Phytochemical composition, GC-MS analysis of *Nigella sativum* and *Eugenia caryophyllus* and its antimicrobial efficacy compared with 2.5% Sodium hypochlorite and 2% Chlorhexidine against *Enterococcus faecalis* and *Candida albicans* - An In vitro study

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ABSTRACT

The aim of developing herbal irrigants to replace chemical irrigants during root canal therapy to prevent microbial contamination and infections was selected as the main objective in the present study. *Nigella sativa(N.sativa)* and *Eugenia caryophyllus(E.caryophyllus)* are selected to determine whether the extracts can be used as natural irrigants after evaluating their antimicrobial activity against the pathogens. Phytochemical analysis and GC-MS analysis was studied to identify the bioactive compounds attributing to the antimicrobial properties. Inhibitory studies were evaluated against *E.faecalis* and *C.albicans* using the standard well diffusion method. The activity was then compared with the antimicrobial activity of chemical irrigant (2% Chlorhexidine(CHX) and 2.5% Sodium hypochlorite(Naocl)). The presence of different phytoconstituents from the ethanolic extracts of *Nigella sativa* and *Eugenia caryophyllus* was presented. GCMS examination of *N.sativa* extract identified the presence of potential bioactive compounds as thymoquinone and thymol; and from *Eugenia caryophyllus* extract caryophyllene and eugenol acetate is found to be the important bioactive compounds attributing for the antimicrobial properties. Antimicrobial activity of *N.sativa* extract exhibited 18mm and 19mm of zones, and *Eugenia*

caryophyllus **extracts exhibited 17mm and 19mm against the *E. faecalis and C. albicans*, respectively. About 17mm and 19mm of inhibitory zones were found evident for 2.5% Sodium hypochlorite, and about 19mm and 20mm of inhibitory zones were obtained for 2% Chlorhexidine against the respective organisms. The overall results showed that herbal irrigants were equally potent to the chemical irrigants in inhibiting the growth of pathogens tested in the study. Hence, these herbal medicines from natural sources can be used alternatively to the chemical irrigants in the near future after performing some optimization studies and clinical trials.**

Keywords: Root canal irrigants, *Nigella sativa, Eugenia caryophyllus,* 2.5% Sodium hypochlorite, 2% Chlorhexidine
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INTRODUCTION

Root canal infections are caused due to contamination and failure in endodontic treatments. The contaminated root canal is a significant source of bacteria and candidal growth or biofilms. *Enterobacter faecalis* and *Candida albicans* are reported to be among the causative agents of root canal infections. *Enterobacter faecalis* are the common isolates from the failed endodontic treatment cases (Stuart et al., 2006); and *Candida albicans* is found to be resistant to chemical irrigants used in apical periodontitis (Chávez de Paz et al., 2003). *Enterobacter faecalis* and *Candida albicans* survive as commensals along with other organisms where scant nutrients are available, hence leading to form biofilm. In the biofilm, the organisms adhere and metabolize even in harsh conditions (Prabhakar et al., 2010). The biofilm producers can invade dentinal tubules after protecting themselves from intracanal medication. Both pathogens colonize the tubules and reinfect the obturated root canal (Love, 2001).

Several irrigants have been suggested for use in combination with mechanical preparation. 2.5% NaOCl and 2% CHX are two popular intracanal irrigants with good antibacterial activity (Zehnder, 2006). 2.5% NaOCl is the most commonly used root canal irrigant. Due to the creation of hypochlorous acid (HOCl) while contacting with organic matter, it acts as a strong antimicrobial irrigant (SIQUEIRA JR et al., 1997). However, NaOCl has some drawbacks like corrosiveness to devices (Sim et al., 2001), cytotoxic when it contacts periapical tissues and unpleasant taste (Marais, 2000). 2% Chlorhexidine (CHX) is another antimicrobial irrigant used with 2.5% Sodium hypochlorite during therapy; CHX is reported to be less toxic (Gomes et al., 2001). Lin et al. highlighted that it is bacteriostatic at low concentrations and bactericidal at higher concentrations (Lin et al., 2003). However, Green et al. identified that it showed cytotoxic reactions on cornea endothelial cells (Green et al., 1980).

The search for a novel root canal irrigant from natural and plant-based sources was considered significant in recent research works. According to Oncag et al., the aim of developing herbal irrigants with broad-spectrum antimicrobial activity, anti-inflammatory and antioxidant activity without any adverse reactions at the oral sites is essential for successful root canal therapy (Oncag et al., 2006).Hence in the present research, *Eugenia caryophyllus* and *Nigella sativa* is selected to determine whether the extracts from these herbs shall be used as natural irrigants after evaluating their antimicrobial activity against the pathogens.

The common name of *Eugenia caryophyllus* is clove, and it grows abundantly in Maluku Islands, Indonesia. The plants are harvested in different Asian countries like Indonesia, India, Malaysia and Srilanka. The clove bud oil of Eugenia consists of eugenitin, eugenol, beta- rhamnetin, caryophyllene, crategolic acid and kaempferol (Mittal et al., 2014) (Gaylor et al., 2014). *N. sativa* is very often referred as black seed or black cumin. It is found abundant in Asia (Pakistan and India) and some African countries bordering the Mediterranean Sea. Some of the pharmacologically active constituents of cumin seed extracts are thymoquinone (TQ), dithymoquinone, thymol, and thymohydroquinone (Jansen, 1981, Atta ur et al., 1985).

The alkaloids present in the black seed such as nigellicine, nigellimine, and nigellidine, have unclear pharmacological effects. (Burits and Bucar, 2000). These compounds from both herbal extracts were reported to contain a wide range of medicinal value that attributes to the treatment of infections caused by microbes associated with root canal contamination, microbial inflammations and pain.

The goal of this experiment was to evaluate the antibacterial effects of 2% Chlorhexidine and 2.5 percent sodium-hypochlorite with the extracts of *Eugenia caryophyllus* and *Nigella sativa* towards *E.faecalis* and *C.albicans*.

MATERIALS AND METHODS

Sourcing of *Nigella sativum* and *Eugenia caryophyllus*

Nigella sativum and *Eugenia caryophyllus* were sourced from Tamil-Nadu Agriculture University, koyamputhtoor, India.

Drying and milling

Collected plants were pre-treated by washing using clean water and dried at room temperature (shade conditions) for complete drying. After drying under shade conditions, the Nigella seeds and Eugenia clove buds were dried at 50°C for 2 hours. Dried plants were finely powdered, sieved in a manual sieve and stored at room temperature.

Test organisms – Collection of *E. faecalis* and *C.albicans*

Test organisms used in this study are usually associated with failed endodontic treatment (*Enterococcus faecalis* and *Candida albicans*), collected from Micro laboratory, Coimbatore, Tamil Nadu, India.

Extraction of bioactive constituents from selected plants - *Nigella sativum* and *Eugenia caryophyllus* using Soxhlet method.

The soxhlet extraction method was implied for collecting the extracts using different solvents from the selected plant sources. Finely powdered *Nigella sativa* seeds and *Eugenia caryophyllus* buds were extracted separately in this method. The powder was placed inside the thimble of the apparatus, and the entire set-up was made for the extraction process under controlled conditions. The extraction solvent (ethanol or chloroform) was taken in the round bottom flask. The vapours were evaporated into thimble, and using a condenser vapours were condensed, and dripped back into the flask as extracts. The siphon arm extract was placed back into the bottom flask, and the operation was repeated three times to obtain complete extracts.The extraction process was initially started at the temperature of 60^0C and slowly raised to 100^0C. The total procedure was carried out for 4 to 6 hours. The obtained extract was added into the separating funnel and shaken well for 2min. The lower part containing the bioactive constituents were separated from the funnel and stored at room temperature for further testing.

Phytochemical analysis of *Nigella sativum* and *Eugenia caryophyllus*

The phytochemical screening of the ethanol and chloroform extracts of *Nigella sativa* and *Eugenia caryophyllus* was determined using established methods for the existence or non existence of various phyto-constituents such as carbohydrates, terpenoids, aminoacid, glycoside, saponin, steroid, phenol, flvonoid, tannin and alkaloid.

Gas Chromatography-Mass Spectroscopy examination of *Nigella sativa* and *Eugenia caryophyllus* extracts

Essential oil constituents from *Nigella sativa* and *Eugenia caryophyllus* extracts were analyzed by Gas Chromatography Mass Spectroscopy examination. On a Varian 3800 gas chromatograph interfaced to a Varian Saturn ion trap 2200 spectrometer, total ion monitoring mode was used for the GCMS. The VF5 capillary-column was employed, which has a length of 30 meters, a diameter of 0.25 millimeters, and a film thickness of 0.25 millimeters.

With a solvent delay time of 3 minutes and an injection volume of 0.2 l, a 20 percent split injection method was chosen.

The earliest column temperature was set at 50 degree centigrade for one minute, then gradually increased to 200 degree centigrade at an 8 degree centigrade per minute rate until it reached 280 degree centigrade at a rate of 10 degree centigrade per minute. The temperature of the injection port was set to 250 degree centigrade. At a constant flow rate of 1 millilitre per minute, helium was used as the carrier gas.

Peak area normalization was used to calculate the relative percentages of Nigella sativa and Eugenia caryophyllus ingredients. The retention indices of essential oil components were compared to a homologous series of n.alkane C8 - C20 on the VF5 column (capillary) with identical operating circumstances, and the GCMS spectra from the Wiley 6.0 MS data and literature data were computer matched.

Comparison of antimicrobial activity of *Nigella sativa, Eugenia caryophyllus* with 2.5% Naocl and 2% CHX

The anti-microbial ability of *N.sativa* and *E.caryophyllus* was compared with the ability of 2.5% Naocl and 2% CHX. The ability was examined towards the tested microorganisms (*Enterococcus faecalis* and *Candida albicans*) by the method of well diffusion. Sterile Mueller-Hinton Agar (MHA) (Composition g/L: Acidic hydro-lysate Casein-17.5gram, Starch- 1.5gram, Nacl-5.0gram, Agar-17.0 g, plates with final pH of 7.0 ± 0.2 were solidified after preparation. Around 0.1 percent of inoculum suspensions of the test organism (*Enterococcus faecalis* and *Candida albicans*) were uniformly swabbed separately on agar surface.

Under sterile conditions, 6mm wells were cut on the agar surface of each MHA plate. About 20µl of herbal extracts were loaded into the well, and the plates were incubated at 37°C for 24h. The antimicrobial activity was evaluated in terms of the zone of inhibition around the wells in all the inoculated MHA plates. The clear inhibition zones were measured and recorded in millimetres.

Each MHA plate had 6 millimeter wells cut into the agar surface under sterile circumstances. A total of 20 micro liters of herbal extracts were poured into each well, and agar-plates incubated for 24 hours at 37 degrees Celsius. In all of the infected Muller hinton agar plates, the antimicrobial activity was measured in terms of inhibition zones around the wells. The clear inhibition zones were measured in millimetre and documented. In parallel, 2.5% Sodium hypochlorite and 2% Chlorhexidine were also evaluated separately against the test organism (Estrela et al., 2003).

RESULTS

Phytochemical analysis of *Nigella sativa* and *Eugenia caryophyllus* extract

The presence or absence of different phytoconstituents from ethanol and chloroform extracts of *Nigella sativa* are presented in Table-1 and Table-2, respectively. In Fig. 1 and Fig. 2, the phytochemical constituents of respective ethanol and chloroform extracts of *Nigella sativa* showed the presence or absence of phytoconstituents based on the colour obtained for the tested extracts.

Similarly, the ethanol and chloroform extracts of *Eugenia caryophyllus* also confirmed the presence of significant phytocompounds. In Table-3, the ethanol extract and Table-4, chloroform extract showing the phytochemical constituents, were presented. In Fig. 3 and Fig. 4, the phytochemical constituents of respective ethanol and chloroform extracts of *Eugenia caryophyllus* extracts showed the presence or absence of phytoconstituents based on the colour obtained for the tested extracts.

Among the two extracts, ethanolic extracts of both plants showed more phytocompounds. Hence ethanolic extracts were selected for further studies in this present research.

Identification of bioactive compounds - GCMS analysis of *N.sativa* extract

Bio-active components of the ethanol extracts of *N.sativa* responsible for the antimicrobial activity were recognised by GC-MS technique. Spectrum revealed the presence of several bioactive compounds, and the identified compounds were tabulated.

GC-MS analysis of *Nigella sativa* extract representing the identified compounds attributing to different biological properties including antimicrobial property with its retention time was illustrated in Fig. 5. The potential bioactive compounds identified were found to be 5-Hydroxy methyl furfural, Thymoquinine and Thymol. The reported biological properties of the compounds were also discussed according to Duke's Ethnobotanical and phytochemistry database. In Table-5, the identified compounds representing the types of biological properties were presented.

Identification of bioactive compounds - GC-MS analysis of *Eugenia caryophyllus*

Bioactive compounds in the ethanol extracts of *Eugenia caryophyllus* responsible for the antimicrobial activity were recognised by GCMS technique. Spectrum recognised the existence of several bioactive components, and the identified components were tabulated.

Fig. 6 represented the GC-MS spectrum of *Eugenia caryophyllus* extract with its retention time. The potential bio-active components identified from the GCMS analysis were Copaene, Caryophyllene and Eugenol acetate. In Table-6, the identified bioactive compounds and the molecular formula with respect to their retention time were presented, along with the reported biological properties of the compounds.

Comparison of Anti-microbial activity of the *N.sativa, E. caryophyllus* against *E. faecalis and C.albicans* compared with 2.5% Naocl and 2% CHX.

The anti-microbial ability of the *N.sativa*, *Eugenia caryophyllus* against *Enterococcus faecalis, and Candida albicans* were compared with 2.5% Naocl and 2% CHX in the present research. The comparison was done to determine whether the individual effect of plant extracts was significant in retarding the growth of selected dental pathogens in comparison with the efficacy of chemical irrigants. The inhibitory zones of *Nigella sativa*, *Eugenia caryophyllus* showed promising results against the test organisms.

In Fig. 7, the antimicrobial activity of the *N.sativa*, *E. caryophyllus* against *Enterococcus faecalis and Candida albicans* was presented with clear inhibitory zones. About 18mm and 19mm of inhibitory zones were obtained for the ethanol extracts of *Nigella sativa* against *Enterococcus faecalis and Candida albicans*. The almost similar size of inhibitory zones (17mm and 19mm) were recorded for the ethanol extracts of *Eugenia caryophyllus* against the respective test organisms.

In Fig. 8, the antimicrobial activity of 2.5% Naocl and 2% CHX against *E. faecalis and C.albicans* was presented with clear inhibitory zones. About 17mm and 19mm of inhibitory zones were recorded for 2.5% Sodium hypochlorite, and about 19mm and 20mm of inhibitory zones were recorded for 2% Chlorhexidine. The obtained values were found similar when compared to the activity proposed by the plant extracts used in the present study. These comparative values of inhibitory zones obtained for all the test solutions against both organisms were presented in Table-7 and Fig. 9.

DISCUSSION

Chemical irrigants, 2.5% Naocl and 2% CHX are the standard root canal disinfectant solutions used. As reported, the chemical irrigants are used for the prevention of microbial contamination; it is also considered cytotoxic to the periapical tissues and endothelial cells. In the developmental field of dentistry, no ideal root canal irrigant replaces these chemical disinfectants. The chemical reactions induced by intra-canal irrigants, increase in antimicrobial resistant strains and adverse reactions due to synthetic drugs paved way for the search for new intra-canal medicaments (Jain and Ranjan, 2014). These novel drugs are recently searched from the vintage herbs and herbal products in order to

ensure safety, efficacy, enhanced shelf life and availability (Palombo, 2011). Hence in the present study, *Nigella sativa* and *Eugenia caryophyllus* are selected to determine whether the extracts shall be used as natural irrigants after evaluating their phytochemical compounds and antimicrobial activity against *Enterococcus faecalis* and *Candida albicans*.

Phytochemical examination of *N.sativa* and *E.caryophyllus*

The qualitative phytochemical examination of *N.sativa* and *E.caryophyllus* was determined in the present research for chloroform and ethanol extracts. During the analysis, the presence and absence of different phytochemical constituents were found evident based on the type of solvent extracts tested. Ethanol extracts showed most of the compounds when compared to the chloroform extracts.

The phytochemicals such as saponin, tannin, terpenoids, steroids, glycosides and flavonoids were found in the ethanol extracts of *Nigella sativa* (Table-1). In contrast, the phytochemicals such as terpenoids, steroids, glycosides and flavonoids alone were found evident in the chloroform extracts of Nigella *sativa* (Table-2). The phytochemicals such as saponin, terpenoids, glycosides, amino acids, alkaloids, and flavonoids were found in the ethanol extracts of *eugenia caryophyllus* (Table-3). In comparison, the phytochemicals such as saponin, tannin, glycosides, alkaloids and flavonoids alone were found evident in the chloroform extracts of eugenia *caryophyllus* (Table-4).

The phytochemical analysis was conducted to investigate the biological activities and medicinal capacity and to exhibit broad spectrum abilities like antimicrobial, analgesic, antiinflammatory, and antioxidant properties. It was reported in one of the studies that most of these medicinal capabilities are due to existence of thymoquinone in solvent extracts of *Nigella sativa*, which is the major active chemical component of this plant seed extracts (Belgaumi et al., 2020).

The literature review highlighted the antimicrobial properties of eugenol and caryophyllene against dental microbes. Eugenol is known to be the principal volatile constituent of *Eugenia caryophyllus*. It consists of wide spectrum of therapeutic properties, including anesthetic, anti-septic, antimicrobial, analgesic, anti-inflammatory, and antioxidant activities. Bioactive components like eugenol, caryophyllene, and limonene have been reported earlier from the solvent extracts of *Eugenia caryophyllus* (Jimoh et al., 2017, Das et al., 2018). Madhavan et al. demonstrated the oil extracts containing these compounds enhances the antibacterial activity against *Enterobacter faecalis* (Madhavan, 2015). In the present study, the presence of thymoquinone and eugenol from the ethanolic extracts of *Nigella sativa* and *Eugenia caryophyllus* were confirmed in the GC-MS analysis, respectively.

GCMS analysis of *N.sativa* and *E.caryophyllus* extracts

GCMS analysis of *N.sativa* extracts representing the identified compounds attributing to the antimicrobial property is presented in Table-5 and Fig. 5.

The potential bioactive components identified from extracts of *N.sativa* in the present study are 5-Hydroxy methyl furfural, thymoquinone and thymol. According to Duke's Ethnobotanical and phytochemistry database, the identified compounds are responsible for antimicrobial activity. The antimicrobial property of 5-Hydroxy methyl furfural, Thymoquinone, Thymol, Tetra decanoic acid ethyl ester, n-Hexadecenoic acid and 9,12-Octadecadienoic acid has verified in earlier studies (Fan et al., 2021) (Marchese et al., 2016) (Idowu, 2017) (Krishnan et al., 2016).

Thymoquinone, thymol, hexadecenoic acid and octadecadienoic acid with some other bioactive constituents were found from the extracts of *Nigella sativa* in different percentages depending upon extraction methods. Among all the compounds, thymoquinone and thymol were responsible for the highest antibacterial activity. In one of the studies, the percentage of thymoquinone was 7.8%, and thymol was 9.4%. In another study, it was 23.25% and 2.32%, respectively. (Hadi et al., 2016, Aftab et al., 2020) (Sultan et al., 2009, Saleh et al., 2018).

GC-MS analysis of *Eugenia caryophyllus* extracts representing the identified compounds attributing to the antimicrobial property is presented in Table-6 and Fig. 6.

The potential bioactive compounds identified from the extracts of *Eugenia caryophyllus* in the present study are copaene, caryophyllene and eugenol acetate. Earlier GC-MS studies also have revealed similar bioactive compounds with different percentages of copaene, caryophyllene and eugenol acetate (Uddin et al., 2017) (Hemalatha et al., 2016) (Teles et al., 2021) (Das et al., 2018). Caryophyllene and eugenol acetate has shown good antimicrobial action in earlier studies (Fidyt et al., 2016) (Sharma et al.)
Similar compounds examined and reported by other researchers were found well supportive for the GCMS examination of *N.sativa* and *E.caryophyllus* extracts in the present research.

Anti-microbial ability of N.sativa and E.caryophyllus towards Enterococcus faecalis and Candida albicans
Anti-microbial ability of chemical irrigants towards *E. faecalis and C.albicans* was tested separately for the ethanolic extracts of *Nigella sativa* and *Eugenia caryophyllus*. About 18mm and 19mm of inhibitory zones were obtained for the ethanol extracts of *Nigella sativa* against *Enterococcus faecalis and Candida albicans*. The almost similar size of inhibitory zones (17mm and 19mm) was recorded for the ethanol extracts of *Eugenia caryophyllus* against the respective test organisms. The observed results in the present research were found supportive to the research works as per the literature survey; the details are compared and discussed.
In one of the earlier research, the anti-microbial ability of methanol extract of *Nigella sativa* was analyzed using the agar diffusion method against *Enterococcus faecalis*. About 18mm of inhibitory zones were obtained during the analysis (Khan et al., 2013). In an another research, the antifungal ability of the essential oil of the alcoholic extract (96% methanol) of *N. Sativa* was investigated. The zones of inhibition ranged from 8 to 15.5 mm for an alcoholic extract of N. Sativa (Naeini et al., 2017). Similarly, the anti-microbial ability of ethanol extracts of *Nigella sativa* showed a comparatively significant inhibitory zone of 18mm and 19mm against *E. faecalis* and *C.albicans*, respectively in the present study.
A study revealed that ethanolic extract of eugenia caryophyllus possesses a high antimicrobial effect against *Enterococcus faecalis* (Hemalatha et al., 2016). The findings were supportive of our present research findings in terms of the antimicrobial activity of *Eugenia caryophyllus* against *Enterococcus faecalis*.In another study, anti-microbial effect of 32% and 16% ethanol and water extract of *Eugenia caryophyllus* was examined towards *Candida albicans* species and other pathogenic bacteria. All of the microorganisms used in the study were inhibited by the ethanol extracts, with inhibition zones ranging from 8mm to 24mm.The researchers concluded that aqueous and ethanol extracts of *Eugenia caryophyllus* showed good antimicrobial activity against medically significant pathogenic bacteria and *Candida albicans* (Mills-Robertson et al., 2014).
Jimoh et al. recorded around 44mm of inhibitory zone while determining antimicrobial activity of *Eugenia caryophyllus* against *Candida albicans* using the agar diffusion method (Jimoh et al., 2017). However, In our present study, the ethanol extracts of *Eugenia caryophyllus* showed 19mm of inhibitory zones against candida albicans in the agar diffusion method. Even though the values were not close to the work of Jimoh *et al.*, but these findings are similar in terms of inhibitory action of *Eugenia caryophyllus* against *Candida albicans*, and the difference in inhibitory zones may be because of the different methodology adopted and different strains of *Candida albicans* used in our study.

Antimicrobial activity of 2.5% Naocl and 2% CHX against E.faecalis and C. albicans
Anti-microbial ability of chemical irrigants against *E.faecalis and C.albicans* was tested separately for 2.5% Naocl and 2% CHX. About 17mm and 19mm inhibition zones were obtained for the irrigant 2.5% Sodium hypochlorite against *E. faecalis* and *Candida albicans*, respectively. Inhibition zones of about 19mm and 20mm were evident towards the respective test microorganisms for 2% Chlorhexidine. The above results were found well supportive to some of the research works as per the literature survey. The details are discussed below in comparison with the research works.

Jose *et al.*, aimed to compare chemical irrigants (2.5% Sodium hypochlorite and 2% Chlorhexidine) with natural irrigants (guava leaf extract and aloe vera extract) in terms of antimicrobial activity against *E. faecalis* and *C.albicans*. The well diffusion methodology was adopted to ascertain the antimicrobial ability of the test disinfectants. During this analysis, the researchers reported that all test solutions showed good antimicrobial activity against the test organisms (Jose et al., 2016).

In a different study, Sinha *et al.* compared antimicrobial effect of *Azadirachta indica* and *Curcuma longa* against *E. faecalis* with that of 2.5% Naocl and 2% CHX. Standard methods like tube dilution and agar diffusion methodology were employed to evaluate the antibacterial ability of the solutions towards test bacteria. 2% Chlorhexidine exhibited the most significant antimicrobial ability when utilized as root canal irrigants towards the test bacteria (Sinha et al., 2017).

In comparison with the above studies, in our present research also the test organisms were inhibited by the ethanolic extracts of *N.sativa* and *Eugenia caryophyllus*, and chemical irrigants, 2% Chlorhexidine and 2.5% Sodium hypochlorite in terms of qualitative inhibition. This work was found supportive in terms of the antimicrobial activity of herbal extracts in comparison with the chemical irrigants.

CONCLUSION

The aim of developing herbal irrigants to replace chemical irrigant during root canal therapy for inhibition of microbial contamination and infections caused by *E. faecalis* and *C. albicans* was selected as the main objective in the present study. Phytochemical analysis and GC-MS analysis of *Nigella sativa* and *Eugenia caryophyllus* were studied to identify the bioactive compounds attributing to the antimicrobial properties. Inhibitory studies were evaluated against *E. faecalis* and *C.albicans* using the standard well diffusion method. The activity was then compared with the antimicrobial activity of chemical irrigants (2.5% Sodium hypochlorite and 2% Chlorhexidine). The overall results showed that herbal irrigants were equally potent to the chemical irrigant in inhibiting the growth of pathogens tested in the study. Hence these herbal medicines from natural sources can be considered using alternatively with the chemical irrigants in the near future after performing optimization studies and clinical trials.

CONFLICT OF INTEREST

Authors declare no conflict of interest

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Table-1: Phytochemical analysis of *Nigella sativa* extract (Ethanol)

S. No.	Phytochemical constituents	Presence/Absence
1	Saponin	+
2	Tannin	+
3	Terpenoids	+
4	Steroids	+
5	Glycosoides	+
6	Aminoacids	-
7	Alkaloids	-
8	Flavanoids	+

Table-2: Phytochemical analysis of *Nigella sativa* extract (Chloroform extract)

S. No.	Phytochemical constituents	Presence/Absence
1	Saponin	-
2	Tannin	-
3	Terpenoids	+
4	Steroids	+
5	Glycosoides	+
6	Aminoacids	-
7	Alkaloids	-
8	Flavanoids	+

Table-3: Phytochemical analysis of *Eugenia caryophyllus* extract (Ethanol)

S. No.	Phytochemical constituents	Presence/Absence
1	Saponin	+
2	Tannin	-
3	Terpenoids	+
4	Steroids	-
5	Glycosoides	+
6	Aminoacids	+
7	Alkaloids	+
8	Flavanoids	+

Table-4: Phytochemical analysis of *Eugenia caryophyllus* extract (Chloroform extract)

S. No.	Phytochemical constituents	Presence/Absence
1	Saponin	+
2	Tannin	+
3	Terpenoids	-
4	Steroids	-
5	Glycosoides	+
6	Aminoacids	-
7	Alkaloids	+
8	Flavanoids	-

Table-5: Bioactive compounds present in *Nigella sativa* extract

S. No	Retention time (min)	Compound	Molecular formula	Activity
1	1.90	5-Hydroxy methyl furfural	C6H6O3	Anti-bacterial activity, Sickle cell disease, Food flavouring
2	2.83	Thymoquinone	C10H12O2	Anti-microbial, anti-arthritic, anti-cancer, anti-diabetic
3	3.96	Thymol	C10H14O	Anthelminthic, Antiacne, antimicrobial, anti-carcinogenic
4	6.29	p-tert-Butyl catechol	C10H14O2	Food additive
5	8.64	Tetradecanoic acid	C14H28O2	antiviral, candidicide, hypocholesterolemic

6	10.01	Tetradecanoic acid, ethyl ester	C16H32O2	anti-inflamamtory, antimicrobial
7	12.09	Hexadecanoic acid, methyl ester	C17H34O2	Anticancer, antioxidant
8	16.43	n-Hexadecanoic acid	C16H32O2	Antioxidant, antimicrobial, larvicidal
9	18.80	Eicosanoic acid	C20H40O2	Anti-inflammatory
10	23.59	9,12-Octadecadienoic acid (Z,Z)-	C19H34O2	Antioxidant, antimicrobial,
11	29.29	9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1 [(acetyloxy)methyl]ethyl ester, (Z,Z,Z)-	C25H40O6	antioxidant, anti-inflammatory, hepatoprotective

Table-6: Bioactive compounds present in *Eugenia caryophyllus* extract

S. No	Retention time (min)	Compound	Molecular formula	Activity
1	7.87	Alpha.- cadinol	$C_{15}H_{26}O$	Anti-Bacterial, Anti- Fungal
2	11.56	Copaene	$C_{15}H_{24}$	Carminative, antioxidant, anti-inflammatory, anti-cancer
3	14.57	Caryophyllene	$C_{15}H_{24}$	Anti-cancer, antioxidant, antimicrobial
4	16.38	Eugenol acetate	$C_{12}H_{14}O_3$	Antimicrobial, antioxidant
5	21.82	Eicosanoic acid	$C_{20}H_{40}O_2$	Anti- Cancer activity
6	25.64	Eugenol	$C_{10}H_{12}O_2$	Anti-cancer, anti-viral, antimicrobial
7	35.63	18- Nonadecen-1- OL	$C_{19}H_{38}O$	Antimicrobial activity, uses in pharmaceuticals
8	38.59	Bicyclo [4.1.0] Heptane, 7-pentyl	$C_{12}H_{22}$	Anti-microbial activity

Table-7: Antimicrobial activity of the *Nigella sativa, Eugenia caryophyllus* compared with 2.5% Sodium hypochlorite and 2% Chlorhexidine

Organisms	Zone of Inhibition (mm)			
	Nigella sativa	*Eugenia caryophyllus*	2.5% Sodium hypochlorite	2% Chlorhexidine
Enterobacter faecalis	18	17	17	19
Candida albicans	19	19	19	20

Fig. 1: Phytochemical analysis of *Nigella sativum* extract (Ethanol)

Fig. 2: Phytochemical analysis of *Nigella sativum* extract (Chloroform)

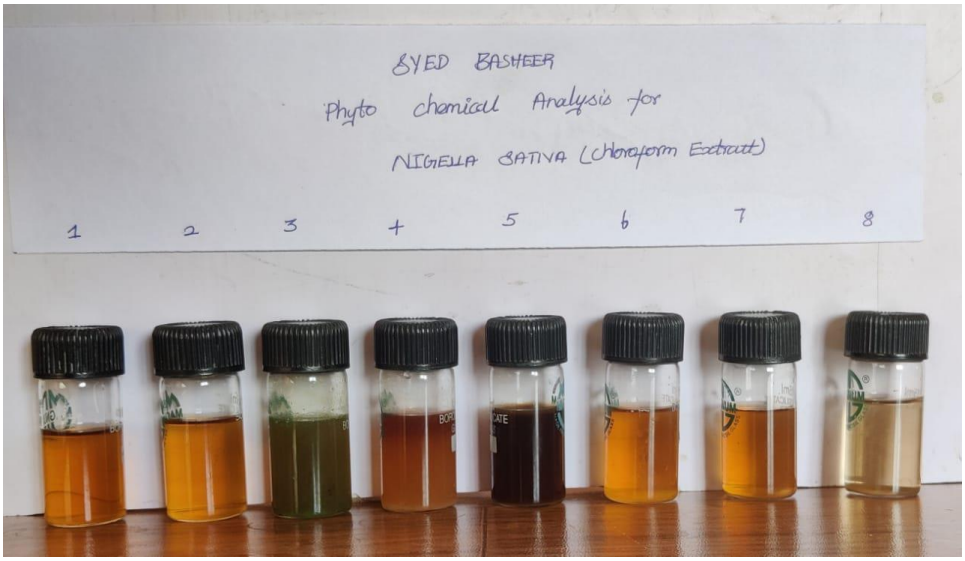

Fig. 3: Phytochemical analysis of *Eugenia caryophyllus* extract (Ethanol)

Fig. 4: Phytochemical analysis of *Eugenia caryophyllus* extract (Chloroform)

Fig. 5: GC-MS spectra of *Nigella sativa* extract

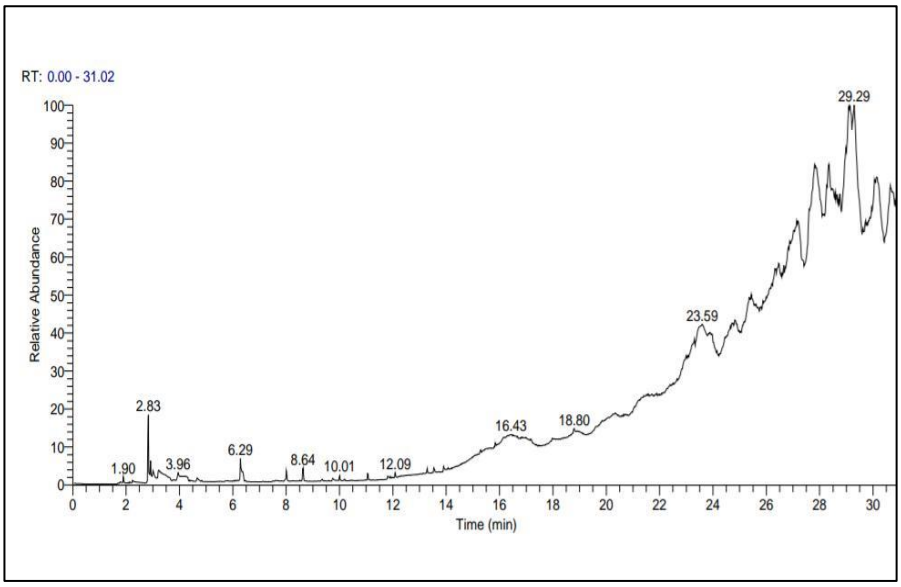

Fig. 6: GC-MS spectrum of *Eugenia caryophyllus* extract

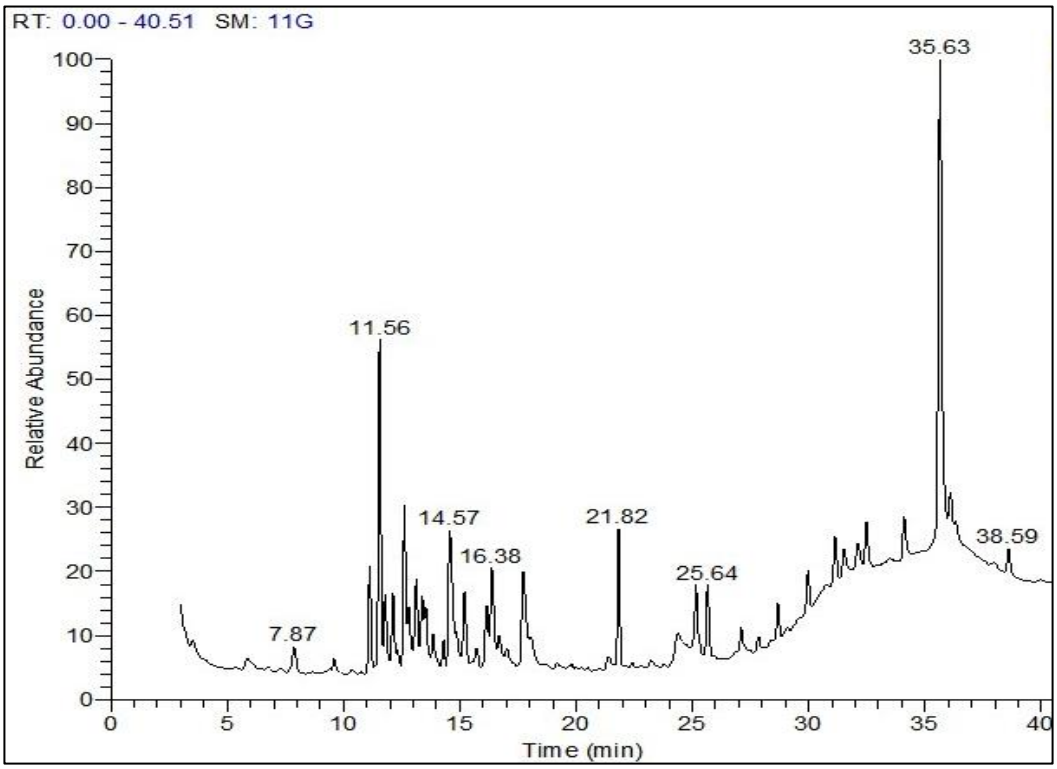

Fig. 7: Antimicrobial activity of *Nigella sativa and Eugenia caryophyllus*

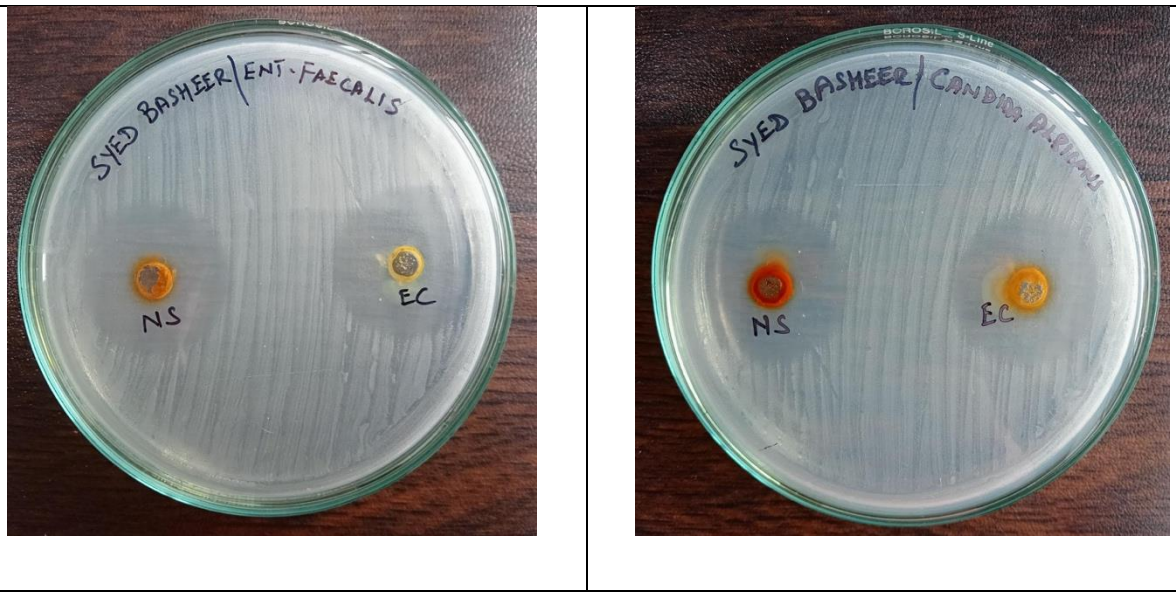

Fig. 8: Antimicrobial activity of Chemical irrigants

Enterococcus faecalis

Candida albicans

Fig. 9: Antimicrobial activity of the *Nigella sativa*, *Eugenia caryophyllus* compared with 2.5% Sodium hypochlorite and 2% Chlorhexidine