



# Nematicidal and antimicrobial activities of methanol extracts of 17 plants, of importance in ethnopharmacology, obtained from the Arabian Peninsula

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## ABSTRACT

**Aim/Background:** The development of resistance to synthetic drugs by target organisms is a major challenge facing medicine, yet locked within plants are phytochemicals used in herbal medicine (especially in the Arabian Peninsula) that may find application in this regard. In pursuit of unlocking these “hidden treasures,” the methanol extracts of leaves, aerial parts, fruits, and resins of 17 plants used in the Arabian Peninsula were screened for antimicrobial activities. **Materials and Methods:** The nematicidal, antibacterial, and antifungal activities were determined using appropriate assays. *Steinernema feltiae*, *Staphylococcus carnosus*, *Escherichia coli*, and *Saccharomyces cerevisiae* were used as test organisms. Concentrations of the extracts ranging from 0.5 to 20 mg/ml were tested and appropriate statistical tests performed on the data generated.

**Results:** The results show that extracts from *Solanum incanum*, *Chenopodium murale*, *Commiphora myrrha*, *Anthemis nobilis*, and *Achillea biebersteinii* were the most active and had very high activities against two or more of the test organisms at low concentrations. Extracts of the leaves of *S. incanum* and resins of *Ferula asafoetida* were the most active nematicides, with significant activity at 0.5 mg/ml. Extracts of *C. myrrha* and *C. murale* had the most active antibacterial activity with inhibition zones of 12-15 mm and minimum inhibitory concentrations (MICs) of 2.5 mg/ml for both bacteria. Extracts of the leaves of *A. biebersteinii* were the most active fungicide, giving an MIC of 1.5 mg/ml. **Conclusion:** The results validate the use of these plants in ethnopharmacology, and open new vistas of opportunities for the development of cheap but effective agents that may be useful against infectious diseases.

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## INTRODUCTION

Although significant progress has been achieved during the last 50 years in fighting infectious diseases, they still remain

an important cause of morbidity and mortality globally [1]. Infections cause an estimated 50% of all deaths in tropical countries, where as much as three million preschool children die each year solely due to infections of the gastrointestinal tract [2].

Besides bacteria and fungi, nematodes transmitted from the soil cause diseases which affect 25% of the world's population, again mostly in the tropics. They are known to lead to anemia and to cause retarded physical and mental growth [3,4]. The negative effects of nematodes on agricultural livestock are also well-documented [5,6]. As for bacteria enteropathogenic strains of Gram-negative *Escherichia coli* are known to cause acute and chronic diarrhea, vomiting, and fever in infants [7]. The Gram-positive bacterium *Staphylococcus aureus* can multiply and spread widely in tissues resulting in enteric infections, boils, skin sepsis, endocarditis, and pneumonia. Their heat-stable endotoxins cause diarrhea, fever, abdominal cramps, and vomiting with an attendant electrolyte imbalance [8]. Owing to their ability to thrive better in warm, humid environments, fungal infections are equally problematic and more rampant in the tropics and sub-tropics than any other place in the world. They cause diseases ranging from superficial mycoses, cutaneous mycoses, sub-cutaneous mycoses, and systemic mycoses; and are usually very difficult to treat [9]. These organisms also cause diseases in domestic and farm animals resulting in massive economic losses.

Unfortunately, many drugs currently available for the treatment of infections are expensive and often not readily available or are easily counterfeited. Furthermore, the development of resistances to these drugs is a major setback to their continued use in humans and livestock [10-12].

Interestingly, the tropics where most of these infections are rampant are also amazingly rich in a diversity of plants and fungi. Given the WHO report that medicines derived from plants serve the health needs of approximately 80% of people globally [13], it is important to screen plants that are used in ethnopharmacology and ethnomedicine for activities against nematodes, bacteria, and fungi. Such plants may provide new and, above all, inexpensive and locally available drugs and improve the health of people in economically under-developed or developing countries.

In the light of the above, the nematicidal and antimicrobial properties of methanol extracts of 17 plants used in ethnopharmacology and ethnomedicine around the tropics and sub-tropics, and particularly in Saudi Arabia and Yemen were investigated [Figure 1]. The primary aim of this investigation has been to uncover phytochemical products that can be produced locally and in sufficient commercial quantities to be used in improving Medicine and Agriculture, especially in some of the developing economies of the world. Details of the plants, the parts harvested and their uses in folk medicine have been obtained from published literature, and traditional users of the plants [14-16] are summarized in Table 1.

## MATERIALS AND METHODS

### Plant Materials

The plant materials were collected between the months of March and April 2014 at different locations in Al Baha town, and its outskirts, Saudi Arabia. *Dendrosicyos socotrana* and *Dracaena cinnabari* were collected from the island of Socotra between November and December 2014. Those plants were identified taxonomically at the Department of Botany, Faculty of Science, Aden University, Republic of Yemen. Voucher specimens of the plant materials were deposited at the Pharmacognosy Department, Faculty of Clinical Pharmacy, Al Baha University, Saudi Arabia for the Saudi plants and at the Department of Botany, Faculty of Science, Aden University, Yemen for *D. socotrana* and *D. cinnabari*.

### Preparation of Plant Extracts

The plant parts harvested were air-dried under the shade at ambient temperature and powdered with a blender. The powdered plant material (10 g) was extracted with absolute methanol (4 × 100 ml). The extractions were carried out at room temperature with the constant shaking of the extraction set-up. Thereafter, the mixtures were filtered, and the filtrate



**Figure 1:** Map of Arabian Peninsula indicating the regions of plant collection

**Table 1: Medicinal plants selected as part of this study, ethnobotanical information and relevant characteristics**

Species	Plant family (voucher no)	Part tested (yield in %)	Local name	Traditional uses
<i>Achillea biebersteinii</i> Afan.	Asteraceae (CP-101)	Fl (1.5)	Thafra	Antispasmodic, and for <sup>1</sup> kidney inflammation
<i>Achillea biebersteinii</i> Afan.	Asteraceae (CP-102)	L (2.6)	Thafra	As antispasmodic, and for inflammation
<i>Calotropis procera</i> (Aiton) W.T. Aiton	Asclepiadaceae (CP-091)	L (4.2)	Alashur	For treating leprosy and filariasis
<i>Chenopodium murale</i> L.	Amaranthaceae (CP-081)	F (7.3)	Jkheara	Leishmaniasis <sup>1</sup>
<i>Dendrosicyos socotrana</i> Balf.f.	Cucurbitaceae (CP-14-1)	L (4.3)	Al-kheyar	Severe constipation <sup>2</sup>
<i>Dodonaea viscosa</i> Jacq.	Sapindaceae (CP-061)	L (3.5)	Shath	For treating chronic ulcer, burns, leishmaniasis <sup>2</sup>
<i>Dracaena cinnabari</i> Balf.f.	Asparagaceae (CP-17-1)	Re (3.5)	Dam Al-akhween	Antispasmodic, wound healing <sup>2</sup>
<i>Euphorbia helioscopia</i> L.	Euphorbiaceae (CP051)	AP (4.2)	Al-dehin	Antiseptic
<i>Lavandula dentata</i> L.	Lamiaceae (CP-041)	AP (2.9)	Al-shiah	As antispasmodic, antiseptic when the leaves are chewed <sup>1</sup>
<i>Pulicaria crispa</i> SCH.BIP	Asteraceae (CP-102)	AP (3.1)	Arararabi	Antimalarial, stomach disorders <sup>2</sup>
<i>Punica granatum</i> L.	Punicaceae (CP-011)	Fl (2.5)	Al-roman	Anthelmintic, antiseptic <sup>2</sup>
<i>Ruta chalepensis</i> L.	Rutaceae (CP-121)	L (5.2)	Al-shathab	Antimicrobial <sup>2</sup>
<i>Solanum incanum</i> L.	Solanaceae (CP-131)	F (7.6)	al-hadak	Antiseptic <sup>2</sup>
<i>Solanum incanum</i> L.	Solanaceae (CP-132)	L (3.9)	Al-hadak	Wounds, paste from fruits for treating leishmaniasis <sup>2</sup>
<i>Verbesina encelioides</i> (Cav.) Benth and Hook. F. ex a. Gray	Asteraceae (CP-021)	L (3.7)	Safeara	Wounds, skin diseases <sup>2</sup>
<i>Withania somnifera</i> (L.) Dunal	Solanaceae (CP-011)	F (4.6)	Alobeb	Chronic dermatitis <sup>2</sup>
<i>Withania somnifera</i>	Solanaceae (CP 012)	L (8.5)	Alobeb	Chronic dermatitis <sup>2</sup>

AP, aerial parts, F: fruits, L: leaves, Re: resins, Fl: Flowers, <sup>1</sup>Information obtained from Ali *et al.*, 1999, Ali *et al.*, 2001, Fatimi *et al.*, 2005, Gashash 2012 and <sup>2</sup>interviewing with local people

evaporated to dryness *in vacuo* at 40°C to yield the methanol extracts subsequently used as a part of our studies. The yields of each dried extract were calculated in %. The resulting dried crude extracts were stored at 4°C until they were analyzed for nematocidal and antimicrobial properties.

### Nematicidal Activity

*Steinernema feltiae* was purchased from Sautter and Stepper GmbH (Ammerbuch, Germany), as a powder cake product and stored in the dark at 4°C. Fresh samples were ordered before each experiment, and each opened batch was discarded after 6 days. Prior to each experiment, a homogeneous mixture of nematodes was prepared by suspending 200 mg of powder cake in 50 ml of distilled water at 27°C to revive the nematodes. Contained therein, the suspension was allowed to stand at room temperature though with occasional rocking and in moderate light for 30 min. Thereafter, the viability of the nematodes in suspension was determined with a microscope at four-fold magnification (TR 200, VWR International, Belgium). A viability of more than 80% was considered optimal and seen as a prerequisite for each experiment.

Each plant extract (100 mg) was dissolved in 5 ml of 2% DMSO to yield a 20 mg/ml stock solution. From this stock solution, a series of dilutions in water was prepared with 0.5, 1, 3, 5, 10, and 15 mg/ml solutions which were then used for the experiments. To each well in the 96-well plate, 10 µl of the nematode suspension was added (which usually contains 30-40 nematodes per well). Thereafter, 100 µl of each concentration of the plant extracts was added to each well. The control experiment was performed with the DMSO/water vehicle in place of the extracts. The well plates were then assessed immediately for viability under the microscope before incubation in the dark at room temperature for 24 h. After 24 h, 50 µl of distilled water at

50°C was added to each well to stimulate the movement of the nematodes. Thereafter, live and dead nematodes were counted under the microscope (magnification × 4). Each concentration was tested in three different wells per experiment, and each experiment was repeated three times to yield a total of nine repeats per individual experiment.

The viability of the nematodes was expressed as percentages. The viability values were calculated using the equation:

$$\text{Viability (\%)} = \left[ \frac{V_{24h}}{V_{0h}} \right] \times 100$$

Where,  $V_{24h}$  is number of live nematodes after 24 h and  $V_{0h}$  is the number of live nematodes at 0 h.

### Antimicrobial Activity

Two bacterial strains, *Staphylococcus carnosus* TM 300 and *E. coli* K2 (representing Gram-positive and Gram-negative bacteria species, respectively) as well as the fungus *Saccharomyces cerevisiae* were used as representative model organisms for the antimicrobial investigations.

The disc diffusion assay [17] was used to determine antimicrobial activities of the extracts investigated. Nutrient Luria-Bertani and Yeast Extract-Peptone-Dextrose (YPD) (Sigma-Aldrich, Steinheim, Germany) were used as media. Sterile plates were inoculated evenly using sterile swab sticks. Sterile qualitative filter paper discs of 6 mm diameter (VWR International GmbH-Darmstadt, Deutschland, ref. No. 601110, lot. 06513) were impregnated with 20 µl of each extract solution (equivalent to 4 mg/disc). The paper discs were allowed to dry

before being gently placed on the surface of the inoculated agar plates, at positions that were equidistant from each disc. The plates were kept for 3 h in a refrigerator to enable pre-diffusion of the substances into the media. A mixture of Penicillin-Streptomycin-Amphotericin-B was used as positive control while the solvent (methanol) was used as negative control. Plates inoculated with bacteria and yeasts were incubated for 18-24 h at 37°C. Inhibition zone diameters around each disc (diameter of inhibition zone plus diameter of the disc) were measured and recorded at the end of the incubation time [18]. An average zone of inhibition was calculated for the three replicates.

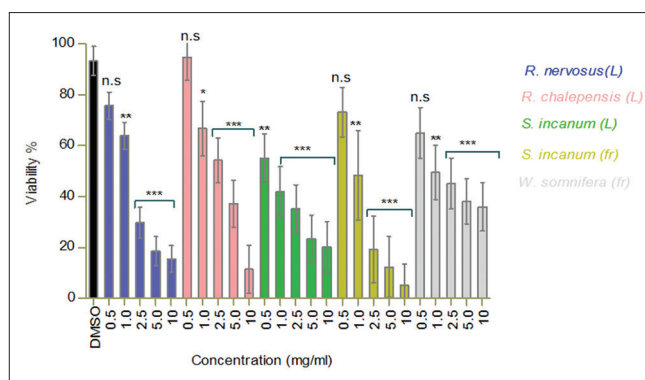
The MIC was determined using the broth microdilution method of Mann and Markham [19], with slight modifications. Fresh cultures of bacteria on LB agar and yeast on YPD agar were prepared and incubated for 18-24 h. From these cultures, inocula were prepared by suspending colonies of the respective organisms in sterile 0.85% NaCl solution and then adjusted to 0.5 of the McFarland standard ( $1.5 \times 10^8$  CFU/ml for bacteria and  $1.5 \times 10^6$  CFU/ml for yeast). Different concentrations (0.5, 1.0, 2.5, 5.0, and 10 mg/ml) of the plant extracts were added to the LB or YPD broth in 96-well plates, and the inocula were subsequently added to each well. Thereafter, the plates were incubated at 37°C for 18-24 h. Antibacterial activity was detected by adding 20  $\mu$ L of 0.01% sodium resazurin (Sigma) and incubating the plates for 1 h. A change from blue to pink indicates a reduction of resazurin and, therefore, bacterial growth. The assay was conducted in triplicate, and three independent experiments were performed on different occasions. The MIC value was defined as the minimum concentration of test sample that inhibited the selected organism's growth, i.e., at which no growth was observed. The reference antibiotics used were penicillin-streptomycin-amphotericin B (Sigma-Aldrich, Steinheim, Germany).

### Statistics

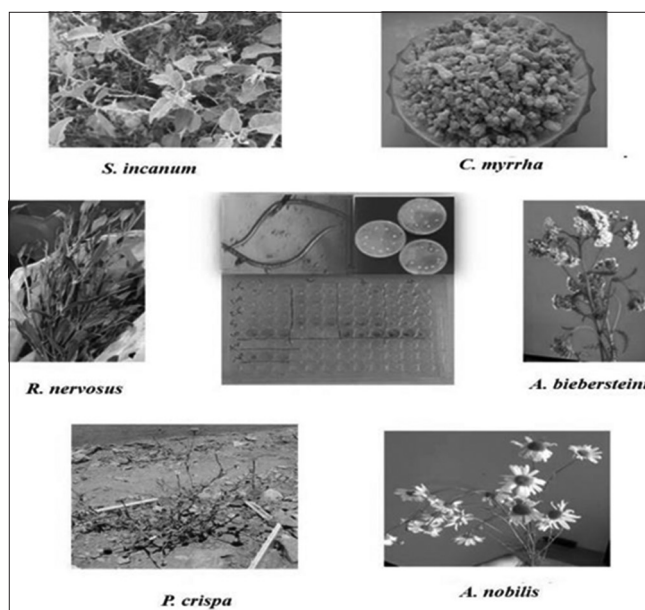
The data generated was subjected to descriptive statistical analysis and the results are presented as mean  $\pm$  standard error of mean. Differences between the means (test versus control) were assessed for statistical significance using the one-way ANOVA test with the significance threshold fixed at  $P < 0.05$ . The GraphPad Prism software (GraphPad Inc., USA) was used for all statistical analyzes. The results are presented in Tables 1-3 and Figures 1-3 and statistical significances in the Figures 1-3 are marked as \*, \*\*, or \*\*\* when the  $P < 0.05$ ,  $< 0.01$  or  $< 0.001$ , respectively.

### RESULTS

For all plant materials under investigation, suitable extracts could be obtained in good quality and yield. Table 1 briefly summarizes the individual yields of extraction for the different extracts. It should be emphasized that the fruits of *Withania somnifera* yielded the most extract (8.5%) while the leaves of *Achillea biebersteinii* yielded the least (2.6%) [Table 1]. The average yield was approximately 5%. The extracts obtained were subsequently investigated for biological activity, first against the nematode *S. feltiae*,



**Figure 2:** Nematicidal activity of the most active plant extracts against the model nematode *S. feltiae*



**Figure 3:** The plants which exhibited the highest toxicity against the selected microorganisms

and subsequently against different bacteria and the fungus *S. cerevisiae*.

### Nematicidal Activity

In the case of nematodes, a distinctively different activity could be observed for the methanol extracts of aerial parts of *Chenopodium murale*, *Pulicaria crispa*, *Euphorbia helioscopia*, and *Lavandula dentata*, leaves of *Dodonaea viscosa*, *Verbesina encelioides*, and *A. biebersteinii*; leaves of *W. somnifera*, *Calotropis procera*, *D. socotrana*, *Rumex nervosus*, *Ruta chalepensis*, and *Solanum incanum*; resins of *C. myrrha* and *D. cinnabari*; fruits of *S. incanum* and *W. somnifera*; flowers of *Punica granatum*, *A. biebersteinii*, and *Anthemis nobilis*. These activities are summarized in Table 2.

While some extracts showed considerable activity against *S. feltiae*, others were hardly active. The extract from the leaves of *S. incanum* was the most active as it resulted in statistically significant mortality of the nematodes at the

lowest concentration tested (0.5 mg/ml) [Figure 2]. Purely for comparison: this concentration corresponds to 2 mM of a chemically pure compound with a molecular weight of 250 g/mol. The next most active extracts in order of activity were those from *S. incanum* and *W. somnifera* fruits, *R. nervosus* leaves, *P. crispa* aerial parts, and resins of *C. myrrha*, each showing statistically significant nematocidal activity at a concentration of 1 mg/ml. This was followed by extracts from *E. helioscopia*, *D. viscosa*, *A. biebersteinii*, *P. granatum*, *D. socotrana*, and *D. cinnabari*, each with statistically significant nematocidal activity at a concentration of 2.5 mg/ml. In contrast, extracts from *C. murale* (10 mg/ml),

*L. dentata* (10 mg/ml), and *C. procera* (20 mg/ml) were hardly effective as nematocides.

### Antimicrobial Activity

The extracts were then tested for their activity against two bacteria, *S. carnosus* and *E. coli*, which are representative of Gram-positive and Gram-negative bacteria, respectively. An activity profile similar to one in nematodes could be observed. In the case of *S. carnosus*, the extract from the fruits of *S. incanum*, but also extracts of the resins of *D. cinnabari* and *C. myrrha* and the aerial parts of *E. helioscopia* resulted in high zones of inhibition and low MIC values against this strain of *Staphylococcus*. *E. coli* appeared to be even more sensitive against a wider range of extracts, such as extracts obtained from the leaves of *C. murale*, *S. incanum*, *D. socotrana*, *A. biebersteinii*, and *V. encelioides*, from the flowers of *P. granatum* and *A. nobilis*, the resins of *C. myrrha*, and the aerial parts of *P. crispa*. All of these extracts resulted in high zones of inhibition and low MIC values against *E. coli*. Ultimately, extracts from the resins of *C. myrrha*, the leaves of *D. socotrana* and *S. incanum*, and from the flowers of *A. nobilis*, provided the best antibacterial activity for both Gram-positive and negative bacteria [Table 3].

A very similar picture also emerged with the single-cell fungus *S. cerevisiae* [Table 3]. Since the broth microdilution method is known to be more reliable and useful in testing plant extracts for activity [19], we have collected data via both, the disc diffusion and the microdilution method. Indeed, in our hands, the latter was also more reliable with regard to toxicity against *S. cerevisiae*. Extracts of the leaves of *A. biebersteinii*, *C. murale*, and *R. chalepensis*, of the aerial parts of *P. crispa*, of the flowers of *A. nobilis* and of the fruits of *S. incanum* inhibited the growth of *S. cerevisiae* at low concentrations. Other extracts such as the

**Table 2: Nematocidal activity of plant extracts against *S. feltiae***

Species	Concentration of extract (mg/ml)				
	0.5	1.0	2.5	5.0	10.0
<i>Achillea biebersteinii</i>	-	-	**	**	***
<i>Anthemis nobilis</i>	-	-	-	*	***
<i>Calotropis procera</i>	-	-	-	-	-
<i>Chenopodium murale</i>	-	-	-	-	**
<i>Commiphora myrrh</i>	-	*	**	***	***
<i>Dendrosicyos socotrana</i>	-	-	-	*	**
<i>Dodonaea viscosa</i>	-	-	**	***	***
<i>Dracaena cinnabari</i>	-	-	*	**	***
<i>Euphorbia helioscopia</i>	-	-	***	***	***
<i>Lavandula dentata</i>	-	-	-	-	***
<i>Pulicaria crispa</i>	-	*	**	***	***
<i>Punica granatum</i>	-	-	*	**	***
<i>Rumex nervosus</i>	-	**	***	***	***
<i>Ruta chalepensis</i>	-	*	***	***	***
<i>Solanum incanum</i>	***	***	***	***	***
<i>Solanum incanum</i> (fruits)	-	**	***	***	***
<i>Verbesina encelioides</i>	-	-	-	**	**
<i>Withania somnifera</i>	nt	-	-	*	**
<i>Withania somnifera</i> (fruits)	-	**	***	***	***

nt stands for "not tested;" \*, \*\*, and \*\*\* show significant differences at  $P < 0.05$ ,  $< 0.01$  and  $< 0.001$ , respectively *S. feltiae*: *Steinernema feltiae*

**Table 3: Antimicrobial activity of plant extracts against *S. carnosus* and *E. coli***

Botanical name	Inhibition zones (mm)			MIC (mg/ml)		
	<i>S. carnosus</i>	<i>E. coli</i>	<i>S. carnosus</i>	<i>S. carnosus</i>	<i>E. coli</i>	<i>S. carnosus</i>
<i>Achillea biebersteinii</i>	8±2	13±4	-	4.0	2.5	1.5
<i>Anthemis nobilis</i>	8±3	15±4	-	4.0	2.5	2.5
<i>Calotropis procera</i>	-	-	-	-	-	-
<i>Chenopodium murale</i>	15±5	13±4	-	2.5	2.5	2.5
<i>Commiphora myrrha</i>	12±3	13±6	-	2.5	2.5	-
<i>Dendrosicyos socotrana</i>	8±2	12±2	-	4.0	2.5	5.0
<i>Dodonaea viscosa</i>	-	-	-	-	-	-
<i>Dracaena cinnabari</i>	13±4	15±2	-	2.5	4.0	-
<i>Euphorbia helioscopia</i>	12±2	15±3	-	2.5	2.5	-
<i>Lavandula dentata</i>	-	-	-	-	-	10.0
<i>Pulicaria crispa</i>	-	11±4	-	-	2.5	2.5
<i>Punica granatum</i>	8±2	6±1	-	4.0	2.5	5.0
<i>Rumex nervosus</i>	-	5±1	-	-	4	5.0
<i>Ruta chalepensis</i>	-	15±3	-	-	2.5	2.5
<i>Solanum incanum</i>	8±2	13±4	-	4.0	2.5	10.0
<i>Solanum incanum</i> (fruits)	12±2	15±4	-	2.5	2.5	2.5
<i>Verbesina encelioides</i>	-	11±2	-	-	2.5	-
<i>Withania somnifera</i>	-	10±2	-	-	4.0	5.0
<i>Withania somnifera</i> (fruits)	-	10±3	-	-	4.0	-
6 mg penicillin, 6 mg streptomycin and 25 µg amphotericin B	30±1	30±2	-	0.01	0.01	0.03

*S. cerevisiae*, *E. coli* and *S. carnosus* represent *Staphylococcus carnosus* TM 300; *Escherichia coli* K2; and *Saccharomyces cerevisiae*; Negative control did not show any activity

ones of the leaves of *R. nervosus* and *W. somnifera* and the flowers of *P. granatum* also inhibited the growth of *S. cerevisiae*, yet to a lesser extent. The extracts from the other plants showed little or no effect with respect to the growth of yeast.

Overall, extracts from 14 of the 17 plants under investigation exhibited high activity against at least one organism tested while the other three plants, *D. viscosa*, *C. procera*, and *L. dentata* showed only limited activities against all of the organisms tested. Among the active plants, *S. incanum* clearly attracted the most attention. Intriguingly, extracts of the fruits of *S. incanum* appeared to possess high activity against all four organisms tested, while the leaves of this plant had limited activity against *S. cerevisiae* and *S. carnosus*. Preparations from other plants were also rather active such as extracts of the aerial parts of *P. crispa* and extracts of the resins of *C. myrrha*, which yielded high activities against three of the four organisms tested. Less active but still of interest due to possible harvesting and processing where the flowers of *A. nobilis* and leaves of *A. biebersteinii*, which showed high activities against two of the four organisms tested.

## DISCUSSION

Although synthetic nematicides and antimicrobials used in Medicine and Agriculture are effective and rapid-acting, the challenges of resistance to these agents by microorganisms, and the concerns to human health and the environment raised by their use in Agriculture, have spurred research efforts at developing “green” plant-based or plant derived alternatives. In fact, various phytochemicals are known to be safe to both humans and the environment. When used in Agriculture, as in the case of nematicides, they are “biodegradable” and usually do not persist in the fields for longer periods of time than is really necessary [20]. Naturally, therefore, research efforts have been geared toward plants that are used in Folk Medicine and Agriculture from different cultures, with a view to identifying those that can be used to develop “green” phyto-protectants, antimicrobials, and pesticides. Many of these plants contain a cocktail of phytochemicals, true treasure chests for bio-activity against the myriad of microorganisms that pose challenges to Medicine and Agriculture, especially in developing countries of the tropics.

Our study has, therefore, investigated the nematicidal and antimicrobial properties of methanol extracts of leaves, aerial parts, and resins from 17 plants used in traditional ethnopharmacology and ethnomedicine in Saudi Arabia, Yemen and neighboring countries of the Arabian Peninsula. Deliberately, methanol was used as the solvent of extraction based on our experience over the years in working with plant products and reports from other researchers working on similar subjects [21]. Indeed, several studies have shown that methanol is the solvent of choice for the extraction of antimicrobial constituents of plants [22-25]. Compared to ethanol, methanol is also less controversial culturally.

Interestingly, extracts from five of the plants studied [Figure 3] - namely *S. incanum*, *P. crispa*, *C. myrrha*, *A. biebersteinii*,

and *A. nobilis* - exhibited high activities at low concentrations against two or more of the organisms tested. *S. incanum*, in particular, which is also known as Jericho tomato, attracted our particular interest, as several parts of this plant seem to be extraordinarily toxic. Though there are reports of toxicity of the plant, such reports should spur on research especially as the plant is particularly promising for several reasons. First, it grows readily, widely and requires little care. Second, it can be harvested and processed easily. Third, it is not used for any other purposes, hence has little “value.” Finally, its extract is amenable to further purification and/or modification, which may improve activity significantly. Indeed, the literature available to date on the most active plants show that *S. incanum* is rich in phytochemicals such as incanumine, solasodine, carpesterol,  $\beta$ -sitosterol, stigmasterol, and khasianine [22]. Lin *et al.* [26] also reported the presence of quercetin, kaempferol, and astragalin in parts of the plant. Besides, members of the *Solanaceae* family have been known for a long time to possess antibiotic activity, which is likely due to the presence of glycosides and alkaloids [27].

Ultimately, a partially purified, stabilized, and appropriately conserved extract of the fruits and/or leaves of the Jericho tomato may well be suitable for applications in the field of Agriculture and possibly also Medicine. Within this context, possible toxic effects on humans and higher animals need to be considered and addressed in earnest, and the possibility of synergism in the action of the individual chemical components contained within the different extracts may have to be accounted for.

Indeed, the activity observed for extracts of *S. incanum*, and the various other extracts may arise from a variety of chemical components and biochemical mechanisms. Though we do not currently possess data on the mechanisms of action, it is known, for instance, that plant extracts often exert their lethal effects through the disruption of cell membrane permeability in organisms that come in contact with them [28]. Within this context, variations observed in the effectiveness of the extracts in killing the nematodes, bacteria, or fungi may indeed be explained by the biological differences which exist between the organisms, for instance, differences in cell wall structure and composition. In fact, it has been reported in other studies, and corroborated by this study, that plant extracts often show a higher activity against bacteria compared to fungi, and this may, in part, be due to differences in the cell wall synthesis and structure [25,29,30]. Specific phytochemicals, such as tannins, furthermore have the capacity to bind to and subsequently denature or disrupt proteins, and if such proteins are vital structural or catabolic proteins, would result in the death of the organism [31-33]. There are obviously many other possible mechanisms and mode(s) of action associated with the plethora of phytochemicals found in those plants.

While we cannot list all of the ingredients contained within our most active extracts and their suspected mode(s) of action, it is worth mentioning a few. Many potent phytochemicals have been found in *P. crispa*, especially sesquiterpene lactones and guaianolide sesquiterpenes [32,33]. Possibly, the *in vitro* antimicrobial activity and known anti-leishmanicidal activities

of the methanol extract of this particular plant are due to the presence of these phytochemicals [34,35]. Similarly, the genus *Commiphora* in general and *C. myrrha*, in particular, is a true hot(s) pot of biologically active secondary metabolites, with more than 300 of them identified and many of them associated with a pronounced activity against a variety of different microorganisms [36,37]. Those include flavonoids, alkaloids, tannins, glycosides, steroids, saponins, and terpenoids, and among them biologically highly active molecules such as myrracadinol A, B, and C, and myrracalamene A, B, and C, and triacont-1-ene [37]. Similarly, flowers of *A. nobilis*, a plant referred to in German language as “Alles zutraut,” meaning “capable of anything” [38], have been used for a long time and are documented in more than 27 national pharmacopoeias. Indeed, this Chuck Norris of medicinal plants has been studied for centuries, and over a century ago, in 1914, Power and Jun [39] reported that the flowers contain essential oils, anthemene, anthemol, and anthesterol. More recently, the terpenoids: bisabolol, chamazulene, and sesquiterpenes; the flavonoids: apigenin, luteolin, and quercetin; and the coumarins: umbelliferone and scopoletin-7-glucoside, have been described as biologically active constituents of *A. nobilis*. Other active substances contained within that particular plant include angelic and tiglic acid esters, anthemiacid, choline, tannin, polysaccharides, phenolic, and fatty acids [40,41]. Finally, the extracts of *A. biebersteinii* contain large quantities of  $\beta$ -sitosterol, stigmaterol, sesquiterpene lactones, guaianolide, germacranolide, and flavonoids [42]. This list of phytochemicals for the five most active plants is clearly not exhaustive and is without prejudice to the apparent rich phytochemical constitution of the other ten plants that did show at least some activity against at least one of the organisms studied. Ultimately, it is, therefore, permissible to speculate that the nematocidal and antimicrobial activities observed in this study derive from the rich milieu of phytochemicals found in these very active plant extracts. These aspects now require further attention.

This study is limited by our inability to access a wider spectrum of organisms and test the activities of these extracts on them. It is hoped that these results will spur interest in these plants and provide the impetus to widen the screening and identification of the active constituents. Work on the latter is currently ongoing in our laboratory. Again, the nematocidal assays would have benefitted from a positive control. This would have afforded us a window to compare the data appropriately. We, however, feel that given the activity observed at concentrations as low as 1 mg/mL in these crude extracts, the purified extracts/fractions may have profound activity and testing them will necessarily require a positive control. In making the above assertion, we are mindful of interactions between individual constituents of plant extracts which may attenuate or accentuate the activity of their parent crude extract.

## CONCLUSIONS

A future search for potent phytochemicals as an integral part of future “green” Medicine and Agriculture should consider the five plants identified by us as most active in more detail, with a particular focus on *S. incanum*. Isolating and characterizing

the active compounds contained therein and elucidating the mode(s) of action will indeed be very important yet also challenging. It may also be useful to develop agents based on combinations of different extracts such as combined extracts of the fruits and leaves of *S. incanum*. Eventually, it may also be feasible to blend active phytochemicals from different plants such as *S. incanum* with *A. biebersteinii* and *R. nervosus* with *C. myrrha*. Moving on from natural products to synthetic chemistry, one may also envisage the design and development of synthetic analogues of the natural compounds, yet this task will be more challenging scientifically and also does not address the matter of local availability at a low cost. After all, the plants selected by us are readily available, easy to cultivate and harvest which renders their utilization cost effective in the Arabian Peninsula and parts of Africa.

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