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Research Article

Evaluation of Anthelmintic and Antimicrobial Activity of the Extract of the Root of the Plant *Jasminum multiflorum*(Andr.)

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ABSTRACT

As per qualitative evaluation in different solvents of the root of the plant satisfies the presence of cardiac glycosides along with trace quantities of steroid and saponins. Among them the petroleum ether extract of the root of the plant *Jasminum multiflorum* was evaluated for anthelmintic activity and the ethanolic extract was evaluated for antimicrobial activity.^{1,2} Traditionally this species are used in indolent ulcer, pitta and inflammation. Only few CNS activity are reported on ethanolic extract of aerial part of the plant, though the root of the plant is more potent as per folkore claim. The petroleum ether extract was investigated for anthelmintic activity using earthworm (*Pheretima posthuma*) at different concentration (5mg/ml – 50 mg/ml). As standard albendazole suspension (10mg/ml) and 3% solution of normal saline was used as control. The death and paralysis time were recorded and compared. Extract exhibit significant anthelmintic activity at (100mg/ml) concentration and found effective.^{1,3,4} The ethanolic fraction of the root of the plant was collected and evaporated to dryness under vacuum to avoid the presence of even less quantity of ethanol in the extract. Two Gram (+ve) bacteria namely *Bacillus subtilis*, *Staphylococcus aureus* and two Gram (-ve) bacteria namely *Escherichia coli* and *Klebsiella pneumonia* were selected for the estimation of antimicrobial activity depending on zone of inhibition. It was seen that 100mg/ml concentration of the extract showed maximum activity against *Klebsiella pneumonia* with a zone of inhibition 0.5mm and for others it ranged from 0.3 to 0.4 mm.^{1,4,5}

Keywords: *Jasminum multiflorum*; anthelmintic; antimicrobial; zone of inhibition

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1. INTRODUCTION

'Worm' is a term which comes from a Greek word to describe 'Helminth'. Both tropical and sub-tropical countries are mainly affected by the intestinal worm & different strategies are targeted officially. Among the huge population of world, majority of them are being suffered by this intestinal infection. The gastro intestinal agent is being resistant when exposed to the drug from synthetic origin for which focus has been given to control such infection by the agent obtained from natural sources. As per WHO, about 2 billion people are going to suffer for parasite infection in near future (2025). Not only are that, in developing countries, there are 57% of population are suffering from such infection. Nausea, vomiting, diarrhoea, abdominal pain is the general symptoms occur due to such infections. Though such reported data reveals the severity of such infection; Very little research work has been occurring to control such worms. Not only that, the evaluation of highly effective agent is very less also.

The major route of pathogenic process of such worms occurs mainly through food.^{5,6}

There is an essential need to establish new drug molecule in this aspect because of the resistant is being developing by the worm to synthetic agent. Now to promote traditionally used anthelmintic plant, proper study and evolution of active principle from that source are needed. In this research, the petroleum ether fraction of the root of the plant *J. multiflorum* was evaluated to identify the potentiality of the plant in this aspect.^{5,6,7}

Infection procedure of round worm may be described by the following figure.

Types of Worms

The tropical and sub-tropical countries are severely infected by mainly three types of worm, namely, Tape-worm (cestodes), Round-worm (nematodes) and flukes (trematodes).



Tapeworms

Round-worm

Flukes

Classification of the Agent

According to the similar types of chemical structure & mechanism of action, the different anthelmintic agents are^{7,10}:-

- Benzimidazole derivatives: Since 1960, this is used. (Example. Mebendazole, Albendazole)
- Diethylcarbamazine: Since 1940, this is used.
- Piperazine citrate: Applied to children
- Praziquantel: Derivative of Isoquinoline

2. MATERIALS AND METHOD

As per the literature survey different portion of different plants were successfully evaluated for anthelmintic activity. Not only that the aerial parts of *Jasminum multiflorum* showed anthelmintic activity in dose dependent manner^{8,9}.

The worm *Pheretima posthuma* resembles with worm of human, helminthic infestation and their control. As per folklore claim the plant may be used of the treatment of helminthic infestation in human.

So I have selected the study to observe the action of petroleum ether extract of the root of the plant on *Pheretima posthuma* in varying dose dependent manner.

The petroleum ether extract of the roots of the plant is evaluated for its anthelmintic activity the preparation, collection and extraction and the percentage of yield of petroleum ether extract of *Jasminum multiflorum* was 0.14, respectively to dried starting materials. Evaluation of anthelmintic activities: Anthelmintic activities were evaluated for petroleum ether extract of *Jasminum multiflorum*.

As *Pheretima posthuma* possess similar morphological properties with intestinal round worm so it is selected for the study. It is collected from the horticulture department of Kolkata. The worm which were selected that is of 8±1cm. then these worms were divided into 5 groups namely control, standard and test extract (T₁, T₂, T₃) and each group contains 3 worms. The worms of control group were treated with 5% Tween 60 in normal saline, for standard Albendazole (10mg/ml) was taken and for test group petroleum ether extract of the root of *Jasminum multiflorum* (5,10, 25 and 50mg/ml) in normal saline containing 5% of Tween 60 were taken. After that for the next 4hours they were observed for paralysis and death and the results is given in observation table.

Table 1:

Types of dose		No of worms	Time interval for paralysis	Time interval for death
Control	5%tween 60 in normal saline	3	—	—
Standard	Albendazole 10mg/ml	3	17 ±1.00	39 ±0.70
Test	5mg/ml	3	91±1.73	148 ±2.64
	10mg/ml	3	65.66±1.55	128.33 ±1.52
	25mg/ml	3	36±1.51	63±2.07
	50mg/ml	3	24±0.93	50 ±2.08

Evaluation of Antimicrobial Activity

Though the medicines from synthetic sources are mainly screened for antimicrobial property; Now-a-days screening of natural products for such activity is being done in several cases. Although the scope of administration of medicine directly from extract of different plant material are not accepted. But in near future, by the application of more advanced technique, use of such natural product may takes place. The agent which is useful to stop growth or to kill the microorganism is commonly known as antibacterial agent & importantly such agents are grouped according to the microorganism against which they shows the activity. Just like, antibiotics are effective against bacteria while antifungals are effective against fungi. The non-selective antimicrobials are known as disinfectant, while the purpose to prevent infection by the utilization of antimicrobial drug which is termed as antimicrobial prophylaxis. Though there are some difficulties during such screening like hydrophobicity of essential oil, or diffusion of lipophilic oil

components through Agar. Among the different procedures, Nutrient agar used for such study & screening obeyed Disk diffusion method. This research work is mainly done to observe whether the ethanolic extract of the root of *J. multiflorum* shown any activity against bacteria or not & here the zone of inhibition of bacteria were observed only.^{11,12}

2.1 Experimental

Collection and recognition of the root of *J. multiflorum*; as described previously (In introductory chapter) it was done.

2.2 Preparation of Plant Extract

At first the plants were gathered from different locations of Kolkata and North 24 parganas in the month of July and August 2015

The roots of the plant were cut and collected after proper washing under constant tap water in the laboratory. Then

the roots were subjected to air dry under shade condition for next one week. Then the roots were grinded and powdered. Then the powder was sieved through mesh no. 40 then the different extract was subjected for the different experimental work. In current evaluation the ethanolic extract was subjected to dryness to avoid the presence of trace quantity of alcohol and subjected for the evaluation of antimicrobial activity.^{14,16}

2.3 Microorganism Taken

The strains were taken from the School of Environmental Studies, Jadavpur University. The stock culture was maintained on nutrient agar media at 37°C. and those microorganisms were used in the study.

Agar Dilution Method for Microorganism

1. For 30mins allow 20ml nutrient agar plates to dry at 37°C.
2. By using sterile glass spreader, spread 500 µL natural selected extract product on to the top of the agar by using pipette.
3. The plate was dried at room temperature for 30mins.
4. Spread 500 µL of the selected bacteria and was put on the extracted solution treated with nutrient agar plate.
5. Incubate the plates over night at 37°C and count the bacterial colonies. The agar absorption assay produces the latest consistent and irreproducible result.
6. Overlay 5ml agar containing 2% extracted solution, with and without 0.02% tween 80, on 15ml of molten nutrient agar plates.
7. At 37° C the bacteria was incubated at the surface of agar plate and allowed for producing the colony.
8. Bacterial growth is measured taking 0 as standard corresponding to the growth of the plates.

Incorporation of the extract into agar, in the presence or absence of tween 80, results in inconsistent growth of bacteria and therefore un-reproducible result.^{11,10}

- Gram (+)ve bacteria - *Bacillus subtilis*, *Staphylococcus aureus*.
- Gram (-)ve bacteria - *Escherichia coli*, *Klebsiella pneumonia*.

2.4 Purification of cylinder (cups)

Porcelain beads of uniform size were selected inspite of stainless steel on. Then these beads were boiled in concentrated HCl for 15mins, followed by washing with distilled water. Then these are putted in crucible and warmed at 600c for 30mins.

2.5 Composition of culture media to study zone of inhibition

Table 2: Composition of the moderate, Nutrient Agar

Peptone	5.0gm
Sodium Chloride	5.0gm
Beef Extract	1.5gm
Yeast extract	1.5gm
Agar	1.5gm
Distilled water up to	1000 ml
pH	7.4 ± 0.2

Nutrient broth was used for the preparation of inoculums of the bacteria and nutrient agar used for screening method Composition of the medium, Nutrient Agar^{14,16}.

2.6 For Zone of Inhibition

- Laminar air flow chamber was first wiped properly with ethanol and then switched on the air flowing unit and UV light. After 2-3mins switched off the UV light.
- 24 cleaned and sterilized petri-dish were taken and kept under the laminar air flow chamber.
- Petri-dishes were noticed correspondingly to the microorganism and the concentration of the standard and test drugs.
- Then Antibiotic assay media no.1 was prepared (as per above mentioned procedure)
- 20ml of the media is put to each petri-dish and kept it for some time to solidify.
- After it gets solidified, four holes were bored (2 hole for standard drug concentration & 2 hole for test drug concentration) in the solidified media by the help of borer in each 32 petri-dish.
- The sterilized cotton was taken and dipped into the nutrient broth containing microorganism^{16,19} (prepared previously).
- The wipe each media with cotton, dipped into the nutrient broth containing microorganism.
- After that standard and aqueous extract of variable concentration was poured into the respective holes.
- Then the petri-dish were covered and kept into the incubator for 24 hours at 37.5°C.
- After 24 hours the petri-dish were taken out and the observation were done.
- For Ethanolic extract same experimental procedure was followed.



Figure 1: *Bacillus subtilis*



Figure 2: *Escherichia coli*

3. RESULTS AND CONCLUSION

The different macerated extract in different solvents shows the presence of cardiac glycoside in polar solvent furthermore the plant material may be evaluated to observe the effect of cardiac glycoside as active constituents but in now the current research work was evaluated to observe anthelmintic activity of the root of the plant *Jasminum multiflorum* and the antimicrobial activity was evaluated for the ethanolic extract of the root of the plant *Jasminum multiflorum*.

The anthelmintic activity of the title compound on *Pheritima posthuma* is exhibited in the table given below. The data

reveals that the roots of petroleum ether extract at the dose level of 5, 10, 25 and 50 mg/ml showed significant anthelmintic activity compare to reference standard Albendazole. However petroleum ether extract of *Jasminum multiflorum* showed the effect at 50mg/ml concentration about to comparable with the reference standard.

100mg/ml concentration of the drug has shown maximum activity against *Klebsiella pneumonia* whose zone of inhibition was 0.5mm. For others the zone of inhibition ranges from 0.4 to 0.3 mm.

So finally the plant may be identified and evaluated for its folklore claim.

Table 3:

Test Drug	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Klebsiella pneumonia</i>	<i>Escherichia coli</i>
Amoxicillin(100µg/ml)	0.3mm	0.5mm	0.2mm	0.4mm
<i>J. multiflorum</i> (25mg/ml)	0.2mm	0.2mm	0.2mm	0.3mm
<i>J. multiflorum</i> (50 mg/ml)	0.2mm	0.2mm	0.4mm	0.3mm
<i>J. multiflorum</i> (100 mg/ml)	0.4mm	0.3mm	0.5mm	0.4mm
Control	00	00	00	00

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