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In Vitro Sensitivity of Some Mycolic Acid-containing Actinomycetes to *Nigella Sativa* Extracts

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Abstract *Nigella sativa* L. (black seed or cumin) is an herbaceous plant, used for centuries for the treatment of various ailments, including infectious diseases. This study investigates the *in vitro* effect of extract of *N. sativa* against representative of mycolic acid containing actinomycetes notably members of the genera *Gordonia, Mycobacterium, Nocardia* and *Rhodococcus*. Methanolic and petroleum ether extracts of *N. sativa* at different concentrations (0.3 mg/ mL, 0.6 mg/ mL, 1.25 mg/ mL, 2.5 mg/ mL and 5 mg/ mL) were used to impregnate filter paper disks. The disks were incorporated onto Diagnostic Sensitivity Test agar, plates inoculated with test strains and incubated aerobically for up to seven days at 37 ℃. Inhibition zones around discs were measured in millimetres. At 0.6 mg/ mL all test strains showed inhibition zones of 1.5 cm except *Mycobacterium phlei* and *R. rhodochrous*. Petroleum ether extract was found to be more effective than the methanolic extracts since relatively higher concentrations from the latter were needed to achieve similar inhibitions zones produced by petroleum ether extracts. It be concluded that extracts of *N. sativa* is a promising candidate for the *in vivo* application since it is a natural food additive with no reported harmful effect.

Keywords *Nigella Sativa,* Actinomycetes, Antibacterial Agents, Mycolic Acids

1. Introduction

Many cultures around the world have strong beliefs in the use of many different plants as medicine which has rendered some cultures, up to date, almost completely dependent on the use of plants in medicine rather than common commercial medical products. *Nigella sativa* L., belongs to the family *Ranunculaceae*, is a small genus of annual herb found in many regions including the African countries. Its seeds are angular, dark gray in color and are locally known as "black cumin". Its oil is generally regarded as safe and are considered carminative, stimulant, diuretic, emmenagogue, galactagogue and other healing effects[1, 6].

Different crude extracts of *N. sativa* have been tested for their antimicrobial effectiveness and found to have promising effects against some organisms[3, 9, 12].

The aim of this study was to investigate the *in vitro* effect of petroleum ether and methanolic extract of *N. sativa* on representative mycolic acid containing actinomycetes notably *Nocardia africana* which has been recently described in Sudan[8]. Also, strains from the genera: *Gordonia, Mycobacterium, Nocardia* and *Rhodococcus* were tested.

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2. Methodology

Fifty grams of air dried and coarsely powdered of clean seeds of *N. sativa* were extracted in Soxhlet apparatus to obtain methanolic and petroleum ether extracts[5]. The extracts were filtered, and the filtrates were vaporized to dryness, and weighed in order to determine the % yield of the extracts, following the formula: % yield = (weight of extract/ weight of ground plant material) × 100. The stock solutions of the crude methanolic and petroleum ether extracts were prepared by diluting the dried extracts with 50% methanol to obtain the desired final concentrations of 0.3 mg/ mL, 0.6 mg/ mL, 1.25 mg/ mL, 2.5 mg/ mL and 5 mg/ mL. These concentrations were used to impregnate filter paper disks (10 mm diameters). Disk impregnated into 50% ethanol were used as control.

Antibiotic susceptibility testing was performed following the Clinical and Laboratory Standards Institute (CLSI) guidelines as described previously[13]. The dried disks containing different concentrations and the controls were incorporated onto Diagnostic Sensitivity Test agar (Difco) onto which bacteria was inoculated, then incubated aerobically for up to seven days at 37 °C. Inhibition zones around discs were measured in millimetres.

3. Results

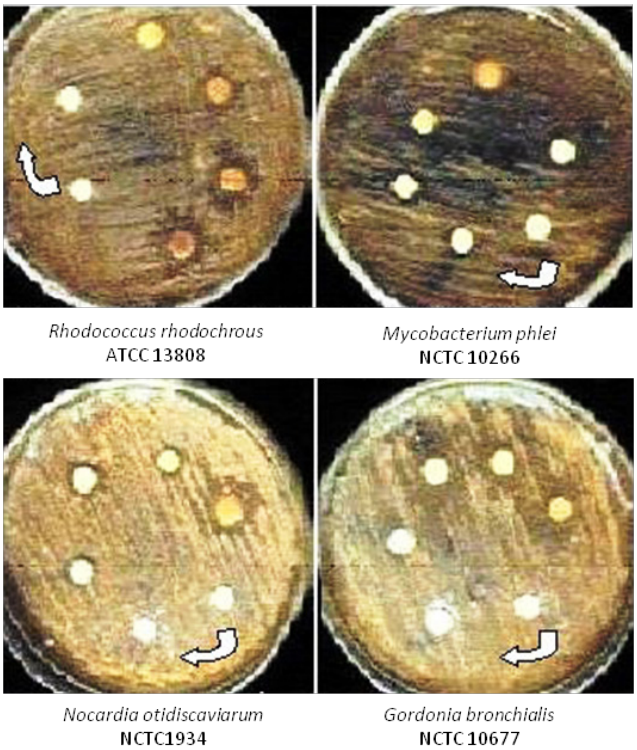

Figure 1. In vitro sensitivity of selected mycolic acid containing actinomycetes to petroleum ether extract of *Nigella sativa* using disc method on DST agar. Arrows indicate the direction of increasing concentration: 312.5µg/ml, 650µg/ml, 1,250µg/ml, 2,500µg/ml, 5,000µg/ml, and 10,000µg/m

Table 1. *In vitro* sensitivity of selected mycolic acid containing actinomycetes to disk impregnated at different concentration of methanolic and petroleum ether extracts of *Nigella sativa*

	Petroleum ether extract					Methanolic extract				
Strains	312.5µ m/ ml	625 µm/ ml	1250 µm/ ml	2500 µm/ ml	5000 µm/ ml	312.5 µm/ ml	625 µm/ ml	1250 µm/ ml	2500 µm/ ml	5000 µm/ ml
Gordonia branchialis NCTC 10677	+	+++	+++	++++	++++	+	++	+++	+++	++++
Gordonia sp. SD156	+	+++	+++	++++	++++	-	+	++	+++	++++
Mycobacterium farcinogenes NCTC 10955	+	+++	+++	++++	++++	-	+	++	++	++++
M. phlei NCTC 10266	-	+	+	++	++	-	-	+	+	++
M. senegalense NCTC 10956	+	+++	+++	++++	++++	-	+	++	++	++++
M. senegalense N717	+	+++	+++	++++	++++	-	+	++	+++	++++
M. smegmatis NCTC8159	++	+++	+++	++++	++++	+	++	+++		++++
Nocardia africana SD880 (NCTC 13182)	++	+++	+++	++++	++++	-	-	+	+	++
N. africana SD910 (NCTC 13183)	+	+++	+++	++++	++++	-	+	++	++	++++
N. africana SD925 (NCTC 13184)	+	+++	+++	++++	++++	-	-	+	+	++
N. otidiscaviarum NCTC1934	+	++	++	+++	++++	-	-	+	++	+++
R. rhodochrous ATCC 13808	-	-	+	++	++++	-	-	++	++	+++

++++, 2 Cm inhibitory zone diameter; +++,1.5 cm; ++, 1 cm ; +, 0.5 cm; -, no inhibition.
ATCC, American Type Culture Collection, Rockville, Maryland, USA; NCTC, National Collection of Type Culture, London, UK; SDs, M.E. Hamid laboratory numbers

The effect of *N. sativa* in terms of inhibition zones is shown in Figure 1 and Table 1. At 312.5 µg /ml, 0.5 cm inhibition zone noticed in all test strains except *Rhodococcus rhodochrous*. At 625 µg/ml all test strains showed inhibition zones of 1.5 cm (+++) except *Mycobacterium phlei* and *R. rhodochrous*. Petroleum ether extract was found to be more effective than the methanolic extracts since relatively higher concentrations from the latter were needed to achieve similar inhibitions zoned produced by petroleum ether extracts.

4. Discussion

Mycolic acid containing actinomycetes (MACA) are a wide group of bacteria belonging to the genera *Mycobacterium, Nocardia, Gordonia* and *Rhodococcus*[7]. It includes some species that cause diseases such as tuberculosis, mastitis, and pulmonary infections[10]. MACA are known to be extremely tough and resistant to many therapeutic agents, both in man and animals[2, 4]. *Mycobacterium* is the main strong acid fast organisms, *Gordonia* and *Nocardia* are week acid fast whereas *Rhodococcus* is a non acid fast[11].

Throughout this study, the effects of the petroleum ether extract of *N. sativa* on the selected organisms were found good (Table 1). Organisms were inhibited at different concentrations. The most susceptible organisms were *G. bronchialis* and *N. otidiscaviarum*, and the inhibitory zones of 0.5 cm have been noticed on disk impregnated with the 312.5 µg/ml concentration.

On the other hand *M. phlei* showed 0.5 cm inhibition zone at 625µg/ml, while in case of *R. rhodochrous* is was 1250 µg/ml. These organisms can therefore be considered the most resistant to the anti microbial activity of *N. sativa* petroleum ether extract among tested strains.

From these comparisons, it is clear that the petroleum ether extract of *N. sativa*, as an antimicrobial agent, is more effective than the methanolic extract. Furthermore, it should be noted that the effect of the petroleum ether extract on *N. otidiscaviarum* and *G. bronchialis* was stronger than on *M. phlei* and *R. rhodochrous* (Table 1).

The study concluded that extract of *N. sativa* is a promising candidate for the in vivo application since it is a natural food additive with no reported harmful effect. The study needed to be supported by a wider in vitro application, not against MACA, but perhaps against a range of gram positive and gram negative organisms.

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