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# *In vitro* biological screening of crude extract and different fractions of *Mirabilis jalapa*

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**Abstract**

The objectives of the current project were to analyze the phytotoxic, insecticidal and anti-termites activities of MJME (*Mirabilis jalapa* Methanolic Extract) and it successive solvent fractions. Methanol preparation and its different fractions were utilized to assess the phytotoxic, insecticidal and anti-termites activities of the selected plant. The MJME and its various fractions expressed low to good potentials against the *Lemna minor* at various concentrations. These fractions were also investigated for their insecticidal potentials, eliciting good activity against the tested insects. The crud extract and its various fractions were also tested for their anti-termites activity expressed good results. Phytotoxic, insecticidal and anti-termites activities of MJME and its various fractions were significant. Extensive researches are need of the hour to collect pure constituents practiced in the synthesis of novel and effective medications.

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## Introduction

Man suffered from many pathological conditions across the globe since ancient times. Approximately 57 million peoples are affected from infection diseases every year. The rising rate of drug resistance attracted the scientists to design and manufacture effective and economical novel products. Medications available from natural sources i.e plants, animals and microorganisms possess limited adverse reactions and are useful for the management of a wide range of abnormalities (Idrees *et al.*, 2018). Natural products have been the most effective source of potential drug leads (Mishra *et al.*, 2011).

Various types of medicinal herbs are present in Pakistan and in the homeland occupies 8$^{th}$ position regarding the export of these precious plants (Sheikh *et al.*, 2005). The population in different developing countries are still relying on the conventional medications to manage various ailments. Even people in developed countries are using traditional medicines to alleviate their sufferings. Different allopathic products are prepared from the derivatives of natural constituents. The present study is exploring novel and effective drugs from this amazing and valuable plant. Literature studies indicate that till now very insufficient studies are performed on the current research project. For finding the biological potentials of the selected plant, the crude extract and its solvent fractions were investigated for 4 type of activities. Statistics showed that up to 35000 to 70000 plant species are practiced in herbal therapy around the globe (khan *et al.*, 2008). Literature surveys estimated that approximately 67% of the medicines have their origin in plants (Ahmad *et al.*, 2011).

Wild plants possess many valuable medicinal herbs practiced in herbal treatment. These medicinal plants are important in pharmaceutical industries because they contain many therapeutic constituents necessary in the synthesis of efficacious medications. Besides this, plants are the important tool for the promotion of man culture in the world. Some plant species have strong nutritional value, as a result these are recommended to cure various deficiency diseases. Ginger, garlic and carrot etc are included in this category.

Plants contain various types of natural constituents such as alkaloids, flavonoids, terpenoids, saponins, carbohydrates and steroids, eliciting important characteristics and are significant as natural medications in many abnormal conditions such as piles, skin, cardiac and inflammatory abnormalities in man (Yari *et al.*, 2011).

The plant *Mirabilis jalapa* is chosen for the present study also known as Four O Clock, Night Beauty and Gul –e- abbas is an important member of Family *Nyctaginaceae* (Bakht *et al.*, 2011). This species is usually occured in different parts of the world like America, Isreal, Sri Lanka, India and Pakistan. This plant carries immense importance to manage different abnormalities. The people in China utilize it to control diabetes (Yıldırım *et al.*, 2000). *M.jalapa* is useful in various kidney diseases. Different organs have valuable therapeutic properties for example bark of the plant is a rich source of energy (Newman *et al.*, 2000). Leaves are effective to control constipation. Eye inflammation, jaundice, and Hepatitis can be cured by taking juice of the leaves (Chetty *et al.*, 2010). Literature studies expressed that very little research have been accomplished on the selected plant so far. MJME and its successive fractions like MJHF, MJEta and aqueous were investigated.

## Material and methods

### *Plant materials*

The selected plant was collected from various areas of Charsadda in 2016.The plant was identified at Botany Department, University of Peshawar.

### *Extract Preparation*

The chosen plant was washed using clean water to eliminate dirts and dry in shade at 37$^{o}$C and convert to find powder using grinder. The materials were then dipped in methonal. The crude preparation was filtered using Whatmann filter paper and concentrated using evaporator. The crude extract was then stored for further exploration.

### *Fractionation*

The crude preparation (1000g) of the *M. jalapa* was mixed in 700ml clean water and then dissolved in

various solvents such as n-hexane, chloroform and ethyl acetate to acquire the respective fractions.

*Phytotoxic Activity*

MJME and its different solvent fractions were analyzed to find out phytotoxic potentials against *L. minor*. Micropipettes, clean flasks, plant growth chamber, plant samples and the chemical methanol were utilized in these experimentations.

*Procedure*

Mc Laughlin procedure was applied to undertaken the said bioassay (McLaughlin *et al*., 1991). The following steps were taken.

A. Stock solution from the plant samples were synthesized in methanol and E- media for the development of *L. minor*.

B. From these stock solutions the tests samples at 10, 100 and 1000µg/ml were transferred to the flasks and kept at 37°C in order to evaporate the organic solvent.

C. 20ml of the E media was poured to every flask.

D. 16 healthy *L. minor* were chosen and kept in the respective flasks.

E. The Flasks having the plant as well as E-media were incubated at temperature of 27 ± 1 °C in growth chamber for a week duration. After this the results were recorded.

*Insecticidal Activity*

The materials, chemicals and equipments needed for the current investigations are; various selected insects such as *Tribolium castanum*, *Rhizoperthica dominica* and *Callosobruchus analis* were utilized. Methanol, growth chamber, test samples, Petri dishes, micro pipettes, filter papers and brush were used in these experiments. The *Khan et al.,* protocols were employed (Khan *et al*., 2008). The filter papers equal to the size of Petri plates were kept in these plates. The selected plant samples stock solution was poured to these plates. These dishes were then kept at 37°C for the evaporation of solvent. Healthy insects from the *T. castanum*, *R. dominica*, and *C. analis* were chosen and placed in Petri dishes having the test samples and control. Methanol and Permethrin were run as negative and positive control, respectively. Afterwards these plates were kept at 27°C for 1 day. Then the percent mortality was calculated.

% Mortality= 100- No. of living insects in test / No. of living insects in control × 100

*Anti termites Activity*

MJME and its succeeding fractions were analyzed for the anti-termites potentials applying *Salihah et al.,* protocols (Salihah *et al*., 1993). Filter papers of equal size to the Petri plates were placed in these Petri dishes. Stock solution of the *M. jalapa* samples were transferred to these plates and kept at 37 °C for some time, in order that evaporation of organic solvent occurred. Then these dishes were kept in desiccator. The plates were keenly checked after first, second and third day. The Eta and MeOH were used as negative control over here. The investigations were performed in triplicate and termites died every day was noted.

**Results**

*Phytotoxic Activity*

The MJME and its different fractions expressed low to good activities against *L. minor* at different concentrations of 10,100 and 1000µg/ml. Findings of the phytotoxic potentials of methanol preparation and its successive fractions are reflected in Table 1.a, b.

**Table 1.a.** Phytotoxic Activity of MJME and its various fractions.

| Name of plant | Conc. of sample µg/ml | No. of fronds | | | | Conc. of standard *µg/ml |
|---|---|---|---|---|---|---|
| | | MJME | MJHF | MJEta | Aqueous | |
| *L. minor* | 1000 | 7 | 5 | 10 | 10 | 0.015 |
| | 100 | 14 | 12 | 13 | 11 | |
| | 10 | 17 | 15 | 12 | 10 | |

**Table 1.b.** Percent growth regulation of *L. minor*.

| Conc. of sample µg/ml | % growth regulation | | | | |
|---|---|---|---|---|---|
| | MJME | MJHF | MJEta | Aqueous | Standard |
| 1000 | 48 | 59.00 | 44.26 | 40 | 100 |
| 100 | 7.82 | 13.02 | 27.0 | 19.2 | 100 |
| 10 | 00 | 00 | 00 | 00 | 100 |

* Paraquat was labeled as standard

*Insecticidal Activity*

The MJME and its various fractions like MJHF, MJEta and aqueous were assessed for the insecticidal potentials. The MJME expressed 30%, 20% and 30% insecticidal activity to *T. castanum*, *C. analis* and *R. dominica*, correspondingly. The percent potentials of MJHF was noted as 100%, 30% and 40% against *T. castanum*, *R. dominica* and *C. analis*, respectively. MJEta showed 40% potentials against *T. castanum* and *R. dominica* while the same gave 30% action against *C. analis*. The aqueous fraction did not produce any result against *T. castanum* and *C.analis* and elicited 20% potentials against *R. dominica*. Table 2, 3 and 4 expressed the relevant results.

**Table 2.** Insecticidal Activity of methanol extract and different fractions.

| Test sample | | Percent mortality |
|---|---|---|
| MJME | *T. castanum* | 30 |
| MJHF | | 100 |
| MJEta | | 40 |
| Aqueous | | 00 |

**Table 3.** Insecticidal potentials of crude preparation and solvent fractions.

| Test sample | | Percent mortality |
|---|---|---|
| MJME | *C. analis* | 20 |
| MJHF | | 40 |
| MJEta | | 30 |
| Aqueous | | 00 |

**Table 4.** Insecticidal Activity of MJME and its successive fractions.

| Test sample | | Percent mortality |
|---|---|---|
| MJME | *R. dominica* | 30 |
| MJHF | | 30 |
| MJEta | | 40 |
| Aqueous | | 20 |

*Anti termites Activity*

MJME and its successive fractions were examined for their anti termites potentials. After 72 hours no termite was survived. The collected results expressed that the crude preparation and its different fractions elicited excellent activity against the tested termites. The results are obtainable from Table 5.

**Table 5.** Anti termites Activity of MJME and its successive fractions.

| Sample | No. of termites utilized | Day | Average termites killed |
|---|---|---|---|
| MJME | 25 | 1st | 13 |
| | | 2nd | 16 |
| | | 3rd | 13 |
| MJEta | | 1st | 17 |
| | | 2nd | 11 |
| | | 3rd | 24 |
| Aqueous | | 1st | 8 |
| | | 2nd | 6 |
| | | 3rd | 4 |

**Discussion**

The present project was set to examine the phytotoxic, insecticidal, and anti-termites activities of MJME and its solvent fractions. A wide range of studies have been done and reported various biological potentials of crude extract and various fractions of many important medicinal plants. Very little studies are available concern with the various activities of the selected plant.

Synthetic insecticides bear many harmful effects on the surrounding environment. So, demand in environment friendly natural insecticides are increasing day by day (Khattak *et al.*, 1985). The insecticidal action of MJME and its different fractions were performed against *T. castanum*, *C. analis* and *R. dominica*, expressing significant results. A same kind of study was undertaking by *Ahmad et al.,* on the *Myrsinea africana* expressed low or remained inactive against these insects. Another important study showed that n-hexane fraction of *Sarcococca saligna* elicited good potentials against *C. analis* and moderate action against *T. castanum*. Ethyl acetate and $CHCl_3$ fractions expressed moderate activity against *C. analis* and *T. castanum*, respectively (Ahmad *et al.*, 2011).

Natural herbicides are environment friendly. *L. minor* is a small aquatic herb, very reactive to bio-organic substances (Rehman *et al.*, 1991). Hence, phytotoxic activity is undertaken to note the phytotoxic and anti-tumor natural constituents. There is a huge possibility to find novel stimulants for the plant growth.

The phytotoxic results of MJME and its different fractions were evaluated and expressed low to

excellent activities against the same plant at various concentrations of 10, 100 and 1000µg/ml. Many researches have been accomplished to record the phytotoxic potentials of crude preparation and fungal metabolites. *Pencillium* metabolites at 10 & 100µg/ml concentrations inhibited *L. minor* growth by 38% while 1000µg/ml stopped the growth of *L. minor* by 82%. However, *Aspergillus* metabolites at 10, 100 and 1000µg /ml hindered the growth of the same plant by 12.5%, 19% and 50% respectively (Idrees *et al.*, 2018).

Similarly, results of the anti termites studies expressed that few termites were killed on day 1 of the experiment, on day 2 nearly half of the termites were alive while on the day 3, all the termites were killed. The present research project expressed that *M. jalapa* is an excellent candidate to obtain effective and biologically active phytocompounds because the methanolic preparation expressed significant activities.

## Conclusion

From the current project, it is clear that MJME and its various fractions elicited good phytotoxic, insecticidal, and anti-termites activities. Comprehensive studies in this regard will be beneficial to isolate active phytochemicals, which are to be utilized in the synthesis of possible novel products.

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