EVALUATION OF ANTHELMINTIC ACTIVITY OF DIFFERENT FRACTIONS OF *SYZYGIUM CUMINI* L. LEAVES

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خلاصہ

جامن Myrtaceae فیملی کاایک جاناماناپوداہے۔جامن کواس کی anthelmintic صلاحیت کی وجہ سے بھی جاناجاتاہے جس میں یہ کیڑوں کو مفلوج کر کے مارتا ہے۔جامن کی اسی صلاحیت کو جانچنے کے لیےاس کے پتوں کا نچوڑ نکال کر کیینچوے، ٹیپ ورم اور ریڈ ورم پر استعمال کیا گیا۔یہ تجربہ لاہور کالج فار ویمن یونیورسٹی لاہور کے شعبہ نباتات میں تین مرتبہ دہرایا گیا جس میں مختلف صلاحیت کے نچوڑ(100,50,25,12.5,6.25%)،albendazong (10%)اور10% نمک کا محلول استعمال کیا گیا۔حاصل کردہ ڈیٹاپر Ducan's Multiple Range Test لگایا گیا۔استعمال کیے گئے سارے نچوڑ کارآمد ثابت ہوے۔جامن کے سب سے گاڑھے نچوڑنے آٹھ منٹ میں تمام کیڑوں کو مفلوج کر دیا جبکہ پندرہ منٹ میں سارے مار دیے۔اس کے برعکس نمک کے محلول سے نہ کوئی کیڑا مفلوج ہوااور نہ ہی مرا۔پس حالیہ تجربہ سے ثابت ہوا جامن کے محلول سے کیڑوں کو باآسانی مارا جاسکتا ہے۔

Abstract

Syzygium cumini L. (Jamun), member of family "Myrtaceae" is a well-known medicinal plant. It is also known for its anthelmintic activity by which it can paralyze the worms leading to their death. Current study was conducted to evaluate anthelmintic activity of methanolic extract of *S. cumini* L. leaves against earthworms (*Lumbricus terestris*), Tape worms (*Monnizia expansa*) and Red worms (*Haemonchus contortus*). Research was conducted at Department of Botany, Lahore College for Women University, Lahore. The experiment was repeated thrice times for accuracy and then average data was statistically analyzed using Ducan's Multiple Range Test. Various concentrations of each extract (100, 50, 25, 12.5 and 6.25 %), Albendazole (10%) and saline solution (10%) were used as treatments. All the tested concentrations were significant to kill the worms used in experiment. Maximum activity of methanolic extract of *S. cumini* leaves was 8 minutes as time of paralysis, 15 minutes as time of death at dose of 100 mg/mL. Saline solution used as control, no paralysis and death of worms was observed in this case. The result of current study indicated that all tested extract of *S. cumini* L. show anthelmintic activity in a dose depended inhibition. The effectiveness of test sample was found to be reversible to time of paralysis and death time of worms. Albendazole (10 mg/mL) was used as reference. All the concentration of *S. cumini* L. extract possessed good anthelmintic activity as compared to Albendazole. Thus, the study concluded that the methanolic extracts of *S.cumini* L. have good anthelmintic activity against all the worms used in this particular.

Key words: Albendazole, Anthelmintic, *Lumbricus terestris*, *Monnizia expansa*, *Haemonchus contortus*, *Syzygium cumini*

Introduction

Helminth diseases are one of the most prevalent diseases especially in third world countries. Worm-like parasites such as flukes, roundworms and tapeworm (Townson *et al*., 2001). According to the World Health Organization about two billion people are affected by parasitic worm infections (Mehta *et al*., 2012; Sutar *et al*., 2010). This parasitic disease cause significant loss for human and animals leading to anaemia, pneumonia, malnutrition etc. Large economic losses include reduced weight grains, reduced milk production in dairy animals, suppressed wool production, decreased quality of wool and reduced animal productivity (Mehlhorn, 2008). Personal and environmental hygiene, poor sanitary conditions, and lack of clean water, poverty and illiteracy are the major factors considering the spread of helminthiasis (Kumar *et al*., 2001).

Antihelminthics are the agents or drugs that are used to expel or destroy parasitic worms from the body, by paralyzing or killing them (Rahman *et al*., 1999). Anthelmintic drugs include Thiabendazole, Albendazole, Mebendazole, Niclosamide, Oxamniquine, Praziquante, Pyrantel and Pamoate. These drugs are acceptable due to their high efficacy against nematodes and low toxicity toward animals. Virtually, all grazing animals are

infected with gastrointestinal parasites (Zajac and Coboy, 2006). Although, these drugs are used to kill the worms but they also have some side effects (Devi *et al*., 2009). The commercial anthelmintic drugs are very expensive; some parasites may also have resistance against these drugs. Plants extracts are used because of fewer side effects, low-cost and easy availability (Aswar *et al*., 2008). Plants based medicines are of good potential to cure many diseases of men and animals especially grazing animals. Anthelmintic activity of some plants has also been reported (Iqbal *et al*., 2009).

Current study is focused on *Syzygium cumini* L. to assess the anthelmintic activity leaves against earthworms (*Lumbricusterestris*), Tape worms (*Monniziaexpansa*) and Red worms (*Haemonchuscontortus*). *S cumini* L. commonly known as Jamun belongs to family Myrtaceae, is an evergreen tropical tree mostly grows in the south-east Asia (Mitra *et al*., 2012).

In Previous literature survey, no reports were found on the anthelmintic activity of the leaves extracts of *S cumini* L. This has encouraged us to explore the anthelmintic activity of *S cumini* L. leaves extracts.

Material and Method

Collection of Plant Material:

Syzygium cumini L. leaves were collected from Govt. College for Women Gulberg, Lahore. *S. cumini* L. leaves were identified by Mrs. Aaliya Iqbal Butt, Head of department of the Botany, Govt. College for Women Gulberg, Lahore. It was washed and shade dried. The dried plant material was ground in an electric chopper to make fine powder.

Preparation of plant Extract:

50 g of powdered plant material was subjected to maceration by taking sufficient amount of methanol in 1-liter beaker and, then kept for seven days. Methanolic extract obtained was filtered and concentrated by evaporation. The extract was used for the preparation of stock solution to check the anthelmintic activity.

Experimental Model

Adult earthworms (*Lumbricus terrestris*) were used and collected from the moist soil of Jam-e-Shirin Park Gulberg, Lahore. Redworms and tapeworms were collected from slaughterhouse, Saddar, Lahore. All the worms were identified, washed with saline water to remove all the faecal matter.

Anthelmintic activity:

To test anthelmintic activity, three test groups were taken, each containing 10 worms of equal size. Different concentrations of methanolic extracts of *S. cumini* L. leaves were prepared in saline solution to obtain different concentrations 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25mg/mL concentrations. Albendazole was used as a reference drug. Among three groups, one group was considered as control group and tested with normal saline water. All the required concentrations of leaves extract were freshly prepared before starting the experiment. 10mL of test solution was poured in petri-dishes and experiments were performed in duplicates to confirm the results. In each petri-plate worms of almost equal size were used and paralysis along with death time of each worm were recorded at room temperature at different intervals of time. Paralysis was considered at the time when no movement of worms was observed even in normal saline. Death was recorded when there was no mobility of worms along with disappearing of color of worm.

Statistical Analysis

All the data was analyzed by ANOVA followed by Ducan's Multiple Range Test using software COSTAT.

Table 1: Percentage of organism killed by different extracts

Sample	Conc. of extract used mg/mL	No. of worms used	No. of *L. terrestris* killed (%)	No. of *M. expansa*killed (%)	No. of *H. contortus*killed (%)
S. cumini L. (Crude extract)	100	10	100	100	100
	50	10	100	100	100
	25	10	90	100	100
	12.5	10	70	90	90
	6.25	10	60	80	50
Albendazole	10	10	100	100	100
Saline solution	10	10	0	0	0

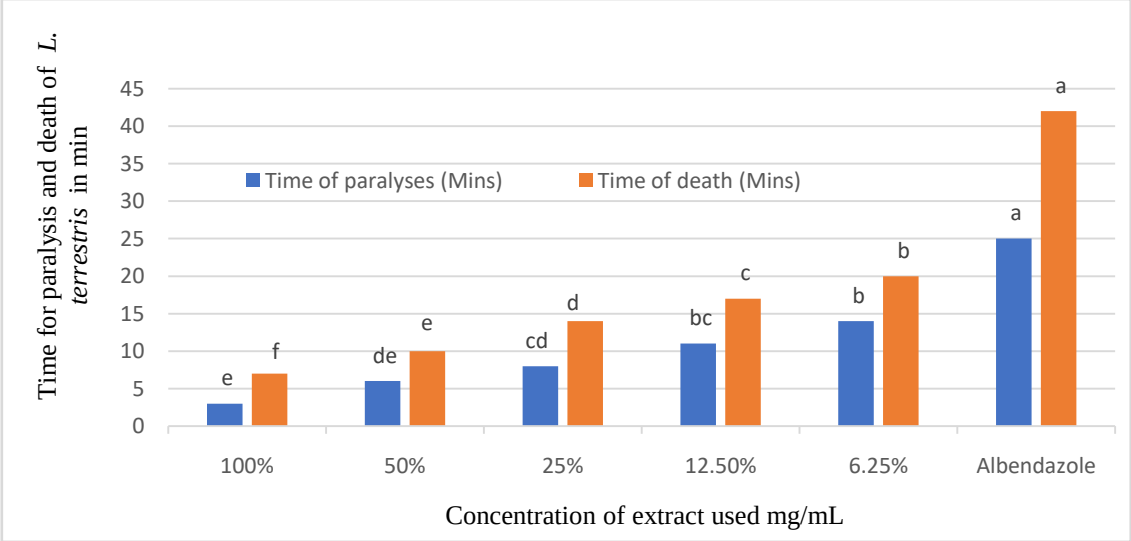

Fig 1. Time of paralysis and death of Earth worms (*L. terrestri*) for *S. cumini* L. leaves extract

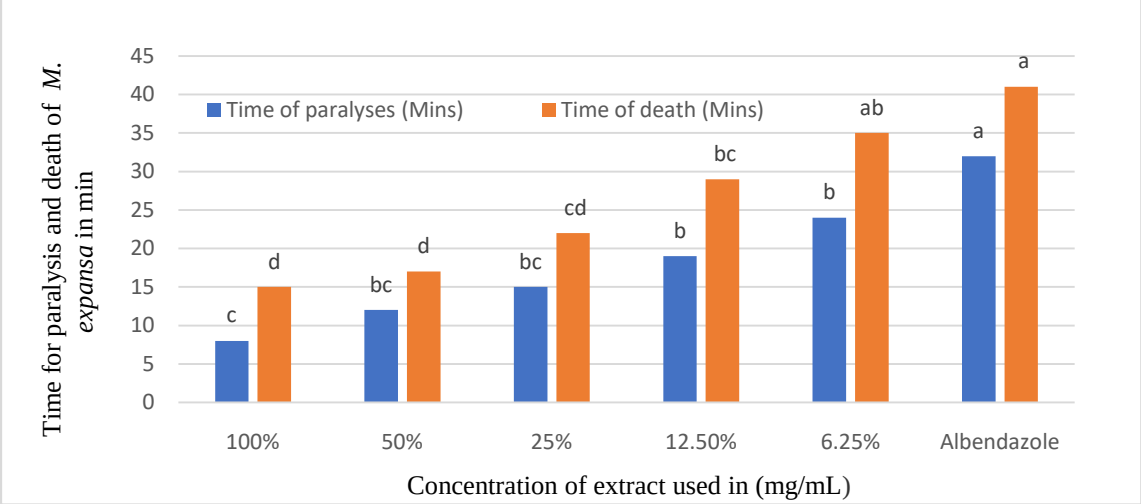

Fig 2. Time of paralysis and death of Tape worms (*M. expansa*) for *S. cumini* L. leaves extract

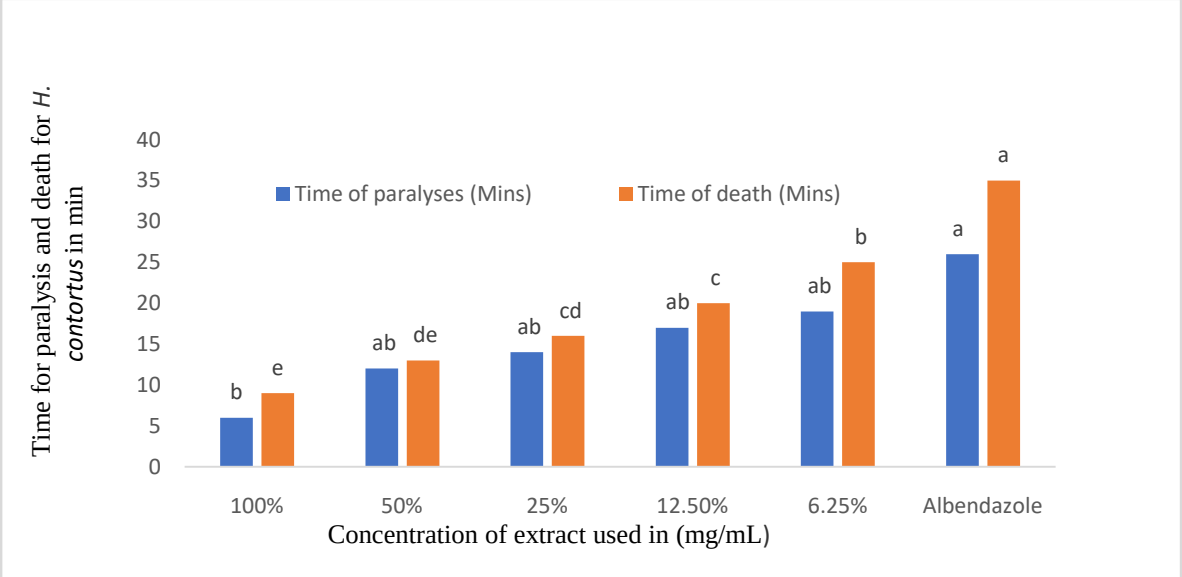

Fig 3. Time of paralysis and death of Red worms (*H. contortus*) for *S. cumini* L. leaves extract.

Results and Discussion

From the observation (Table 1) it was observed that, earthworms, tape worms and red worms lost their motility on the exposure to leaves extracts of *S cumini* L. Each crude extract containing 6.5, 12.5, 25, 50 and 100 mg/mL, produced dose-dependent paralytic effect, which ultimately progressed to death.

6.5 and 12.5 mg/mL of methanolic extract produced paralysis of *Lumbricucterrestri*within 11 and 14 min. respectively. Mortality was noted with 6.25 mg/mL of methanol extract within 20 minutes [Fig. 1].

100 and 50 mg/mL of methanol extract produced paralysis of *Monniezia expansa*at 8 and 12 minutes respectively and the mortality was occurred at 15 and 17 minutes respectively [Fig. 2].

Methanol extract also produced dose-dependent paralysis of *Haemonchus contortus*at concentration of 100 and 50 mg/mL, paralysis was observed at 9 and 13 minutes respectively, while lower mortality time was 9 minutes at 100 mg/mL concentrations [Fig. 3].

To determine the anthelmintic activity of *S. cumini* L. scientifically, the methanolic extract from the leaves of this plant was collected. In order to examine the anthelmintic activity of *S. cumini*, earthworm, red worm and tape worm were used. All the concentration of plant extract showed significant anthelmintic activity. The results of plant extracts were compared with standard drug Albendazole, in the case of standard drug worms were just paralyzed but remained alive when placed in fresh water.

S. cumini is a valuable medicinal plant in Ayurveda and Unani system of medication for having healing properties. Intake of *S. cumini* is a cheaper and beneficial source to control diabetes. *S. cumini* seed has gastro-protective, anti-cancer and anti-viral properties. It is most effective in case of peptic ulcer as it helps to promote mucosal defensive factors as well as antioxidant status and decrease lipid peroxidation. Leaves of this plant are used to cure constipation, diabetes, dermopathy, fever, gastropathy, leucorrhoea, stomachalgia and strangury (Srivastava and Chandra, 2013). The leaves also help to strengthen teeth and gums (Damasceno *et al*., 2002).

Phytochemical analysis of the leaves of *S. cumini* revealed that it contains acylated flavonol glycosides, esterase, galloyl carboxylase, myricetin, myricetin 3-O-4-acetyl-L-rhamnopyranoside, quercetin, tannin, and triterpenoids (Ayyanar and Subhan, 2012). It was reported that tannins are responsible for anthelmintic activities in *S. cumini* (Shrestha *et al*., 2009). This may be due to tannins that bind with proteins in the gastrointestinal tract of parasite (Athanasiadou *et al*., 2001) or glycoprotein on the cuticle of host animal and finally cause death (Thompson and Geary, 1995). The anthelmintic activity of the methanolic extract against earthworms, red worm and tape worm suggested that it is an effective source against parasitic infections. Further studies are needed to identify the mechanism responsible for the anthelmintic activity and to study the pharmacological actions.

Conclusion

The methanolic extract of the *Syzygium cumini* L. (Jamun) leaves was more effective even at lower concentrations in causing paralysis and death of earth worms, tape worms and red worms than the standard drug, Albendazole. It can be concluded that active constitutents that are presents in the methanolic extract of leaves of *S. cumini* L. are responsible for anthelmintic activity.

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