



Phytochemical Analysis and Nematicidal Activity of Ethanolic Leaf Extracts of *Datura metel*, *Datura innoxia* and *Brugmansia suaveolens* Against *Meloidogyne incognita*

Aiyadurai Nandakumar¹, Muthu Mayil Vaganan², Palaniyandi Sundararaju²
and Rajangam Udayakumar^{1*}

¹Post Graduate and Research Department of Biochemistry, Government Arts College (Autonomous),
Kumbakonam - 612 001, Tamilnadu, India.

²ICAR-National Research Centre for Banana, Thayanur Post, Thogamalai Road, Tiruchirappalli - 620
102, Tamilnadu, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author AN performed the research work and wrote the initial draft of manuscript. Author MMV helped in the phytochemical study and managed the literature searches. Author PS helped in the nematicidal study and managed the statistical analysis. The corresponding author RU designed the research problem and corrected the final format of manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJOB/2017/34241

Editor(s):

(1) Jayanta Kumar Patra, Assistant Professor, Research Institute of Biotechnology & Medical Converged Science, Dongguk University, Ilsandong, Republic of Korea.

Reviewers:

(1) Azhari Hamid Nour, International University of Africa, Sudan.

(2) Muhammad Shahzad Aslam, Bioprocess Engineering University, Malaysia.

(3) Noriah Bidin, Universiti Teknologi Malaysia, Malaysia.

(4) Eliton da Silva Vasconcelos, Federal University of São Carlos, Brazil.

Complete Peer review History: <http://www.sciencejournal.com/review-history/19602>

Original Research Article

Received 20th May 2017
Accepted 11th June 2017
Published 19th June 2017

ABSTRACT

The present study was aimed to screen the phytochemicals and quantification of alkaloids, phenolic compounds and flavonoids and to evaluate the nematicidal activity of ethanolic leaf extracts of *Datura metel*, *Datura innoxia* and *Brugmansia suaveolens* against *Meloidogyne incognita*. Phytochemical screening of leaf extracts of *D. metel*, *D. innoxia* and *B. suaveolens* was carried out by qualitative analysis and the results revealed that the presence of bioactive compounds like alkaloids, steroids, flavonoids, terpenoids, phenolic compounds, tannins, anthroquinone glycosides,

*Corresponding author: E-mail: udayabiochem@yahoo.co.in;

saponins and triterpenes. The quantitative phytochemical analysis showed that the maximum amount of alkaloids 12.12 mg/g in leaf of *D. innoxia*, phenolic compounds 5.675 mg/g in leaf of *D. metel* and flavonoids 4.945 mg/g in leaf of *B. suaveolens*. The nematicidal activity of extracts of *D. metel*, *D. innoxia* and *B. suaveolens* were studied against the root knot nematode *M. incognita*. For nematicidal activity, the leaf extracts were used at different concentrations like 250 ppm, 500 ppm, 750 ppm and 1000 ppm in different exposure time like 24 hrs, 48 hrs and 72 hrs. The *in vitro* nematicidal activity results suggested that the extracts of *D. metel*, *D. innoxia* and *B. suaveolens* were possessed nematicidal activity. From this study, we concluded that the extracts of *D. metel*, *D. innoxia* and *B. suaveolens* contain phytochemicals and the plants were exhibited nematicidal activity against root knot nematode *M. incognita*. The present study may be useful for the identification and isolation of novel active nematicidal compounds from the leaf of *D. metel*, *D. innoxia* and *B. suaveolens*.

Keywords: *Datura metel*; *Datura innoxia*; *Brugmansia suaveolens*; *Meloidogyne incognita*; alkaloids; phenolic compounds; flavonoids.

1. INTRODUCTION

Plant-derived substances have recently become a great interest owing to their versatile applications. Medicinal plants are the local heritage with global importance and the world is endowed with a rich wealth of medicinal plants [1]. Secondary metabolites are the substances produced by plants as defense chemicals. They include alkaloids, flavonoids, essential oils, phenols, saponins etc. Recently, many pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants, but the information available is quite meagre [2]. Plant pathogens consist of viruses, bacteria, parasitic green algae, protozoans, fungi and nematodes [3]. Plants may represent a source of natural pesticides [4]. Phytochemicals that extracted from different parts of plant such as alkaloids, glycosides, limonoids, quassinoids and phenolics become new and promising tools as fungicides, bactericides and nematicides [5]. Phytonematodes are root feeders, which completing their life cycles in or on the root zone or even shoot zone in some cases. The most wide spread plant nematodes that parasitize crops are the root-knot nematodes which consist of more than 150 species. Among the root-knot nematodes, *M. javanica*, *M. incognita*, *M. arenaria* and *M. hapla* are of major agronomic importance, being responsible for 95% of infestations [6] and responsible for at least 90% of all damage caused by nematodes [7]. Plant parasitic nematodes, especially root-knot nematodes (*Meloidogyne* species), are among the most important soil borne pathogens of economically important agricultural crops, affecting both the quantity and quality of marketable yields [8,9].

Plant parasitic nematodes cause diseases to nearly all crop of economic importance with estimated losses of US\$ 125 billion per year worldwide [10]. *Meloidogyne* species root knot nematode is one of the most harmful nematode pests in both tropical and subtropical crop production regions and cause extensive economic damage worldwide [11]. The presence of root-knot nematodes causes the deformation of root system when galls are formed; nutrient and water uptake by plant roots are then affected [12]. *M. incognita* has been reported to constitute about 47% of the total root-knot nematodes population [13]. There is increasing interest in the development and adoption of environmental friendly tactics for managing nematodes, particularly as fumigants and other chemical nematicides become more limited [14]. Organic amendments offer an alternative or supplementing control tactic to chemical or cultural control of plant-parasitic nematodes, but confirmation of their potential effect requires more detailed studies [15].

Biological control of root knot nematode is a promising method of management, especially by the bacterial endospore-forming parasite *Pasteuria penetrans* [16]. The applications of natural products are of priority to control the root-knot nematodes infecting vegetable crops. Several workers have reported different plant parts for their nematicidal properties against plant parasitic nematodes which were used as soil amendments or as extracts [17,18]. The nematicidal properties of natural products have been identified by testing the effect of plant parts and extracts (leaves, stem, fruits and seeds), oil extracts, plant exudates and plant volatiles on nematodes that infect plants [19,20]. Various nematicidal compounds of plant origin, including

triglycerides, sesquiterpenes, alkaloids, steroids, tannins, diterpenes, flavonoids, phenolics and many essential oils, have been identified in this way [21]. Numerous plant species, representing 57 families, have been found to contain biologically active phytochemicals which are able to control plant diseases [22]. Alcoholic and aqueous extracts from as many 39 plant species have been shown to possess nematicidal activity against *M. incognita*. Additionally, many of these plant extracts have been reported to possess broad-spectrum activity against diverse insect pests and pathogens [23]. The effective nematicidal activity was reported in extract of *Gliricidia sepium* [24] and *Azadirachta indica* (Neem), *Withania somnifera* (Ashwagandha), *Tagetes erecta* (Marigold) and *Eucalyptus citriodora* (Eucalyptus) against nematodes (*M. incognita*) associated with papaya (*Carica papaya*) [25].

D. metel, *D. innoxia* and *B. suaveolens* belongs to the family Solanaceae are distributed worldwide. All species of *Datura* and *Brugmansia* contain certain biologically active tropane alkaloids. The main alkaloids present in the plants are scopolamine and hyoscyamine. The effects of these alkaloids include stimulation of the central nervous system and simultaneous depression of the peripheral nerves typical for a parasympathomimetic. The medicinal qualities of alkaloids include spasmolytic, antiasthmatic, anticholinergic, narcotic and anesthetic properties [26]. There is no study on *Datura* and *Brugmansia* species in controlling nematodes of crop plants. The use of synthetic nematicidal chemicals for the management of nematodes is an expensive and highly toxic. For the above mentioned reasons, there is a need to search of cheaper and less toxic alternative control measure on nematodes in crop plants. So, the present study was aimed to analyse the phytochemicals and to evaluate the nematicidal activity of extracts of *D. metel*, *D. innoxia* and *B. suaveolens* against root knot nematode *M. incognita*.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Materials

Fresh leaves of *D. metel* and *D. innoxia* were collected from Orathanadu Village, Thanjavur District, Tamilnadu, India and *B. suaveolens* was collected from Ooty, Nilgiris District, Tamilnadu,

India. The collected plants were identified by Rev. Dr. S. John Britto, Director, Rabinet Herbarium and Centre for Molecular Systematics, St. Joseph's College, Tiruchirappalli, Tamilnadu, India and deposited in the herbarium (Voucher specimen number ANK 001, ANK 002 and JN 001). The leaves were washed thoroughly in running tap water and then finally washed with distilled water. The plant materials were dried under shade and then ground well into fine powder. The powdered materials were stored in airtight containers at 4°C until the time of use.

2.2 Preparation of Ethanolic Extract for Phytochemical Screening

Fifty gram of leaf powder of *D. metel*, *D. innoxia* and *B. suaveolens* were soaked in 500 mL of ethanol separately and then kept in orbital shaker for 48 hrs at room temperature. After 48 hrs, the mixture was filtered through a clean muslin cloth and the filtrate again filtered by using Whatmann No.1 filter paper. Then the extracts were concentrated and dried in a rotary evaporator at 37°C [27] till a sticky mass was obtained. After evaporation, the dried extracts were stored at 4°C until further use.

2.3 Preliminary Phytochemical Screening

Phytochemical tests for the screening of bioactive constituents in extracts of *D. metel*, *D. innoxia* and *B. suaveolens* were carried out by using the standard procedures [28-30].

2.3.1 Test for alkaloids

A few mL of the extract was taken and a drop of Mayer's reagent was added by the side of the test tube. A creamy or white precipitate formed indicates the presence of alkaloids.

2.3.2 Test for steroids

Two mL of acetic anhydride was added to 0.5 gram of extract with two mL of H₂SO₄. The colour changed from violet to blue or green, which indicates the presence of steroids.

2.3.3 Test for flavonoids

A few drops of 1% aluminium chloride solution was added to a portion of plant extract. A yellow colouration was observed, which indicates the presence of flavonoids.

2.3.4 Test for terpenoids

Five mL of plant extract was mixed with two mL of chloroform and three mL of concentrated H₂SO₄ was carefully added to form a layer. A reddish brown colouration in the interface was formed, which indicates the presence of terpenoids.

2.3.5 Test for phenolic compounds

Fifty milligram of extract was dissolved in five mL of distilled water and then added three mL of 10% lead acetate solution and formed bulky white precipitate, which indicates the presence of phenolic compounds.

2.3.6 Test for tannins

About ten mL of plant extract was taken in a test tube and a few drops of 0.1% ferric chloride was added and observed the brownish green or a blue-black colouration, which indicates the presence of tannins.

2.3.7 Test for cardiac glycosides

Five hundred milligram of extract was diluted to five mL with distilled water and to this two mL of glacial acetic acid and one drop of ferric chloride were added. Then one mL of conc.H₂SO₄ was added. A brown ring at the interface indicates the presence of deoxysugar characteristic of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may form, which indicates the presence of cardiac glycosides.

2.3.8 Test for anthroquinone glycosides

Two hundred milligram of extract was dissolved in diluted H₂SO₄ and boiled. Then it was filtered and cooled. To the filtrate, three mL of benzene or chloroform or ether was added and mixed well. The chloroform layer was separated and two mL of ammonia solution was added and ammonia layer was allowed to form of rose pink (or) cherry red color development, which indicates the presence of anthroquinone glycosides

2.3.9 Test for saponins

Ten mL of plant extract was taken in a test tube and then mixed with five mL of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil

and again shaken vigorously, then observed the formation of emulsion, which indicates the presence of saponins.

2.3.10 Test for triterpenes

Ten milligram of the extract was dissolved in one mL of chloroform and then one mL of acetic anhydride was added and followed the addition of two mL of concentrated H₂SO₄. Formation of reddish violet colour indicates the presence of triterpenes.

2.4 Quantitative Phytochemical Content Analysis

The quantitative determination of phytochemicals such as alkaloids, total phenolic compounds and flavonoids in the extracts of *D. metel*, *D. innoxia* and *B. suaveolens* were analysed by standard methods.

2.4.1 Determination of alkaloids

Five gram of the sample was weighed and transferred to 250 mL beaker and 10% acetic acid in ethanol was added and covered and then allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until precipitation was complete. The whole solution was allowed to settling. Then the precipitate was collected and washed with dilute ammonium hydroxide and filtered. The residue is the alkaloid, which was dried and weighed. All determinations were carried out in triplicates [30,31].

2.4.2 Determination of phenolic compounds

Hundred milligram of extract was weighed accurately and dissolved in 100 mL of triple distilled water (TDW). One mL of this solution was transferred to a test tube, then 0.5 mL of the Folin-Ciocalteu reagent and 1.5 mL of 20% Na₂CO₃ solution were added and ultimately the volume was made up to eight mL with TDW and shaking the content vigorously and finally allowed to stand for 2 hours after which the absorbance was measured at 765 nm. These data were used to estimate the total phenolic content using a standard calibration curve obtained from various diluted concentrations of gallic acid. All determinations were carried out in triplicates [32].

2.4.3 Determination of flavonoids

The method of determination of flavonoids is based on the formation of the flavonoids - aluminium complex which has an absorptive maximum at 415 nm. 100 µL of the plant extract in methanol (10 mg/mL) was mixed with 100 µL of 20% aluminum trichloride in methanol and a drop of acetic acid and then diluted to five mL with methanol. The absorption at 415 nm was read after 40 minutes. Blank samples were prepared from 100 µl of plant extract and a drop of acetic acid, and then diluted to five mL with methanol. The absorption of standard rutin solution (0.5 mg/mL) in methanol was used as above condition. All determinations were carried out in triplicates [33].

2.5 Preparation of Ethanolic Extract for Nematicidal Activity

One kilogram leaf powder of *D. metel*, *D. innoxia* and *B. suaveolens* were taken separately and soaked in 3 liters of ethanol for 15 days at room temperature in the orbital shaker. The ethanolic extracts were filtered and then concentrated under low pressure at 40°C using a rotary evaporator and the final yield of extracts were stored at 4°C prior to use.

2.5.1 Assay of nematicidal activity

M. incognita juveniles were collected from infested banana roots under field condition at Experimental Farm of ICAR-National Research Centre for Banana and identified by Dr. P. Sundararaju, Principal Scientist, ICAR-National Research Centre for Banana, Tiruchirappalli, Tamilnadu, India. From this, the 2nd stage larval suspension in distilled water was prepared for bioassay. The nematicidal activity was carried out using 6 well sterile plates. Two mL of extracts of *D. metel*, *D. innoxia* and *B. suaveolens* was separately prepared in methanol at various concentrations like 250 ppm, 500 ppm, 750 ppm and 1000 ppm and were poured in 6 well sterile plates and then left for 24 hrs for complete removal (evaporation) of organic solvents by air drying at room temperature. After removal of organic solvent and two mL of 2nd stage juveniles of *M. incognita* suspension (50J₂/2 mL) was added. The two mL of distilled water alone without extracts served as control and then incubated at room temperature. Each treatment was replicated three times. Mortality of 2nd stage juveniles of *M. incognita* was recorded after 24

hrs, 48 hrs and 72 hrs. Mortality was confirmed by touching the larvae with a fine needle for the identification of movements [34].

2.6 Statistical Analysis

The results of this study were subjected to statistical analysis and the results were expressed as mean percentage ± standard deviation of three replicates. Mortality percent value of nematicidal activity was calculated and significance was determined by using Duncan's Multiple Range Test (DMRT) at 5% level ($P = .05$).

3. RESULTS AND DISCUSSION

Plants constitute various natural products that are important for medicinal point of view. Phytochemical analysis is of paramount importance in identifying new source of therapeutically and industrially valuable compounds from medicinal plants have been chemically investigated [35]. The screening and quantification of phytochemicals and nematicidal activity of ethanolic leaf extracts of *D. metel*, *D. innoxia* and *B. suaveolens* against root knot nematode *M. incognita* were carried out. The results obtained from phytochemical screening of *D. metel*, *D. innoxia* and *B. suaveolens* were presented in Table 1. The secondary metabolites alkaloids, steroids, flavonoids, terpenoids, phenolic compounds, tannins, anthroquinone glycosides, saponins and triterpenes were present and cardiac glycosides were absent in extracts of *D. metel*, *D. innoxia* and *B. suaveolens*. Phytochemical screening helps to identify the chemical constituents of the plant extracts and also used to search for bioactive agents for starting products used in the synthesis of some useful drugs [36]. Similarly, the phytochemical analysis and antibacterial activity of leaf of *Vitex negundo* and *D. metel* against bacterial pathogens were reported [37,38].

The extracts of *D. metel*, *D. innoxia* and *B. suaveolens* were subjected to quantitative analysis of alkaloids, phenolic compounds and flavonoids and the results were presented in Table 2. The extracts of *D. metel*, *D. innoxia* and *B. suaveolens* showed different amount of phytochemicals. Among the quantification of secondary metabolites, the alkaloids content was maximum in all three tested extracts when compared with other compounds like phenolic compounds and flavonoids. The extract of *D. innoxia* contain alkaloids 12.12 ± 0.0194 mg/g

followed by *D. metel* 7.314 ± 0.0164 mg/g and *B. suaveolens* 5.903 ± 0.0133 mg/g. Phenolic compounds was observed maximum in *D. metel* 5.675 ± 0.0111 mg/g when compared with *D. innoxia* 3.625 ± 0.0147 mg/g and *B. suaveolens* 3.435 ± 0.0110 mg/g. The flavonoids content in extract of *B. suaveolens* 4.945 ± 0.0256 mg/g, *D. innoxia* 4.118 ± 0.0167 mg/g and *D. metel* 3.701 ± 0.0249 mg/g were observed. Similarly, the screening and quantification of phytochemicals in *Dendrobium ovatum* [39] and the alkaloids, phenolic compounds and flavonoids content quantified in many plant species such as *Pteris argyreae*, *Pteris confusa*, *Pteris vittata*, *Pteris biaurita* and *Pteris multiaurita* [40] and the total phenolic content of *Indigofera aspalathoides* [41] were reported. In the present study, the content of alkaloids, phenolic compounds and flavonoids in leaf of *D. metel*, *D. innoxia* and *B. suaveolens* was observed in lesser amount than other plants like *D. ovatum*, *P. argyreae*, *P. confusa*, *P. vittata*, *P. biaurita*, *P. multiaurita* and *I. aspalathoides*. Nematicidal potential of botanicals obtained from *Andrographis affinis* and *Adhatoda vasica* is possibly due to presence of phytochemicals. The secondary metabolites, alkaloids and flavonoids of plants were reported to have ovicidal and nematicidal properties against root knot nematodes [42-44].

Alkaloids play some metabolic role and in control of development in living system. They are also involved in protective function in animals and are used as medicine especially the steroidal alkaloids [45]. Phenolic compounds are characterized by the presence of several phenol groups and act as chemical barriers against invading pathogens and protecting the plant

species [46]. Flavonoids are a group of polyphenolic compounds and they widely distributed in plants. Almost 6000 flavonoids have been identified in plants. Flavonoids are free radical scavengers to the major role of inhibition of hydrolytic, oxidative enzymes, anti-inflammatory action, anticarcinogenic, antifungal, antiviral and antibacterial activities and inhibit the initiation, promotion and progression of tumours [47]. Triterpenoids are a large class of natural isoprenoids present in higher plants, which exhibit a wide range of biological activities. Steroids have been observed to promote nitrogen retention in osteoporosis and in animals with wasting illness [48]. Anthraquinones are considered to be one of the most active agents in metastatic breast cancer. The mechanism of action of tannins is based on their ability to bind proteins thereby inhibiting cell protein synthesis [39]. *D. stramonium* is a species possess various active biomolecules with medicinal and pharmacological properties and are responsible for antibacterial, antiviral and anti-diabetic activities [49] and the analgesic and CNS depression of the species is attributed to the presence of high alkaloids contents. The atropine biomolecules isolated from this species is used for treating parkinson's disease, peptic ulcers, diarrhea and bronchial asthma [50].

Traditionally, leaves of *D. stramonium* are externally used for injuries, wounds, bleeding and pain. Juice of flower petals of *D. stramonium* is used in ear pain and seeds are used as purgative in cough, fever and asthma [51]. The wound healing property of *B. suaveolens* contain bioactive compounds alkaloids, triterpenoids and flavonoids are well known and are attributed to their antimicrobial

Table 1. Phytochemical analysis of ethanolic leaf extracts of *D. metel*, *D. innoxia* and *B. suaveolens*

Name of the phytochemicals	Ethanolic leaf extract		
	<i>D. metel</i>	<i>D. innoxia</i>	<i>B. suaveolens</i>
Alkaloids	+	+	+
Steroids	+	+	+
Flavonoids	+	+	+
Terpenoids	+	+	+
Phenolic compounds	+	+	+
Tannins	+	+	+
Cardiac glycosides	-	-	-
Anthroquinone glycosides	+	+	+
Saponins	+	+	+
Triterpenes	+	+	+

+ Present: - Absent

Table 2. Alkaloids, phenolic compounds and flavonoids content of ethanolic leaf extracts of *D. metel*, *D. innoxia* and *B. suaveolens*

Name of the plant	Name of phytocompound (mg/g)		
	Alkaloids	Phenolic compounds	Flavonoids
<i>D. metel</i>	7.314 ± 0.0164	5.675 ± 0.0111	3.701 ± 0.0249
<i>D. innoxia</i>	12.12 ± 0.0194	3.625 ± 0.0147	4.118 ± 0.0167
<i>B. suaveolens</i>	5.903 ± 0.0133	3.435 ± 0.0110	4.945 ± 0.0256

Values are expressed as mean ± SD of three replicates

property and also responsible for wound contraction and increased rate of epithelialization [52]. The plant *D. metel*, *D. innoxia* and *B. suaveolens* were examined and observed that they possess secondary metabolites including alkaloids and flavonoids with high concentrations, which may serve as potent sources of bioactive molecules are using as remedies for physiological disorders and pathological diseases.

In this study, the nematicidal activity of extracts of *D. metel*, *D. innoxia* and *B. suaveolens* were recorded at different concentrations such as 250 ppm, 500 ppm, 750 ppm and 1000 ppm when exposed to different time intervals like 24 hrs, 48 hrs and 72 hrs. It is seen from the data presented in Tables 3 to 5 revealed that all three tested extracts were showed nematicidal activity against root-knot nematode *M. incognita*. The nematicidal activity of extract of *D. metel* was analyzed and the results were presented in Table 3. The maximum 53% of larval mortality was recorded at 1000 ppm concentration of extract of *D. metel* after 72 hrs of exposure time.

The extract of *D. innoxia* showed nematicidal activity and the results were presented in Table 4. The maximum 44% of larval mortality of *M. incognita* was recorded at 1000 ppm concentration in 72 hrs of exposure time. The nematicidal activity of extracts of *B. suaveolens*

was determined and the results were showed in Table 5. There was observed the maximum 64% of nematicidal activity was recorded at 1000 ppm concentration of extract of *B. suaveolens* in 72 hrs of exposure time. All the three selected plant extracts were showed gradual increase of nematicidal activity to the plant extracts with the increase of concentration and exposure time. The statistical analysis was performed for the determination of the significance between concentrations of extract (250 ppm 500 ppm 750 ppm and 1000 ppm) and nematicidal activity at different time intervals (24 hrs, 48 hrs and 72 hrs).

The significance was calculated by ANOVA using DMRT at 5% level of P value ($P = .05$). Table 3, 4 and 5 showed significant difference in nematicidal activity among different concentrations and various time intervals. So, the nematicidal activity of the present study was concentrations and time dependent. Because the higher concentration of the extract 1000 ppm showed maximum nematicidal activity than lower concentration 250 ppm. Similarly, 72 hrs of exposure time showed maximum nematicidal activity than 24 hrs of exposure time. The significance was also mentioned in the results as superscript letters. The table value possess superscript a, which indicates the maximum nematicidal activity than other values carry alphabetic letters b - k in the superscript.

Table 3. Nematicidal activity of extract of *D. metel*

Concentration of extract (ppm)	Nematicidal activity (%)		
	24 hrs	48 hrs	72 hrs
250	7.33 ± 1.155 ^g	14.00 ± 2.000 ^f	22.00 ± 2.000 ^e
500	14.00 ± 2.000 ^f	22.67 ± 3.055 ^e	32.67 ± 1.155 ^d
750	21.33 ± 3.055 ^e	32.00 ± 3.464 ^d	39.33 ± 2.309 ^c
1000	29.33 ± 4.163 ^d	45.33 ± 3.055 ^b	53.33 ± 5.033 ^a
Control	1.33 ± 1.155 ^h	4.00 ± 2.000 ^g	7.33 ± 2.309 ^g

Values are expressed as mean ± SD of three replicates

Mean values with common letter are not significantly different ($P = .05$) according to Duncan's Multiple Range Test (DMRT)

Table 4. Nematicidal activity of extract of *D. innoxia*

Concentration of extract (ppm)	Nematicidal activity (%)		
	24 hrs	48 hrs	72 hrs
250	5.33 ± 1.155 ^{gh}	10.00 ± 2.000 ^f	17.33 ± 2.309 ^e
500	10.00 ± 2.000 ^f	18.67 ± 1.155 ^{de}	26.00 ± 2.000 ^c
750	16.67 ± 3.055 ^e	25.33 ± 4.163 ^c	33.33 ± 3.055 ^b
1000	22.00 ± 3.464 ^{cd}	34.00 ± 2.000 ^b	44.00 ± 3.464 ^a
Control	1.33 ± 1.155 ^h	4.00 ± 2.000 ^{gh}	7.33 ± 2.309 ^{fg}

Values are expressed as mean ± SD of three replicates

Mean values with common letter are not significantly different ($P = .05$) according to Duncan's Multiple Range Test (DMRT)

Table 5. Nematicidal activity of extract of *B. suaveolens*

Concentration of extract (ppm)	Nematicidal activity (%)		
	24 hrs	48 hrs	72 hrs
250	10.00 ± 4.000 ⁱ	16.67 ± 3.055 ^h	26.67 ± 2.309 ^g
500	20.67 ± 2.309 ^h	28.67 ± 1.155 ^{fg}	38.67 ± 3.055 ^e
750	32.67 ± 3.055 ^f	42.00 ± 3.464 ^{de}	48.67 ± 4.163 ^{bc}
1000	46.67 ± 4.619 ^{cd}	52.67 ± 5.033 ^b	64.00 ± 2.000 ^a
Control	1.33 ± 1.155 ^k	4.00 ± 2.000 ^{jk}	7.33 ± 2.309 ^{ij}

Values are expressed as mean ± SD of three replicates

Mean values with common letter are not significantly different ($P = .05$) according to Duncan's Multiple Range Test (DMRT)

Similarly, the leaf extract of *D. stramonium* showed the highest percentage of juvenile mortality 100% after 12 hrs of exposure time at higher concentration [53]. These reports are in agreement with the present study results. Similarly, the leaf extracts of some tested plants *A. indica*, *Cartha edulis*, *T. minuta* and *W. somnifera* showed juveniles mortality. In this study, a positive correlation was found between the juveniles' mortality and each of the extract concentration and the exposure time. Extracts of *Datura* and *Brugmansia* species were more effective by increasing the exposure time being the most effective extract. The methanol or hexane leaf extracts of *Solenostemma argel*, *Aristolochia bracteolata*, and *Ziziphus spinachristi* and the seeds of *Aregimone mexicana*, *D. stramonium* and *A. indica* showed effective nematicidal activity [54].

The nematicidal activities of leaf of *D. stramonium*, *Peganum harmala*, *D. innoxia*, *A. mexicana* and *Nicotiana glauca* against *M. incognita* was reported [55]. The suppressive effect of some phytochemical compounds on nematodes population has been well documented in several pathosystem [21]. *Datura* species contains rich source of alkaloids like atropine, meteloidine, nicotine, scopolamine, hyoscyamine, terpenoids and flavonoids which have high rate of nematicidal activity [56,57].

Similarly, the Marigold (*Tagetes* spp.) contains polyacetylenes and polythienyls that proved their nematicidal activity [58]. In this study, the secondary metabolites like alkaloids, steroids, flavonoids, terpenoids, phenolic compounds, tannins, anthroquinone glycosides, saponins and triterpenes were observed in the leaf of *D. metel*, *D. innoxia* and *B. suaveolens* and they may be responsible for the nematicidal activity.

4. CONCLUSION

From this study, we concluded that the medicinal plants *D. metel*, *D. innoxia* and *B. suaveolens* possess phytochemicals and nematicidal activity. So, these plants would serve as source for novel nematicidal agents. Further studies are needed to identify and isolate the active nematicidal compounds from these plants, which may be useful as bionematicide for controlling nematode diseases.

ACKNOWLEDGEMENTS

Authors are thankful to the higher authorities of ICAR-National Research Centre for Banana, Tiruchirappalli, Tamilnadu, India for providing laboratory facilities to carried out this research work and also thankful to Mrs.T. Anitha, Technical Assistant, Division of Nematology, ICAR-National Research Centre for Banana,

Tiruchirappalli, Tamilnadu, India for her help in collection and maintenance of nematodes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Suruyavathana M, Usha V, Shanthanayaki M. Studies of phytochemical analysis and antioxidant activity of selected medicinal plants from Kolli hills. *J. Pharm Res.* 2010; 2:260-262.
2. Padmavathy S, Mekala V. Preliminary Phytochemical Investigation of some Medicinal Plants of Western Ghats, the Nilgiris. *Int J of Res in Pharmaceutical and Biomedical Sci.* 2013;4(1):1-5.
3. Agrios GN. *Plant pathology*, 5th ed. Elsevier-Academic Press, San Diego; 2005.
4. Matthiessen JN, Kirkegaard JA. Biofumigation and enhanced biodegradation: opportunity and challenge in soil borne pest and disease management. *Crit. Rev. Plant Sci.* 2006; 25:235-265.
5. Khalil MS. Bright future with nematicidal phytochemicals. *Biol Med J.* 2014;6(2):2-3.
6. Sasser JN, Eisenback JD, Carter CC, Triantaphyllou AC. The international *M.* project its goals and accomplishments. *Ann. Rev. Phytopathol.* 1983;21:271-288.
7. Castagnone Sereno P. Genetic variability in parthenogenetic root-knot nematodes, *M. spp.*, and their ability to overcome plant resistance genes. *Nematology.* 2002; 4:605-608.
8. Peachy JE. Nematodes of tropical crops. Commonwealth Bureau of Helminthology, Technical communication no. 40, St. Albans, U.K; 1969.
9. Netsher C, Sikora RA. Nematode parasites of vegetables. In: Luc M, Sikora RA, Bridge J (eds). *Plant parasitic nematodes in subtropical and tropical agriculture* 2nd ed. International Institute of Parasitology, CAB International, Wallingford. UK. 1993;237-283.
10. Chitwood DJ. Research on plant-parasitic nematode biology conducted by the United States department of agriculture-agricultural research service. *Pest Manag. Sci.* 2003;59(6-7):748-753.
11. Sikora RA, Fernandez E. Nematode parasites of vegetables. In: *Plant parasitic nematodes in subtropical and tropical agriculture*, Ed. Luc M, Sikora RA, Bridge J. CABI Publishing, Wallingford, UK. 2005;319-392.
12. Sikora RA, Greco N, Silva JF. Nematode 14. [IITA] International institute of tropical agriculture, parasites of legumes. In: Luc M, Sikora RA, Bridge J. (Eds.), 2nd Ed. *Plant parasitic nematodes in subtropical and tropical agriculture*. CAB International, Wallingford, UK. 2005;259-392.
13. Sasser JN, Carter CC. Overview of the International *M.* project In: Sasser JN, Carter CC. (Eds.), *an advanced treatise on M. Biolo. Control*. North Carolina State University and United States agency for international development, Graphic Raleigh. 1985;1.
14. Schneider SM, Roskopf EN, Leesch JG, Chellemi DO, Bull CT, Mazzola M. Alternatives to methyl bromide preplant and post harvest. *Pest Manage. Sci.* 2003;59:814-826.
15. Akhtar M, Malik A. Roles of organic soil amendments and soil organisms in the biological control of plant-parasitic nematodes: A review. *Bioresource Technol.* 2000;74:35-47.
16. Chen ZX, Dickson DW. Review of *Pasteuria penetrans*: Biology, ecology, and biological control potential. *J Nematol.* 1998;30:313-340.
17. Susan AH, Noweer EMA. Management of root knot nematode *M. incognita* on eggplant with some plant extracts. *Egypt. J. Phytopathol.* 2005;3:65-72.
18. Ibrahim SK, Traboulsi AT, El-Haj S. Effect of essential oils and plant extract on hatching, migration and mortality of *M. incognita*. *Phytopathol Medit.* 2006; 45:238-246.
19. Qamar F, Begum S, Raza SM, Wahab A, Siddiqui BS. Nematicidal natural products from the aerial parts of *Lantana camara* Linn. *Natural Products Res.* 2005;19:609-613.
20. Parihar K, Rehman B, Siddiqui MA. Nematicidal potential of aqueous extracts of botanicals on *M. incognita in vitro*. *Curr. Nematol.* 2011;22:55-61.
21. Chitwood DJ. Phytochemical based strategies for nematode control. *Annu Rev Phytopathol.* 2002;40:221-229.

22. Sukul NC. Plants antagonistic to plant-parasitic nematodes. Indian J. L.Sci. 1992;12:23-52.
23. Sarma BK, Pandey VB, Mishra GD, Singh UP. Antifungal activity of berberine iodide, a constituent of *Fumaria indica*. Biomedical and Life Sciences. 1999;44:164-166.
24. Rahila Nazli, Mussarat Akhter, Shagufta Ambreen, Abdul Hameed, Nighat Sultana. Insecticidal, nematocidal and antibacterial activities of *Gliricidia sepium*. Pak. J. Bot. 2008;40(6):2625-2629.
25. Khan IA, Sayed M, Shaukat SS, Handoo ZA. Efficacy of four plant extracts on nematodes associated with papaya in Sindh, Pakistan. Nematologia Mediterranea. 2008;36:93-98.
26. Mann J. Murder, magic and medicine. Oxford University Press Oxford. 1996;82-84.
27. Ogu GI, Williams TO, Nwachukwu PU, Igere BE. Antimicrobial and phytochemical evaluation of the leaf, stem bark and root extracts of *Cyathula prostrata* (L) blume against some human pathogens. J Intercult Ethnopharmacol. 2012;1(1): 35-43.
28. Sofowora A. Medicinal plants and traditional medicine in Africa. 2nd ed. Spectrum Books Limited, Ibadan, Nigeria. 1993;1-15.
29. Trease GE, Evans WC. A textbook of Pharmacognosy 13th ed. Bacilluere Tinal Ltd, London; 1989.
30. Harborne J. Phytochemical methods. Chapman and Hall, Ltd London. 1973;49-88.
31. Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in edo and delta states of Nigeria, Global J. Pure and Appl. Sci. 2001;8:203-208.
32. Hagerman A, Muller I, Makkar H. Quantification of tannins in tree foliage. A Laboratory Manual, Vienna: FAO/IAEA. 2000;4-7.
33. Kumaran A, Karunakaran R. Antioxidant and free radical scavenging activity of an aqueous extracts of *Coleus aromaticus*. Food Chem. 2006;97:109-114.
34. Atta-ur-Rahman AM, Khan M, Shabbir M, Abid MI, Chaudhary A, Nasreen MA, Maqbool M, Shameel Sualeh R. Nematicidal activity of marine organisms. Pak J Nematol. 1997;15:95-100.
35. Ambasta SP, Ramachandran K, Kashyapa K, Chand R. Useful plants of India. Publication and information directorate. Council of Scientific and Industrial Research, New Delhi. 1986;433-7.
36. Yakubu M, Akanji MA, Oladiji AT. Aphrodisiac potentials of the aqueous extract of *Fadogia agrestis* (Schweinf. Ex Hiern) stem in male albino rats. Asian J Androl. 2005;7:399-404.
37. Prasanna K, Yuwvaranni S. Preliminary phytochemical screening and antibacterial activity of *D. metel* and *Vitex negundo* against bacterial cold water disease causing organism. Int J Pharm Pharm Sci. 2014;6:230-233.
38. Gargade VA, Kadam DG. Antibacterial activity and phytochemical analysis of some medicinal plants against *Xanthomonas axonopodis* pv. Punicae bacterial blight of pomegranate, Bionano Frontier. 2013;6(1):26-28.
39. Ganapaty S, Ramaiah M, Ysaswini K, Nuthakki VK, Harikrishnareddy D. Quantitative phytochemical estimation and evaluation of hepatoprotective activity of methanolic extract of *Dendrobium ovatum* (L.) Kraenzl. Whole plant against CCl4 induced hepatotoxicity. J Pharmacogn Phytochem. 2013;2(3):113-118.
40. Herin Sheeba Gracelin D, John De Britto A, Benjamin Jeya Rathna Kumar P. Qualitative and quantitative analysis of phytochemicals in five *Pteris* species. Int. J. Pharm. Pharm. Sci. 2013;5(1):105-10.
41. Tamilselvi N, Krishnamoorthy P, Dhamotharan R, Arumugam P, Sagadevan E. Analysis of total phenols, total tannins and screening of phytocomponents in *Indigofera aspalathoides* (Shivanar vembu) Vahl EX DC. J Chem Pharm Res. 2012; 4(6):3259-3262.
42. Knobloch K, Pauli A, Iberi N, Weigand N, Weis HM. Antibacterial and antifungal properties of essential oil components. J Essent Oil Res.1989;1:119-128.
43. Adegbite AA. Comparative effects of carbofuran and water extract of *Chromolaena odorata* on growth, yield and food components of rook-knot nematode infested soybean (*Glycine max* (L) Merrill, Ph.D. thesis, University of Ibadan, Nigeria, (*In press*). 2003;120.
44. Trifonovo Z, Atanasov A. Investigation on the nematicidal effects of some plant extracts on mortality and hatching of *M. incognita* and *Globodera rostochiensis*. Plant Sci. 2009;46:548-554.

45. Petti S, Scully C. Polyphenols, oral health and disease: A review, J Dent. 2009;37(6):413-423.
46. Asha K, Rasika CT, Nirmala RD, Jyoti PS. Antioxidant potential from salem bark of *Juglans regia* L. Ann. Biol. Res. 2011; 2(1);176-180.
47. Jayanthi P, Lalitha P, Shubashini KS. Phytochemical investigation of the extracts and the solvent fractionates of the aqueous extract of *Eichhornia crassipes*. J. Pharm. Res. 2011;4(5):1405-1406.
48. Brahim L, Noureddine G, Samir H, Segni L, Amar Z. Mechanism of action of tannins. Der Pharmacia Lettre. 2010;2(6):142-145.
49. Tyler V, Brady LR, Robbers JE. CNS depression by scopolamine alkaloids. In: Pharmacognosy 7th ed Vet. Hum. Toxicol. 1990;32:355.
50. Sayyed A, Shah A. Phytochemistry, pharmacological and traditional uses of *D. stramonium* L. Review. J Pharmacogn Phytochem. 2014;2(5):123-125.
51. Tsuchiya H, Sato M, Miyazaki T. Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*. J Ethnopharmacol. 1996;50(1):27-34.
52. Annie M, Bounty Rebekah Aviv Y, Venkateswara Rao P, Sudhakar AMS. Identification of aphrodisiac, antioxidant activity in angel *B. root* aqueous extract. The Experiment. 2013;13(1):814-821.
53. Chaudhary KK, Haile A, Ayresea ZG, Semereab G, Weldegergish T. Nematicidal activity of Eritrean weed plants against the root-knot nematode *M. incognita* (Kofoid and White) Chitwood. Nematropica. 2013;43:207-215.
54. Elbadri GA, Lee DW, Park JC, Yu HB, Choo HY. Evaluation of various plant extracts for their nematicidal efficacies against juveniles of *M. incognita*. J. Asia Pac. Entomol. 2008;11:99-102.
55. Saeed MRM, Awadh GAM, Al-Thobhani MA, Aziza Taj Al-Deen. *In vitro* nematicidal activity of ten plant extracts against juveniles of *M. incognita*. Egypt. J. Agronematol. 2015;14(1):78-90.
56. Shahwar D, Abid M, Rehman AU, Maqbool MA, Choudhary MI. Nematicidal compounds from *D. fastuosa* Eds. Rehman AU, Choudhary MA, Sheikhan MS. In the proc. 19th IURC Symposium on the Chemistry of Natural Products. HEJ Res. Inst. of Chemistry, University of Karachi, Pakistan.1995;171-179.
57. Pavela R. Insecticidal activity of certain medicinal plants. Fitoterapia. 2004;75:745-749.
58. Wat CK, Prasad SK, Graham EA, Partington S, Arnason T. Photosensitization of invertebrates by natural polyacetylenes. Biochem. Syst. Eco. 1981;9:59-62.

© 2017 Nandakumar et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://sciedomain.org/review-history/19602>