33±0.33	19.33±0.33
0.63	18.33±0.33	22.33±0.33
0.31	20.33±0.33	29.67±0.33
0.16	25.33±0.33	36.67±0.67
Piperazine adepate (12.00 mg/ml)	5.00±0.58	6.00±0.58

Table 3. Wormicidal activity of acetone extracts of *Melia dubia*

Concentration (mg/ml)	Time (min) for paralysis	Time (min) for mortality
10.00	5.33±0.33	10.00±0.58
5.00	11.67±0.33	19.33±0.33
2.50	18.67±0.33	23.67±0.33
1.25	29.67±0.33	50.67±0.67
0.63	40.67±0.67	62.33±1.45
0.31	62.67±1.45	72.00±1.15
0.16	66.67±0.67	79.33±0.67
Piperazine adepate (12.00 mg/ml)	5.00±0.58	6.00±0.58

M. dubia in the present study showed lower anthelmintic activity against *H. contortus* of sheep. Contrary to this, Das *et al.* (2016) reported anthelminthic activity of methonolic extract of *M. dubia* on the motility of earthworms and showed highest paralytic activity at 6.47 and 10.3 min, followed by death at 9.42 and 16.27 min, respectively at 150 mg/ml. The results greatly vary from one study to other depending upon the type of solvent used and its ability to dissolves the active metabolites from plants. The anthelmintic activity of the plant may be attributed to high content of phenolics, tannins and alkaloids in its extracts. Tannins and phenolics which are the active metabolites of *M. dubia* are known to interfere with energy metabolism of parasite (Athnasiadou *et al.* 2001) and bind to free proteins on the cuticle of the parasite that leads to death. The data on anthelmintic activity of *M. dubia* against *H. contortus* are scanty as much of its medicinal properties were focused on to antiurolithiatic activity of the plant rather than on anthelmintic properties (Vennila and Mariyal 2015).

SUMMARY

It may be concluded that out of 2 plants under study, *Syzygium aromaticum* had higher potential anthelmintic activity (6 times more potential than *Melia dubia*) against *H. contortus* worms of sheep. Extensive studies need to be carried out to identify the pharmacologically active compounds in *S. aromaticum* that are playing key role in killing the worms and reducing the hatchability of eggs. Further validation with *in vivo* trials is of utmost significance.

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REFERENCES

Amulya G, Sudharani R, Shareer M and Gopinath S M. 2016. *In vivo* and *in vitro* detection of anthelmintic resistance against gastrointestinal nematodes of sheep. *Indian Veterinary Journal* **93**(4): 81–83.

Athnasiadou S, Kyriazakis I, Jackson F and Coop R L. 2001. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: *In vitro* and *in vivo* studies. *Veterinary Parasitology* **99**: 205–19.

Bankar, Kumar A and Sandhna P. 2011. Photochemical constituents of *Syzygium aromaticum* L . *International Journal of Current Research* **3**(7): 215–17.

Chaudhry U, Elizabeth M, Muthusamy R and John S. 2015. Genetic evidence for the spread of a benzimidazole resistance mutation across southern India from a single origin in the parasitic nematode *Haemonchus contortus*. *International Journal for Parasitology* **45**: 721–28.

Coles G C, Bauer C, Borgsteede F H, Geerts S, Taylor M A and Waller P J. 1992. World association for the advancement of veterinary parasitology: methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Veterinary Parasitology* **44**: 35– 44.

Dhanraj M K and Veerakumari L. 2015. *In vitro* studies on the effect of ethanol extract of *Syzygium aromaticum* on the carbohydrate metabolism of *Cotylophoron cotylophorum*. *International Journal of Advanced Veterinary Science and Technology* **4**(1): 199–210.

Das K, Someswar D, Thejaswini K, Sabita U and Raman D. 2016. Effect of cultural condition on element contents in raw material vis-à-vis impact of solvent nature on estimation of phytochemicals and screening of anthelmintic activity of *Melia dubia cav. L*. *Annals of Phytomedicine* **5**(2): 58–68.

Hounzangbe-Abote, Paolini M S, Moutairou H and Hoste H. 2005. *In vitro* effects of four tropical plants on three life cycle stages of parasitic nematodes *Haemonchus contortus. Research in Veterinary Science* **78**: 155–60.

Karthikeyan J, Kamalanathan M N, George T and Elangovan V. 2014. Larvicidal and antibacterial efficacy of green synthesized silver nanoparticles using *Melia dubia*. *International Journal of Pharmacy and Pharmaceutical Sciences* **6**(7): 395–99.

Kumar P and Singh V K. 2014. Activity of *Allium sativum*, *Ferula asafoetida* and *Syzygium aromaticum* against *Fasciola gigantica*. *Journal of Biology and Earth Sciences* **4**(1): 57–65.

Sindhu Z, Jonsso N and Iqbal Z. 2012a. Syringe test (modified larval immersion test): A new bioassay for testing acaricidal activity of plant extracts against *Rhipicephalus microplus. Veterinary Parasitoogy* **188**: 362–67.

Sonalkar M Y and Nitave S. 2014. Comparative anthelmintic activity of *Eugenia caryophyllus* and *Uncaria Gambier*. *World Journal of Pharmacy and Pharmaceutical Sciences* **3**(7): 1409–14.

Swargiary A, Abhijit D, Manita D, Noymi B and Ezekiel N. 2016. Phytochemicals, antioxidant and anthelmintic activity of selected traditional wild edible plants of lower Assam. *Indian Journal of Pharmacology* **48**(4): 418–23.

Vennila V and Mariyal A. 2015. *In vitro* analysis of phytochemical and antiurolithiatic activity of various extracts of *Melia dubia* leaves. *World Journal of Pharmacy and Pharmaceutical Sciences* **4**(4): 1277–89.

Yang Y C, Lee S H, Lee W J, Choi D H and Ahn Y J. 2003. Ovicidal and adulticidal effects of *Eugenia cryophyllata* bud and leaf oil compounds on *Pediculus capitis*. *Journal of Agriculture and Food Chemistry* **51**: 4884–88.