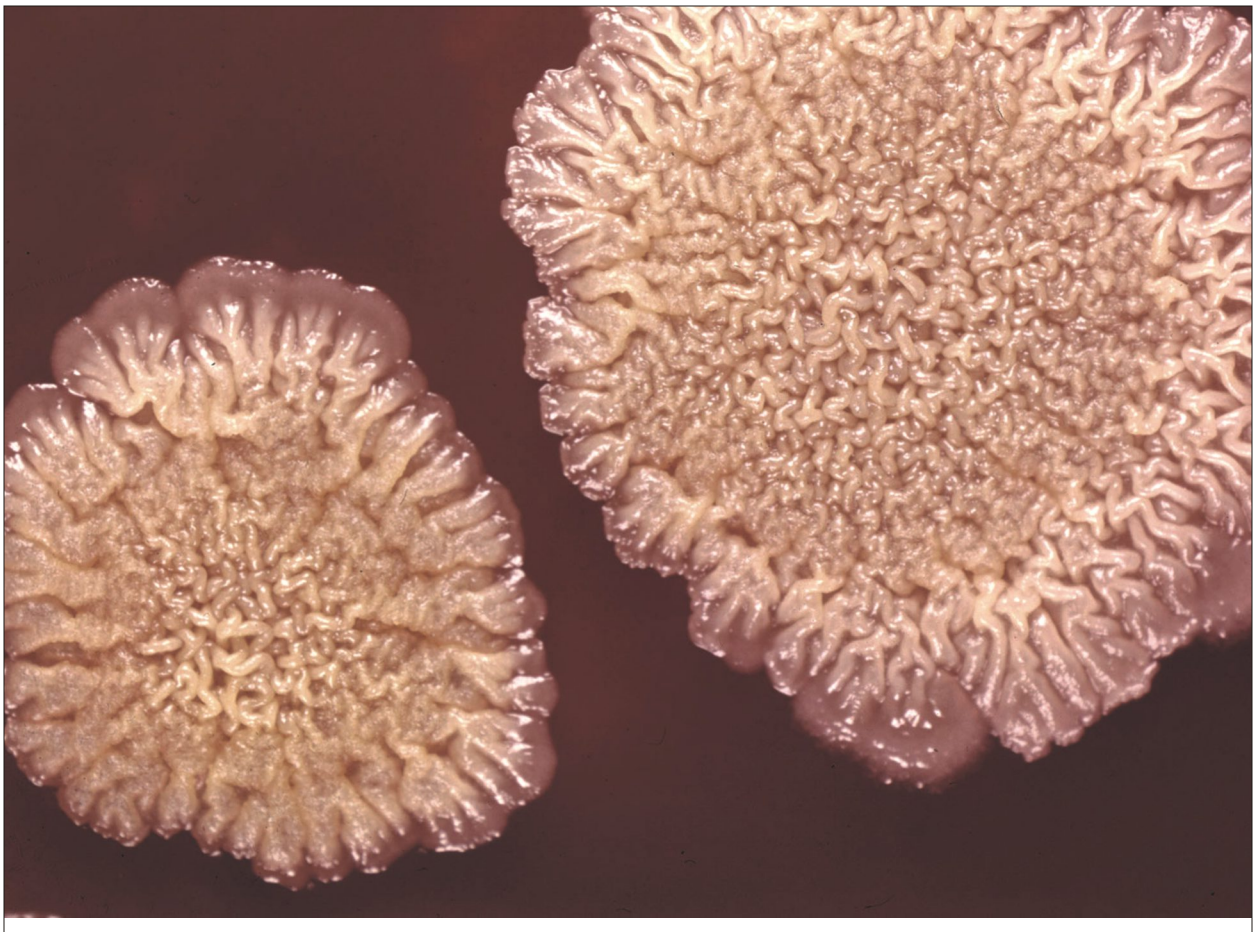

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Research Article
Antimicrobial Activity of Essential Oil Against *Rhizobium (Agrobacterium) vitis* Using Agar Well and Disc Diffusion Methods

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Abstract

Background and Objective: Essential oils have played many important roles in Plant Protection. The aim of this study was to determine antimicrobial effects of some essential oils against mixture of seven different *Rhizobium vitis* isolates. **Materials and Methods:** Typical crown galls were collected from vineyards in the main grape-growing regions in Thrace region. *Rhizobium* spp., were isolated and streaked on RS and PDA medium. After incubation for 48 h at 28°C, *Rhizobium* colonies resembling *A. vitis* were selected and purified three times on PDA. Typical *A. vitis* colonies were streaked and maintained on PDA. After bacterial isolates were tested for colonization morphology and pathogenicity, essential oils were examined for six different concentration to determine antibacterial activity and select suitable method according to disc diffusion and well diffusion methods. The experiments were attempted with five replications, with positive and negative controls. **Results:** As a result, it has been observed that the disk diffusion method has a larger diameter and a more visible zone. According to inhibition zone formation, it has been found that John's wort , thyme and ginger essential oils, respectively are the most effective among the other essential oils and doses tested in disk diffusion and well diffusion methods. The highest antibacterial activity was observed in john's wort at 30 mg mL^{-1} concentration, while black cumin essential oil at 5 mg mL^{-1} concentration was the lowest antibacterial activity against *A. vitis* isolates. Component analysis of antibacterial essential oils were performed by GC/MS. The major volatile compounds in the John's wort, thyme and ginger essential oil were found to be camphor (20.67%), thymol (22.9%) and benzyl alcohol (43.07%), respectively. **Conclusion:** So, John's wort EO showed very high antibacterial activity and disc diffusion method showed more bacterial inhibition.

Key words: *Rhizobium vitis*, essential oil, inhibition zone, John's wort, antimicrobial activity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Plants are constantly exposed to various pathogenic microorganisms in their environment. Diseases caused by plant pathogenic bacteria and fungi cause significant reductions in product yield and severe product losses worldwide[1,2]. Many phytopathogens, including *Rhizobium vitis* (Crown gall), reduce the yield and quality of plants and cause severe economic losses in vineyards and nurseries. *Rhizobium vitis* is a Gram-negative soil-borne bacterial strain specific for *Vitis* spp. Pathogenicity is caused by the formation of large plasmids (pTi) carrying genes for crown gall formation. Genes at different regions of the Ti plasmid are required for the virulence, including transfer plasmid (T-DNA) and virulence (vir) genes. A portion of this TDNA is transferred to the plant nuclear DNA during infection and leads to abnormal cell proliferation in the plant[3]. Subsequently, overexpression of T-DNA genes results in to high levels of indole-3-acetic acid (oxine or IAA) and cytokinins called as opines, which lead to the formation of tumor and amino acid derivatives[4]. *Rhizobium vitis* can produce three types of opines; octopine/cucumopine, nopaline and vitopine[5] that increase the pathogenicity of crown gall. Pathogenicity genes are usually found on large tumors induced by plasmid (pTi). Expression of T-DNA genes in the plant cell and elevated levels of hormone production disturb the regulation of the cell cycle because the plant cell can not regulate the expression of T-DNA genes. These trigger abnormal cell growth leading to gall formation[6-8].

Rhizobium survives systemically in infected grapes and can survive for a long time in contaminating soils, even if the grapevine seedlings are replaced. *Rhizobium vitis* can be spread through asymptomatic and apparently healthy production material as it survives systematically in grapes. Freezing and injury are important in the process of infection. The injury provide an entry mode for the pathogen, it also causes the production of stress-induced compounds by the plant that attracts bacterial cells to these sites. When the bacterium enters a plant cell, it donates some of its DNA to the grape plant.

Infections are observed in cambial cells during wound healing for this reason, the crown crisis damages the vines in wound healing process.

The crown gall management is based on cultural practices to reduce the adverse effects of large-scale injuries[9], if the trunk surface is covered with 50% or more of the disease. However, increasing resistance of microorganisms to traditional chemicals and drugs are a worldwide problem and has accelerated researches for the identification of new broad-spectrum biocides. Also, there are no valid chemical treatments effective for disease control. The efficacy of chemicals such as copper-based compounds, other chemicals and antibiotics is not sufficient and thus not recommended. Biological control of grape crown gall is investigated and provides an effective alternative for protecting graft unions and other injures on grapevines.

Essential oils play an important role in plant protection. Herbs and spices are known to have been used for antimicrobial properties since antiquity[10]. Essential oils are characterized by two or three major components in very high concentrations (20-70%) when compared to other components in trace amounts. The amount of different components of essential oils varies depending on such factors as different plant parts, different plant species, climate and soil conditions.

Essential oils include compounds of volatile terpenoid or terpenoid origin and nitrogen or sulfur derivatives[11]. The principal terpenes are monoterpene and sesquiterpene. The monoterpenes consist of the binding of two isoprene units. Sesquiterpenes consist of three isoprene associations.

The structure and function of sesquiterpenes is similar to monoterpenes[12,13] stated that there may be a relationship between the chemical structure of major compounds and antimicrobial activity. The various factors such as local, climate, plant species[14,15] can also affect the biological and antimicrobial activities of the essential oils[16]. In addition, the duration and temperature of distillation can significantly affect components, also antimicrobial activity[17].

On the other hand, the widespread and unnecessary use of antibiotics has led to spread antibiotic-resistant strains of bacteria. The resistance of microorganisms to antibiotics has caused to search different management methods such as application of plant essential oils to a wide range of Gram-positive and Gram-negative bacteria. It is possible that antimicrobial mechanism of essential oils are due to their composition and the synergistic effect of the compounds with each other.

Although antibacterial efficacy of essential oils has been reported, the mechanism of action has not been researched in great detail. Due to different chemical compounds present in essential oil composition, the antibacterial activity is not attributed to only one specific mechanism, it depends on various targets in the cell[18]. One of the important target of essential oils is hydrophobicity; which allows to be cleaved by lipids present in the bacterial cell membrane causing the more permeable cell structure. Also, because of their lipophilic nature, essential oils can penetrate through the bacterial cell membranes. It ultimately results in the death of bacterial cells

by corrupting metabolic functions and disrupting many cellular activities such as breakdown of membrane integrity, leakage of cellular components and ions, depletion of the ATP and increased bacterial cell membrane permeability[19,20].

When taking into account that researches are not satisfactory in addition to no available and effective chemical treatments for crown gall control, thus essential oils are possible natural alternative and biologically active plant products instead of chemical-based bactericides which might threat food safety, cause accumulation in the food chain and environmental pollution[21]. This study was therefore conducted to investigate the chemical composition of the essential oils and to test their antibacterial efficacy on *Rhizobium vitis* under *in vitro* conditions. Also, black cumin, mustard, ginger and John's wort essential oils haven't been examined against *A. vitis* so far in previous studies. According to our results, it was identified that particularly john's wort essential oil showed maximum antibacterial activity and had a potential to control crown gall.

MATERIALS AND METHODS

Material

Bacterial organism and culture medium: The grapevine trunks and necrotic canes with typical crown galls were collected from vineyards in the main grape-growing regions of Thrace region in July, 2016-March, 2017. Vineyards and nurseries of grapevine (*Vitis* spp.) were inspected for crown gall occurrence in different grapevine growing regions. The gall suspensions were streaked on Roy and Sasser (RS) and potato dextrose agar (PDA) medium.

Plant materials: Essential oils obtained from different parts of black cumin (*Nigella sativa*), mustard (*Sinapis* sp.), john's wort (*Hypericum perforatum*), garlic (*Allium sativum*), thyme (*Thymus vulgaris*) and ginger (*Zingiber officinale*) plants examined for their antibacterial effect were purchased as commercial preparations. It has been ensured that essential oils are 100% pure and reliable trademarks. Essential oils were obtained by distillation method from the seed of black cummin and mustard, flower of the john's wort, root of ginger and clove of garlic.

Method

Isolation of *A. vitis* and preparation of standard inoculum: Samples of crowns, parts of trunks and canes with typical galls from the infected plants were collected and transported to the laboratory in plastic bags within 48 h and *Rhizobium* spp. were isolated as explained by Schaad *et al.*[22]. Plant samples were washed with tap water to remove soil particles. Galls were surface-sterilized by 2.5% sodium hypochlorite for 2 min, rinsed three times with sterile distilled water (SDW) and placed on sterile filter paper the sterile filter paper for drying. The surface of the galls was removed with a scalpel and small fragments were removed from each sample and triturated with pestle for maceration in 8.5% NaCl. After 1 h, the gall suspension was streaked on RS and PDA medium. After incubation for 48 h at 28°C, *Rhizobium* colonies resembling *A. vitis* were selected and purified three times on PDA. Typical *A. vitis* colonies were streaked and maintained on PDA. Pure colonies were transferred with the help of a straight wire and made stable emulsion in a test tube containing saline and inoculum intensity was adjusted 1×10^8 CFU mL^{-1} approximately.

Disc diffusion method: The antibacterial effect of essential oils at different concentrations was individually carried out by the disc diffusion method. Potato dextrose agar (PDA) was poured in plates of 9-10 cm; depth of agar was 3-4 mm. The bacterial inoculum was adjusted to certain concentration, 100 µL of culture (containing 10^8 CFU mL^{-1}) was spread an over the surface of the agar plate using a sterile glass spreader and inoculated onto the entire surface of a PDA plate. The sterile paper discs (6.0 mm) were saturated with 5, 10, 15, 20, 25 and 30 mg mL^{-1} of the tested each essential oils. Oil impregnated discs were put on the center of inoculated surface of the agar plate. It was used 0.02% Tween 80 to emulsify the oils. Tween 80 was added to the essential oil prior to application to the disc. After treatment, agar plates were incubated in PDA at 28°C for 48 h. Antibacterial activity was evaluated by measuring the diameter of the inhibition zone (IZ) surrounding the discs. The assay was repeated five times. Antibacterial activity was determined as the mean zone of inhibition diameters (mm) produced by the essential oil. Streptomycin was used as positive control and sterile distilled water was used as negative control.

Well diffusion method: Antibacterial activity of oils was individually evaluated with well diffusion method[23,24]. The agar plate surface is inoculated by spreading 100 µL of containing 10^8 CFU mL^{-1} test culture with sterile glass spreader. The plate was allowed to dry for 3-5 min. Wells of 5 mm diameter were cut on the surface of the agar with a sterile cork borer. Fifty microliter of 5, 10, 15, 20, 25 and 30 mg mL^{-1} of each essential oil solutions was placed into the wells and the plates were incubated at 28°C for 48 h. After incubation, the diameter of inhibition zone was measured in milimeters. The assay was repeated five times. Streptomycin was used as positive control and sterile distilled water was used as negative control.

Gas chromatography-mass spectrometry: Essential oils were analyzed using GC-MS (Gas Chromatography-Mass Spectrometry) technique. Component analyzes of essential oils were performed using Shimadzu QP2010-Ultra model GC-MS. The components present in the volatile oil were separated according to the holding time of the fused silica capillary and the evaluation procedures were carried out using the GC-MS instrument library. The oven program started with an initial temperature of 60°C held for 5 min and then the oven temperature was heated at 4°C min^{-1} to 260°C and reached 300°C with an increase every 15 min and it was held for 2 min. The injection volume was 1 µL and the injection port temperature was 250°C. Helium gas (40:1 split ratio and 1 mL min^{-1}) was supplied as carrier gas. For GC-MS detection, an electron ionization system was operated in electron impact mode with an ionization energy of 70 eV. The total program duration was 59.67 min.

Statistical analysis: To determine whether there was a statistically significant difference among the results of inhibition zone diameter of applied essential oils, standard analysis of variance (ANOVA) was carried out by using PASW Statistics 18 statistical computer software program. Significant differences between means was determined according to Duncan's multiple range test and values with $p<0.05$ were considered significantly different.

RESULTS

The antibacterial activities of essential oils at different concentrations against mixture of seven different *Rhizobium vitis* isolates obtained from Thrace region vineyards were showed in Table 1 and 2 according to disc diffusion and well diffusion method. The inhibition zones were varied related to different concentrations of essential oils. The inhibitory effects of the essential oils were significantly different at $p<0.05$ (Table 1, 2).

The inhibition zones were varied related to different concentrations of essential oils. According to inhibition zone formation, it has been found that over all, john's wort, thyme and ginger are the most effective essential oils among the other oils at all doses tested in disk diffusion and well diffusion methods. Also, it was observed to be a significant inhibitory effect especially at concentration of 30 mg mL^{-1} of john's wort oil for both methods. Inhibition zone diameter of john's wort, thyme and ginger essential oils at the concentration of 30 mg mL^{-1} was found to be 82, 51 and 54 mm for disc diffusion method and 75, 32 and 33 mm for well diffusion method, respectively (Fig. 2-4).

Table 1: Inhibitory effects of essential oils at different concentrations against mixture of seven different *A. vitis* isolates (inhibition zone diameter in mm) by disc diffusion assay[a]

Essential oils	Concentrations (mg mL^{-1})	Inhibition zone in diameter (mm)
Black cumin	5	14.0^{g}
	10	17.0^{f-i}
	15	18.0^{e-g}
	20	18.3^{f-h}
	25	19.2^{f}
	30	22.2^{cd}
Garlic	5	18.0^{f-h}
	10	18.4^{f-h}
	15	19.2^{f}
	20	21.5^{e-i}
	25	22.5^{e-g}
	30	25.0^{e-g}
John's wort	5	45.5^{def}
	10	56.4^{d}
	15	66.2^{cd}
	20	69.3^{c}
	25	75.2^{b}
	30	82.0^{a}
Mustard	5	19.2^{f}
	10	23.4^{e-g}
	15	24.0^{e-g}
	20	25.7^{e-g}
	25	28.0^{e}
	30	32.3^{d-h}
Thyme	5	33.0^{d-h}
	10	38.4^{d-g}
	15	42.0^{def}
	20	47.1^{de}
	25	48.2^{de}
	30	51.0^{de}
Ginger	5	33.4^{d-h}
	10	35.0^{d-h}
	15	44.7^{def}
	20	50.0^{de}
	25	52.5^{de}
	30	54.0^{d}
Streptomycin	5	28.0^{e}
	10	33.0^{d-h}
	15	42.0^{def}
	20	47.1^{de}
	25	50.0^{de}
	30	54.5^{d}

[a]Values expressed are mean of five replicates. Values given separately for essential oils within each row followed by different letters are significantly different at $p<0.05$

Inhibition zone diameter of black cumin essential oil at the 5 mg mL^{-1} concentration was found to be 11 mm for well diffusion method and 14 mm for disc diffusion method, respectively. Accordingly, increase in the diameter of the inhibition zone was observed on increasing concentrations of the essential oils especially at 30 mg mL^{-1} comparison with the lower concentrations.

Table 2: Inhibitory effects of essential oils at different concentrations against mixture of seven different *A. vitis* isolates (inhibition zone diameter in mm) by well diffusion assay[a]

Essential oils	Concentrations (mg mL^{-1})	Inhibition zone in diameter (mm)
Black cumin	5	11.0^{j}
	10	15.2^{ij}
	15	17.5^{hij}
	20	21.5^{gh}
	25	23.3^{gh}
	30	25.0^{g-i}
Garlic	5	13.4^{ij}
	10	18.0^{hij}
	15	24.1^{g-j}
	20	24.5^{g-j}
	25	26.5^{g-i}
	30	28.2^{g-i}
John's wort	5	35.2^{def}
	10	38.0^{de}
	15	44.5^{d}
	20	58.5^{c}
	25	62.7^{b}
	30	75.0^{a}
Mustard	5	18.2^{hij}
	10	20.0^{h}
	15	20.2^{h}
	20	23.4^{g-j}
	25	27.0^{g-i}
	30	27.5^{g-i}
Thyme	5	23.4^{g-j}
	10	26.4^{g-i}
	15	28.2^{g-i}
	20	30.0^{gh}
	25	30.5^{gh}
	30	32.0^{g}
Ginger	5	20.0^{h}
	10	24.0^{g-i}
	15	25.2^{g-i}
	20	27.0^{g-i}
	25	31.2^{gh}
	30	33.0^{g}
Streptomycin	5	20.2^{h}
	10	28.0^{g-i}
	15	33.0^{g}
	20	40.0^{de}
	25	41.1^{de}
	30	45.0^{d}

[a]Values expressed are mean of five replicates. Values given separately for essential oils within each row followed by different letters are significantly different at $p<0.05$

In all concentrations for disc diffusion method, thyme and ginger showed similar results to streptomycin sulfate in terms of inhibitory effect and the inhibition zone diameter was significantly high. The smaller inhibition zones of black cumin and garlic essential oils were observed at all concentrations when compared to the positive control. However, 25 mg mL^{-1} concentration of mustard showed similar antibacterial effect and inhibition zone diameter to 5 mg mL^{-1} of streptomycin and 30 mg mL^{-1} concentration of mustard showed similar result to 10 mg mL^{-1} of streptomycin.

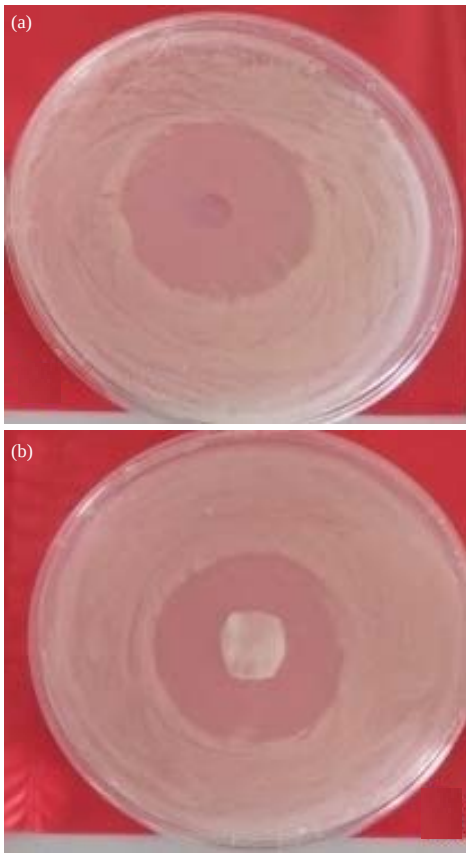


Fig. 1(a-b): Antibacterial effect of streptomycin as positive control examined by (a) Agar well and (b) Disc diffusion method at 30 mg mL^{-1} concentration

In well diffusion method, it was observed that inhibitory effect of thyme and ginger essential oils were lower at high concentrations (20, 25 and 30 mg mL^{-1}) compared to disc diffusion method. Black cumin and garlic essential oils produced low inhibition zone diameter at all concentrations compared to positive control. In mustard essential oil, the inhibition zone diameter was found to be quite low compared to the positive control particularly, even at high concentrations (20, 25 and 30 mg mL^{-1}).

Therefore, when the results are compared with each other for both method, it was observed well diffusion method produced smaller inhibition zones than disc diffusion method (Fig. 1-4).

Consequently, the highest antibacterial activity was observed in john's wort at 30 mg mL^{-1} concentration, while black cumin essential oil at 5 mg mL^{-1} concentration was the lowest antibacterial activity against *A. vitis* isolates. The larger zone of john's wort was found than streptomycin as positive control, particularly at 25 and 30 mg mL^{-1} concentration for both method (Fig. 1, 2).

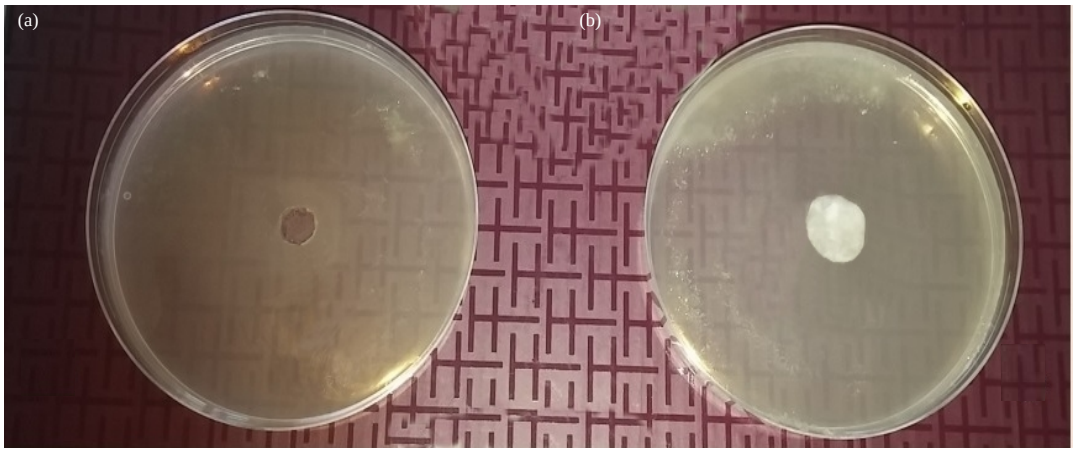


Fig. 2(a-b): Antibacterial effect of john's wort oil examined by (a) Agar well and (b) Disc diffusion method at 30 mg mL^{-1} concentration

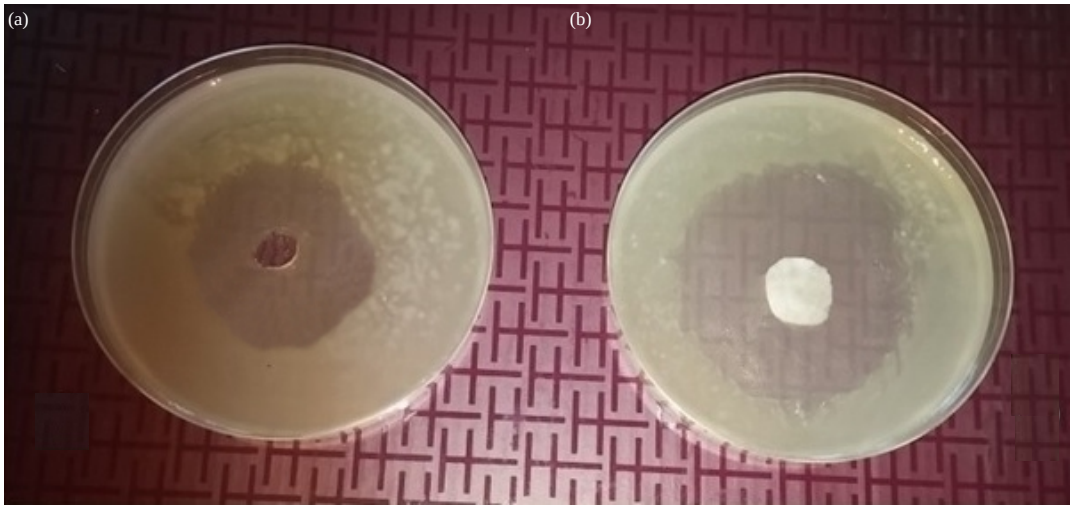


Fig. 3(a-b): Antibacterial effect of thyme oil examined by (a) Agar well and (b) disc diffusion method at 30 mg mL^{-1} concentration

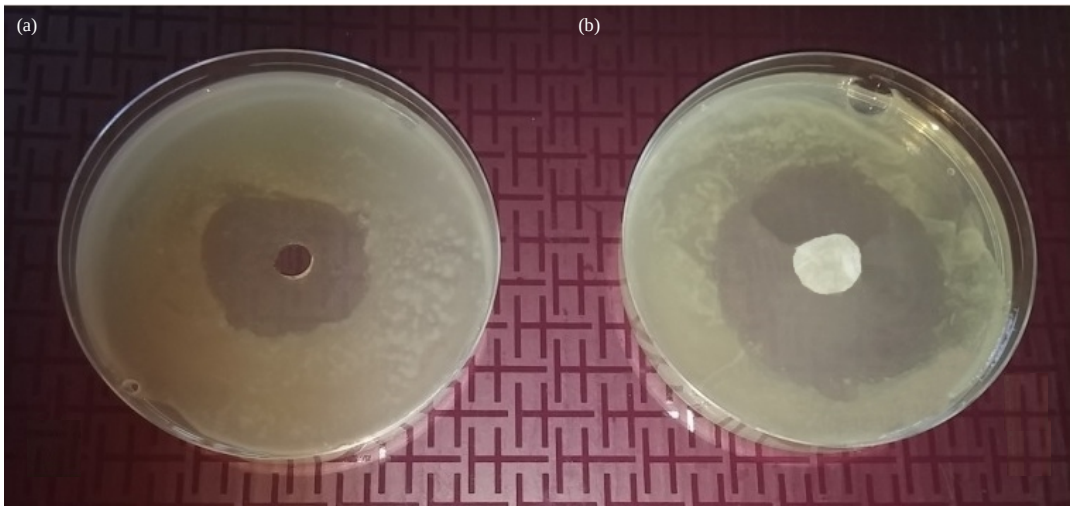
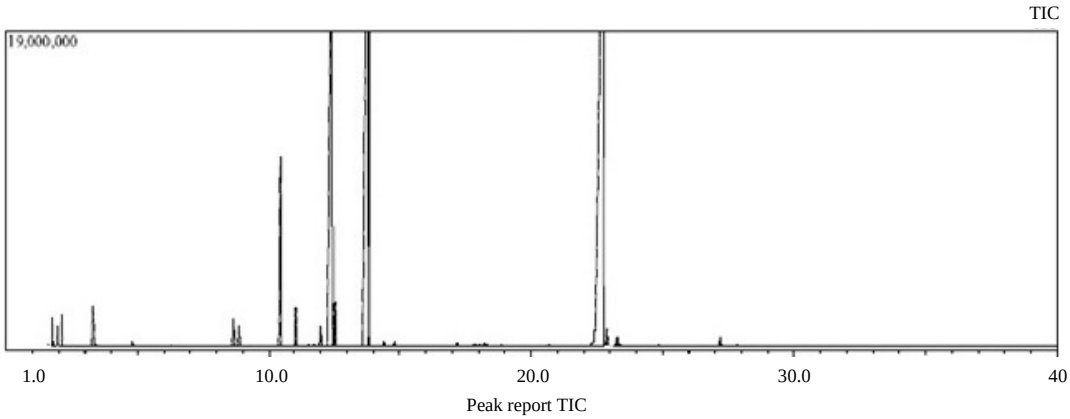
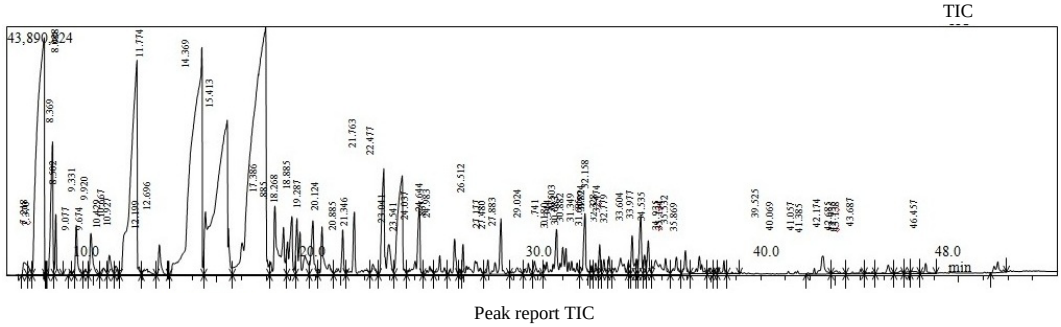


Fig. 4(a-b): Antibacterial effect of ginger oil examined by (a) Agar well and (b) Disc diffusion method at 30 mg mL^{-1} concentration



Peak	R time	Area (%)	Name
1	13.805	4.64	Gamma-terpinene
2	15.235	10.78	Linalool
3	18.194	11.38	4-terpineol
4	18.877	5.54	Alpha- terpineol
5	22.739	22.9	Thymol
6	22.895	17.8	Caryacrol

Fig. 5: Principal components of thyme essential oil



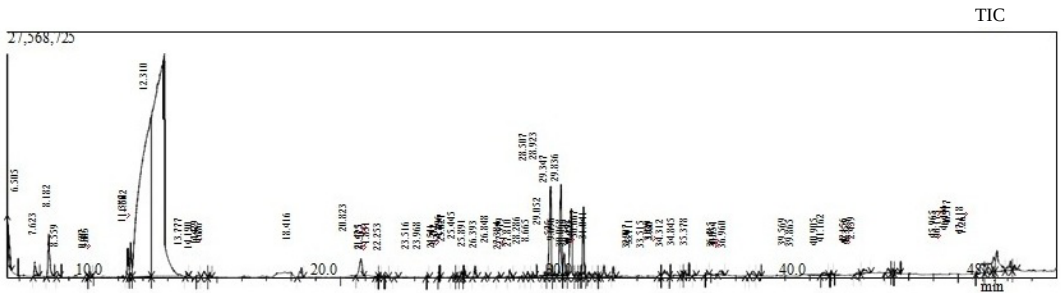
Peak	R time	Area (%)	Name
1	8.028	10.23	Cis-Ocimene
2	8.369	1.80	Camphene
3	11.774	8.48	EUCALYPTOL (1,8-CINEOLE)
4	14.369	14.36	L-fenchone
5	16.962	11.65	Linalool
6	16.962	20.67	Camphor
7	17.386	2.26	Borneol
8	21.763	2.39	ENDOBORNYL ACETATE
9	29.024	2.02	Ledne

Fig. 6: Principal components of John's wort essential oil

According to GCMS analysis, thyme EO contained a high amount of thymol (22.99%) and carvacrol (17.8%), respectively. Other main components of thyme EO were 4-terpineol (11.38%), linalool (10.78%), alpha-terpineol (5.54%), gamma-terpinene (4.64%) (Fig. 5).

John's wort EO was characterized high content of monoterpenes Camphor (20.67%), L-fenchone (14.36 %), linalool (11.65%), Cis-ocimene (10.23%) and 1,8 cineole (8.48%). Other components of john's wort were Endobornyl acetate (2.39%), Borneol (2.26%), Ledene (2.02%) and Camphene (1.80%) (Fig. 6).

Ginger EO contained a high amount of Benzyl alcohol (43.07%) and Bornyl acetate (31.33%). Other main components were ar-curcumene (3.14%), Zingiberene (2.90%), beta-bisalone (2.45%), beta-sesquiphellandrene (1.88%), camphene (1.35%) and oleic acid (1.39%) (Fig. 7).



Peak report TIC

Peak No.	R time	Area (%)	Name
1	8.182	1.35	Camphene
2	12.310	31.33	Bornyl alcohol
3	12.864	43.07	Benzyl alcohol
4	28.507	3.14	Benezene, 1-(1,5-dimethyl-4-hexenyl-4-methyl-(CAS)ar-curcumene
5	28.923	2.90	Zingiberene
6	29.347	2.45	Beta-Bisabolene
7	29.836	1.88	Beta-sesquiphellandrene (CAS)2-methyl-6-(4-methylenecyclohex
8	46.577	1.39	Oleic acid

Fig. 7: Principal components of ginger essential oil

DISCUSSION

John's wort, ginger ve thyme essential oils showed significant antibacterial effect and the activity of essential oils at all doses tested in disk diffusion and well diffusion methods has been linked to the presence of numerous terpenoids and phenolic compounds (thymol, eugenol, carvacrol) that exhibit antibacterial activity. The essential oils with the strongest antibacterial properties in the study include high-order terpenes such as carvacrol, eugenol, linalool, camphor and benzyl alcohol. The essential oils with high antibacterial effect at 30 mg mL^{-1} concentration, particularly John's wort essential oil were supposed to depend on presence of major and minor components and interactions between them, application doses. Generally, inhibition zone diameter significantly indicated an increase parallel to application dose.

Components of essential oils appear to be effective on cytoplasmic membrane-embedded cell proteins[25]. It is known that enzymes such as ATPases are located within the cytoplasmic membrane and are restricted by lipid molecules. Terpenes can disrupt and penetrate the lipid structure of the bacteria cell, it leads to denaturation of proteins, cytoplasmic leakage, cell disruption and ultimately cell death. The decrease in pH related to cell membrane degradation causes lost of DNA transcription, enzyme activity and protein synthesis[26,27].

Thyme EO contained high amounts of thymol and carvacrol, respectively[28] reported that thymol and carvacrol had higher antibacterial activity than streptomycin. Carvacrol and thymol have been shown to affect the outer membrane of Gram-negative bacteria[29]. The antibacterial efficacy of thyme essential oil and its main components such as thymol and carvacrol may be held a controlling effect against Gram-negative plant pathogenic bacteria such as *Rhizobium* spp. Carvacrol is one of the few components of thyme EO that shows disrupting effect on the outer membrane of Gram-negative bacteria[30]. Movement of carvacrol on microbial cells causes structural and functional damage to the membranes[31], that results in increased permeability. It may cause release of lipopolysaccharide [32] and also move on the cytoplasmic membrane to change the transport of ions. Antibacterial activity of carvacrol is supposed to be due to the presence of a hydroxyl group capable of acting as trans-membrane transporter of monovalent cations by transporting H^+ to the cell cytoplasm and transporting K^+ to the back away[33,34]. Antimicrobial activity of thymol may result in structural and functional changes that disrupt the outer and inner of the cytoplasmic membrane[31]; it may also interact with membrane proteins and intracellular targets. Carvacrol may also inhibit the synthesis of flagellin, a microbial protein for bacterial motility and may lead to flagella-free cells that exhibit less motility. However, even cells with flagella may exhibit reduced activity due to the amount of carvacrol, indicating that the compound can decrease the proton motility required to carry out flagellar movement[35]. To maintain optimal membrane function and structure, it has been suggested that cells exposed to carvacrol alter the fatty acid composition of the membrane due to the effect of carvacrol related to fluidity[36,37].

John's wort EO was characterized high content of monoterpenes[28] reported that linalool, camphor and 1,8-cineole had same or slightly higher antibacterial activity than streptomycin. Oxygenated monoterpenes are common components of volatile oils, usually found in high quantities. In this context, the mechanism of antibacterial activity can be attributed to the degradation of cytoplasmic membrane, coagulation of cell contents, dissipation of proton motility, electron flow and active transport[31].

Ginger EO contained a high amount of Benzyl alcohol and Bornyl acetate. Benzyl alcohol monoterpene, major compound in ginger essential oil, is supposed to be responsible for the degradation of lipids, degradation of cell membrane barrier, increased permeability of fungal cells and disruption of the basic enzymes. Also, benzyl alcohol is a strong antimicrobial agent against various microorganisms[38], Salicylic acid, volatile methyl ester of MeSA, acts as a salicylic acid-dependent biosynthetic cascade in a variety of plants leading to systemic acquired resistance (SAR) against bacterial infection[39]. Bornyl acetate, another major component in ginger EO has been known as a highly active antimicrobial agent[40]. Dorman and Deans[29] reported activity against Gram-negative bacteria such as *Rhizobium* for bornyl acetate found the second main component of the oxygenated monoterpene fraction in the study.

CONCLUSION

As a result of the study, John's wort essential oil showed very high antibacterial activity among other essential oils when evaluated according to two methods. Especially, at 30 mg mL^{-1} concentration, it reached maximum antibacterial effect. Ginger and thyme essential oils followed it, respectively. It is supposed that application of herbal preparations containing john's wort essential oil will be significantly promising and applicable for control of crown gall. It was also observed that Agar Well Diffusion Method showed smaller inhibition zone than Disc Diffusion Method.

Consequently, interactions between essential oils and their components need to be investigated comprehensively for improving agricultural applications that are effective, non toxic and non polluting to control crown gall.

SIGNIFICANCE STATEMENT

This study provides an innovation in terms of antibacterial effect of black cumin, mustard, John's wort and ginger essential oils against *Rhizobium vitis*. It is obvious that these essential oils haven't been examined against *Rhizobium vitis* so far in previous studies. We don't claim that this type research is first. We think that it provides an alternative and potential advantages to control crown gall disease. Also, we believe that our study will be promising and provide improvement and acceleration for these type of researches. As a result of the study, John's wort essential oil showed very high antibacterial activity among other essential oils when evaluated according to two methods. Especially, at 30 mg mL^{-1} concentration, it reached maximum antibacterial effect. Ginger and thyme essential oils followed it, respectively. We suppose and hope that application of herbal preparations containing john's wort essential oil will be significantly promising and applicable for control of crown gall.

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