



Nanoencapsulation of Plant Volatile Organic Compounds to Improve Their Biological Activities

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NUFFIELD DEPARTMENT OF
WOMEN'S &
REPRODUCTIVE HEALTH
Medical Sciences Division



Dr Helen E Townley
University Research Lecturer,
William Dodd Fellow

22nd June 2020

Dear Editor,

We would like to submit a review article entitled "*Nanoencapsulation of Plant Volatile Organic Compounds to Improve Their Biological Activities*" for consideration by *Planta Medica*. We believe that this would fall under the category of delivery systems of natural products, which would be of interest to your readers.

In this work, we aimed to demonstrate the biological properties and applicational drawbacks of plant volatile organic compounds (VOCs) and their improved bioavailabilities and biological activities through nanoparticle encapsulation. The manuscript has covered the biosynthesis, analysis, biological activities and limitations of plant VOCs and the antibacterial, antifungal, antiviral and anticancer potentials of plant VOCs loaded nanoformulations.

Given that the biological applications of plant VOCs are substantially hindered by their physicochemical properties despite their excellent potential, we investigated a number of nanoencapsulation related works that have been performed to boost the activities of volatile compounds. We believe that this manuscript would provide readers with valuable information about the use of nanoparticles in enhancing the physicochemical and biological features of bioactive materials. In particular, the comparative explanation of stabilities and bioactivities of plant VOCs before/after nanoencapsulation described in this review might encourage a more widespread use of volatile compounds to treat microbes and human diseases using various nanoparticle systems.

Your Faithfully,

A handwritten signature in blue ink that reads 'Helen Townley'.

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Editor's Comments to Author:

- **Title page: Authors names: please double check: are all authors names spelled correctly? Are they listed in the correct order? Have all contributing authors been mentioned? Note: changes to this part will not be possible after acceptance of the manuscript.**

- **Affiliations: see comment above - please make sure that all affiliations are listed correctly since changes to this part will not be possible later.**

- **For correspondence, please give academic title.**

- **For correspondence, please add a phone and a FAX number if appropriate.**

- **The list of abbreviations should be in alphabetical order.**

The abbreviations have been rearranged into alphabetical order.

- **Abstract: the claim in the abstract about anticancer properties does not seem well founded.**

The sentence:

'They have been proven to exhibit a broad range of biological properties such as antimicrobial and anticancer activities and not to cause serious toxicity to environment.'

Was changed to:

They have been shown to exhibit a broad range of biological properties and have been investigated for antimicrobial and anticancer activities. In addition, they are thought be more environmentally friendly than many other synthetic chemicals [1].

- **Introduction: please add some sentences where information about the method of collecting literature data were explained.**

The sentence added:

'Herein, we have discussed the general features and applicational limitations of plant VOCs and the bioactivities and both free and nanoencapsulated form of the volatile compounds by using many keywords such as "biosynthesis and analysis of plant VOCs", "limitations of essential oil in applications" and "biological activities of plant VOCs and nanoencapsulated plant VOCs" in the Scopus database.'

- **Language: "do not cause serious toxicity to environment". Please add an article.**

The sentence:

'Since plant VOCs do not cause serious toxicity to environment, they have been classified as GRAS (Generally Regarded as Safe) and listed in EAFUS (Everything Added to Foods in the United States) [6].'

Was altered to:

'Since most plant VOCs do not cause the serious toxicity to environment, they have been classified as GRAS (Generally Regarded as Safe) and listed in EAFUS (Everything Added to Foods in the United States) [7].

- "IC₅₀ = 20.48 µg/mL» Please note that the given precision is too high.

'NEs carrying Cuminum cyminum VOCs [154] and ginger and frankincense essential oils [155] were noted to have in vitro antitumour activities against human tongue carcinoma (SAS) cells (IC₅₀ = 1.5 µL/mL) and breast adenocarcinoma (MCF-7) cells (IC₅₀ = 20.48 µg/mL), respectively.'

Was changed to:

'(IC₅₀ = 20.5 µg/mL)'

- References: "Plant VOCs are known to have a diverse array of biological properties including antibacterial, antifungal, antiviral, anticancer, insecticidal, antiinflammatory, antidiabetic, anti-obese, neuroprotective and antihepatotoxic activities [7–9].» Please check the references and preferably cite primary literature as for example the claim "anticancer" does not seem to be well founded. For example [9] cites an article presenting results in animals for "anticancer". [7] cites "The antioxidants of human extracellular fluids" and "DNA repair of oxidative DNA damage in human carcinogenesis: Potential application for cancer risk assessment and prevention", both of which do not seem appropriate.

The sentence:

'Plant VOCs are known to have a diverse array of biological properties including antibacterial, antifungal, antiviral, anticancer, insecticidal, anti-inflammatory, antidiabetic, anti-obese, neuroprotective and antihepatotoxic activities [7–9].'

Was changed to

'Plant VOCs are known to have a diverse array of biological properties including antibacterial [8,9], antifungal [10], antiviral [11], anticancer [12,13], insecticidal [14], anti-inflammatory [15], antidiabetic [16], neuroprotective [17] and antihepatotoxic activities [18].'

- "In vitro anticancer properties». Please rephrase. Cancer is not an in vitro condition. Anticancer properties can only be shown in humans.

The sentence:

'In vitro anticancer assays showed that IC₅₀ against A549 cells of bulk gold, free volatile compounds and gold nanoparticles coated with the VOCs were 87.20 µg/mL, 64.15 µg/mL and 28.37 µg/mL, respectively [111].'

Was changed to

'In vitro antitumour assays showed that IC₅₀ against A549 cells of bulk gold, free volatile compounds and gold nanoparticles coated with the VOCs were 87.20 µg/mL, 64.15 µg/mL and 28.37 µg/mL, respectively [117].'

- Acknowledgement: Please confirm that you have mentioned all organizations that funded your research in the Acknowledgements section of your submission, including grant numbers where appropriate. Note: changes to this paragraph will not be possible after acceptance of the manuscript.

N/A

- Figures: please do not include them into the main manuscript, only upload them as TIFF files.

All the figures were removed from the main manuscript.

- **Figures:** Please reduce the size of the pictures. As indicated on the generated PDF file, the converter can only handle up to 40 mega pixel which is already much too big. please see our instructions for authors for correct format and layout of artwork (<https://www.thieme.de/de/planta-medica/140866.htm>).

The figures have been resized.

Reviewers' Comments to Author:

Reviewer: 1

Comments to the Author

The manuscript "Nanoencapsulation of Plant Volatile Organic Compounds to Improve Their Biological Activities" by Townley has been reviewed. It can not be accepted in this stage but after revision can be accepted for publication. To improve the quality of article following points should be clarified.

1. Page4, lines 16-17; Authors stated "Plant VOCs ... comprise low molecular weight metabolites (<300 Da) with fairly low boiling points (<260 °C)" and cited the article "Dong et al. 2016" but when examined the article it can be notified that this expression was cited from the book chapter by Negre-Zakharov et al. 2009. However, because of VOC is defined as " (VOCs) are organic chemical compounds that evaporate easily at room temperature", reviewer does not agree with using the expression " (<260 °C)" because this point is very high and not based on an experimental data. Authors should re-consider for this state.

The sentence:

'Plant VOCs account for about 1% of plant secondary metabolites and typically comprise low molecular weight metabolites (<300 Da) with fairly low boiling points (<260 °C) [5].'

Was changed to

'Plant VOCs account for about 1% of plant secondary metabolites currently known and typically comprise low molecular weight metabolites (<300 Da) with fairly low boiling points (e.g. 236.8 °C for carvacrol, 225 °C for citral and 198 °C for linalool) [6].'

2. Page 6, lines 31-32; Author defined VOCs as Page 6, lines 33-34; in the expression "...functional groups including phenols, terpenes, ketones, aldehydes ..." terpenes are a big chemical group and not a functional group. The term terpenes should be removed.

The term 'terpene' has been removed.

3. Page 7, lines 3-7; MFCs concentrations should be given as mg/ml because 6250 µg/ml seems not applicable.

The sentence:

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'.. fungicidal concentrations (MFCs) of 781.25 µg/ml and 390.625 µg/ml each, and also substantially hindered aflatoxin B₁ production from those fungi at a concentration of 6250 µg/ml [45].'

Has been amended to:

'..fungicidal concentrations (MFCs) of 0.78 mg/mL and 0.39 mg/mL within 2 days on Muller-Hinton agar plates each, and also substantially hindered aflatoxin B₁ production from those fungi at 6.25 mg/mL and 3.12 mg/mL after the 29 days of incubation in maize respectively [54].'

4. Page 7 lines 22-23; the expression "fugal cell" should be revised as "fungal cell" Page 7 lines 31-32; In the section "Antiviral activity". authors should explain the by which VOCs affected to viruses.

The spelling error has been corrected.

5. Page 9 lines lines 19-21; Author should mention the antioxidant capacity of VOCs with under separate head and not anticancer activity. Currently, to be antioxidative can be accepted as "antioxidant potential" or "antioxidant capacity" and not "activity". Moreover, to be antioxidative can not be defined as anticancer activity. If authors wish to give this properties of VOCs they should give as antioxidant capacity with the under separate title.

Terminology was corrected into "capacity".

The sentence:

'Plant VOCs are able to prevent carcinogenesis through their antimutagenic and antioxidant activities.'

Was changed to

'Plant VOCs are able to prevent carcinogenesis through their antimutagenic and antioxidant capacities.'

The sentence:

'The degradation of VOCs can result in a reduction in their bioactivities such as antioxidant activities.'

Has been changed to read:

'The degradation of VOCs can result in a reduction in their bioactivities, for example, their antioxidant capacity.'

The sentence:

'Indeed plant VOCs used in food preservations have been shown to have decreased antioxidant activities after heating [91–93].'

Has been changed to:

'Indeed plant VOCs used in food preservations have been shown to have decreased antioxidant capacity after heating [97–99].'

Antioxidant potentials of plant VOCs were removed from anticancer activity.

6. Page 8 lines 53-54; the statement ..that VOCs of Salvia species increased Bax/Bcl2.. Which species of Salvia ??

The sentence:

'It had been determined that VOCs of Salvia species increased Bax/Bcl-2 expression ratio in prostate cancer cells [74],...'

Now includes the species names:

'It had been determined that VOCs of three Salvia species, i.e. S. aurea, S. judaica and S. viscosa increased Bax/Bcl-2 expression ratio in prostate cancer cells [79],...'

7. Page 9 lines 21-22; In the expression "The insolubility of plant VOCs may also restrict their applications" .. VOCs are insoluble ?? They can be soluble in organic solvents ??? Authors should mention about solubility of VOCs particularly in analysing section. If VOCs are insoluble in water, how to test in biological or analytical assays ?? Authors should mention in the text. References Dong F, Fu X, Watanabe N, Su X, Yang Z. Recent Advances in the Emission and Functions of Plant Vegetative Volatiles. Molecules 2016; 21: 124. Negre-Zakharov, F.; Long, M.C.; Dudareva, N. Floral scents and fruit aromas inspired by nature. In Plant-Derived Natural Products, Synthesis, Function, and Application; Osbourn, A.E., Lanzotti, V., Eds.; Springer Berlin: Heidelberg, Germany, 2009; pp. 405–431.

The sentence:

'The insolubility of plant VOCs may also restrict their applications.'

Has been changed to:

'The insolubility of plant VOCs in the aqueous phase may also restrict their applications [90].'

Reviewer: 2

Comments to the Author Manuscript Number: PLAMED-2020-06-0655-REV Authors: Mun H., Townley H. Title: Nanoencapsulation of Plant Volatile Organic Compounds to Improve Their Biological Activities

Scientific Content The paper is supposed to review the nanoencapsulation of plant VOCs. But it curiously begins by a lengthy but uncomplete review of VOCs biosynthesis and biological properties. This review is much more detailed in ref. 31 by the same senior author; as here it brings only selected data and practically no information useful to the section on nanoencapsulation, I propose to briefly summarize this part of the manuscript (while keeping and amending Figure 1) and to delete Figure 2. In a few instances, I went back to the original papers cited and found misinterpretation or exaggeration of published data. I suggest to more rigorously review data presented in the original papers. I'm not sure that the use of VOCs to reduce metals into nanoparticles is really an "encapsulation of VOCs" as the redox reaction modifies the original compounds. This should be discussed.

In metal nanoparticles, plant VOCs have been used as reducing agents or coating agents. So, the terminology "coating of VOCs into metal nanoparticles" should be more sensible than "encapsulation of VOCs into metal nanoparticles". However, once VOCs are coated on the nanoparticles, they are protected and also have the increased bioactivities like those

encapsulated in organic nanoparticles. In the manuscript, we referred to those metal nanoparticles as “metal nanoparticles carrying plant VOCs” or “metal nanoparticles coated with plant VOCs”.

1. Page 4, line 11: remove the "and"

The sentence:

‘Many plants release diverse blends of VOCs from nearly all organs to attract pollinators, and to prevent attacks from pathogens and herbivores, and to communicate with the surrounding environment [2–4].’

Was changed to read:

‘Many plants release diverse blends of VOCs from nearly all organs to attract pollinators, to prevent attacks from pathogens and herbivores, and to communicate with the surrounding environment [3–5].’

2. Page 4, line 14: is there any real meaning for this "1%"? Many compounds are still unknown (especially minor and trace metabolites) both in VOCs and other metabolites.

The sentence:

‘Plant VOCs account for about 1% of plant secondary metabolites and typically comprise low molecular weight metabolites (<300 Da) with fairly low boiling points (<260 °C) [5].’

Was changed to:

‘Plant VOCs account for about 1% of plant secondary metabolites currently known and typically comprise low molecular weight metabolites (<300 Da) with fairly low boiling points (e.g. 236.8 °C for carvacrol, 225 °C for citral and 198 °C for linalool) [6].’

3. Page 4, line 20: as some compounds are quite (geno)toxic, please change "Since plant VOCs" into "Since most plant VOCs"; not all essential oils are GRAS 4.

The sentence:

‘Since plant VOCs do not cause serious toxicity to environment, they have been classified as GRAS (Generally Regarded as Safe) and listed in EAFUS (Everything Added to Foods in the United States) [6].’

Has been changed to:

‘Since most plant VOCs do not cause the serious toxicity to environment, they have been classified as GRAS (Generally Regarded as Safe) and listed in EAFUS (Everything Added to Foods in the United States) [7].’

4. Page 4, line 27: what is "anti-obese"????

Since the anti-obesity activity was not seen to be very pronounced in the literature reviewed it has been removed.

The sentence:

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'Plant VOCs are known to have a diverse array of biological properties including antibacterial, antifungal, antiviral, anticancer, insecticidal, anti-inflammatory, antidiabetic, anti-obese, neuroprotective and antihepatotoxic activities [7–9].'

Has been changed to:

'Plant VOCs are known to have a diverse array of biological properties including antibacterial [8,9], antifungal [10], antiviral [11], anticancer [12,13], insecticidal [14], anti-inflammatory [15], antidiabetic [16], neuroprotective [17] and antihepatotoxic activities [18].'

5. Page 4, line 32: replace "lifetimes" by "bioavailability"

'However, many of their biological activities are prevented from wide scale use because of the short lifetimes of the active components due to volatility.'

This sentence refers to the fact that the compounds are volatile and breakdown very quickly into inactive compounds, rather than whether they are bioavailable.

6. Page 5, line 10: replace "oxygenation" by "peroxidation"

The sentence:

'Volatile fatty acid derivatives are generated from C18 fatty acids, i.e. linoleic acid or linolenic acid, which are subjected to the oxygenation at C9 or C13 position by lipoxygenases, leading to the production of two kinds of compounds, the 9-hydroperoxy and 13-hydroperoxy derivatives of polyenoic fatty acids (Fig. 1; yellow-highlighted box) [16].'

Was amended to:

'Volatile fatty acid derivatives are generated from C18 fatty acids, i.e. linoleic acid or linolenic acid, which are subjected to peroxidation at C9 or C13 position by lipoxygenases, leading to the production of two kinds of compounds, the 9-hydroperoxy and 13-hydroperoxy derivatives of polyenoic fatty acids (Fig. 1; yellow-highlighted box) [25].'

7. Page 5, line 25: please delete the sentence "The analytical....[18]" (these are useless in face of a complex mixture); all this is anyway summarized in the beginning of the following sentence and the reference 18 could be placed there: "Although a number of analytical methods including organoleptic, physical, chemical and spectroscopic techniques are available [18]"

The following sentence was deleted:

'The analytical techniques used to identify the plant VOCs are based on their physical and chemical properties such as polarities, melting and freezing points, relative densities, optical rotations, and refractive indices [18].'

8. Page 5, line 36: GC has also the major advantage to be a highly resolutive method.

The sentence:

'Gas chromatography (GC) is thought to be the most suitable platform for analyzing plant VOCs because they are low-molecular weight compounds [21].'

Was changed to:

Gas chromatography (GC) is thought to be the most suitable platform for analysing plant VOCs due to their volatility and low-molecular weights [30].

9. Page 5, line 37: "low molecular weight" is not enough; you need volatility (e.g. underivatized sugars are difficult to analyze by GC).

The sentence:

'Gas chromatography (GC) is thought to be the most suitable platform for analyzing plant VOCs because they are low-molecular weight compounds [21].'

Has been changed to read:

'Gas chromatography (GC) is thought to be the most suitable platform for analysing plant VOCs due to their volatility and low-molecular weights [29]'

10. Page 5, line 39: replace "adopts" by "combines"; note : to separate enantiomers, you need a chiral GC column.

The sentence:

'In particular, multidimensional GC (MDGC), which adopts several columns with different stationary phases, e.g. nonpolar and polar phases, can improve the efficiency of separation and authentication of enantiomeric plant VOCs [30].'

Has been changed to:

'In particular, multidimensional GC (MDGC), which combines several columns with different stationary phases, e.g. nonpolar and polar phases, can improve the efficiency of separation and authentication of enantiomeric plant VOCs [31].'

11. Page 6, line 12: a MIC of 10 mg/mL is far from impressive (e.g. compared to those mentioned on line 26)?? Please check the units; ref. 31 is self-citation and does not bring info on the source of data. Some reports indicate much higher activities: e.g. MICs ranging from 0.08 mg/ml to 0.64 mg/ml for oregano oil (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6182053/>)

12. subsequent: note that the inhibition diameter for a given compound depends on 2 parameters: its antimicrobial activity and its diffusion in agar. A very active compound but poorly diffusing will give a low inhibition zone diameter. Hence it is not appropriate to use diameters to compare the activity of compounds from different chemical classes (with presumably different diffusions in agar), e.g. carvacrol and streptomycin!

The sentence:

'Essential oils extracted from several plants such as Origanum species and Melaleuca alternifolia have highly active VOCs that exert activity against gram-positive and gram-negative bacteria with minimum inhibitory concentrations (MICs) of approximately 10 mg/mL [31]. Soković et al. reported that the inhibition zones (IZs) of carvacrol against gram-positive bacteria (27 - 36 mm) were much bigger than those of streptomycin (12-20 mm).'

Has been amended to read:

'Essential oils extracted from several plants such as Origanum species have highly active VOCs that exert activity against multidrug resistant bacteria including Acinetobacter baumannii and Pseudomonas aeruginosa with minimum inhibitory concentrations (MICs) of 0.08 – 0.64

mg/mL [40]. Soković *et al.* reported that carvacrol possessed higher antibacterial activity towards gram-negative and gram-positive bacteria with a MIC of 0.125 – 1.0 µg/mL than streptomycin (MIC = 1.0 – 3.0 µg/mL).'

Same remark on page 15, line 15

The following sentence was removed:

The loaded Ch-NPs showed a greater inhibition zone against the bacteria than free volatiles, e.g. in Staphylococcus aureus the average inhibition zone increased from 9.7 mm to 11.3 mm, after encapsulation [133].

13. Page 6, line 28: linalool is NOT an aldehyde

We have corrected the sentence:

'Plant VOCs with long complex chain alcohols and aldehydes such as linalool were shown to be effective in inhibiting the growth of gram-positive bacteria including Listeria monocytogenes [33].'

To read:

'Plant VOCs with long complex chain alcohols, and aldehydes such as citral, were shown to be effective in inhibiting the growth of gram-positive bacteria including Listeria monocytogenes [42].'

14. Page 6, line 33: terpene is not a functional group; and what is meant by "oxide"? Ethers? Peroxides?

'Terpenes' has been deleted from the following list:

'Many studies have demonstrated that the functional groups including phenols, terpenes, ketones, aldehydes and oxides of volatile compounds are related with the antimicrobial activities of plant VOCs [34].'

15. Page 7, line 3: if the fungicidal concentration is 781 and 390 µg/mL (remove decimals; these are illusory precision...), how comes that you need 6250 µg/mL to reduce the production of aflatoxins???? Please check figures and units.

The sentence:

'Moghadam et al. reported Ziziphora clinopodioides VOCs inhibited the growth of Aspergillus flavus and Aspergillus parasiticus with minimum fungicidal concentrations (MFCs) of 781.25 µg/ml and 390.625 µg/ml each, and also substantially hindered aflatoxin B1 production from those fungi at a concentration of 6250 µg/ml [45].'

Has been altered to:

'Moghadam et al. reported Ziziphora clinopodioides VOCs inhibited the growth of Aspergillus flavus and Aspergillus parasiticus with minimum fungicidal concentrations (MFCs) of 0.78 mg/mL and 0.39 mg/mL within 2 days on Muller-Hinton agar plates each, and also substantially hindered aflatoxin B1 production from those fungi at 6.25 mg/mL and 3.12 mg/mL after the 29 days of incubation in maize respectively [54].'

16. Page 7, line 22: correct "fugal"

This has been corrected.

17. Page 8, line 8: are these in vitro or in vivo or clinical data?

The sentence:

'β-Caryophyllene was also evaluated to possess strong antimutagenic activity against 2-nitrofluorene, because the compound might inactivate mutagens and block DNA damages [73].'

Was changed to:

'β-Caryophyllene was also evaluated to possess strong antimutagenic activity against 2-nitrofluorene, because the compound might inactivate mutagens and block DNA damages in vitro [74].'

18. Page 8, line 13: what are those "changes of cell membrane permeability"? Ref. 31 is self-citation and does not bring info on the mechanism or source of data. 19.

The following sentence has been removed:

'Plant VOCs are able to prevent carcinogenesis through their antimutagenic and antioxidant activities. These antimutagenic properties may originate from the change of cell membrane permeability that leads to the prevention of mutagen penetration into cells, and inhibition of DNA damage in mammalian cells [31].'

19. Page 9, line 28: chlorophyll is not a good example as they are not present in VOC fractions and so cannot be responsible for "auto-oxidations" such as discussed for fennel seeds VOCs.

The sentence:

'Ultraviolet light and visible light are able to induce the excitation of quantum state of sensitizers such as chlorophylls and the transition energy from an excited state to ground state causes the autooxidation of plant VOCs [85].'

Was altered to read:

'Ultraviolet light and visible light are able to induce the excitation of quantum state of sensitizers such as riboflavin and the transition energy from an excited state to ground state causes the autooxidation of plant VOCs [91].'

Please indicate more likely photosensitizers (also for page 10, line 3).

The sentence:

'Oregano essential oil, whose major ingredients are carvacrol and thymol, was shown to be photodegraded through the excitement of endogenous sensitizers and the formation of singlet oxygen (O₂(¹Δg)) followed by the oxidation of VOCs by O₂(¹Δg) [88].'

Was changed to:

'Oregano essential oil, whose major ingredients are carvacrol and thymol, was shown to be photodegraded through the excitement of endogenous sensitizers such as flavins, NADH/NADPH, and urocanic acid and the formation of singlet oxygen (O₂(¹Δg)) followed by the oxidation of VOCs by O₂(¹Δg) [94].'

20. Page 9, lines 33-42: the original publication indicates that storage of essential oil was done in the light with "Air delivered through a pipette for 10 days"; so the observed effect is not only due to light but also to oxygen. Also in the original publication "The components of this essential oil were extremely unstable throughout storage, even in the dark".

The following sentence has been removed because the study is not a good example of photodegradation.

'Plant VOCs of fennel seed (Foeniculum vulgare) that had been stored under illumination for 4 months underwent a noticeable compositional change due to the dramatic degradation of some of the VOCs. The major VOCs including α -pinene, β -myrcene, limonene, γ -terpinene, and trans-anethol could no longer be detected, whilst eugenol, estragol and fenchone remained in considerable amounts. Trans-anethol, for example, can be oxidized to anisaldehyde or undergo isomerization to cis-anethol [86].'

Instead, the following has been added:

'Maria Beltrame et al. reported that essential oils of Marjoram (Origanum majorana) were likely to undergo the rapid photodegradation and exhibit the dramatic change in their chemical profiles even in 5 min under UV light. The main components of the essential oils degraded by the light were p-diisopropyl-benzene, 2-undecanone and m-diisopropyl-benzene [92].'

21. Page 9, lines 44-49: the presented figures do not allow to indicate "a lower degree of degradation in thyme oil than in rosemary oil", i.e. what was the original content of rosemary oil?

The sentence:

'According to the work done by Turek et al., the α -terpinene component of rosemary oil was reduced to 16.4% upon light exposure for 12 weeks. However, under the same conditions α -terpinene in thyme oil had been reduced from 91.5% to 73.3%, indicating that there is a lower degree of degradation in thyme oil than in rosemary oil.'

Was amended to:

'According to the work done by Turek et al., the α -terpinene component of rosemary oil was reduced to 16.4% upon light exposure for 12 weeks, while there was no decrease of the volatile compound in the dark condition. However, the amount of remaining α -terpinene in thyme oil in the dark and light condition after 12 weeks were 91.5% and 73.3% respectively, indicating that there is a lower degree of photodegradation in thyme oil than in rosemary oil.'

22. Page 10, line 54: in fact Semperio analysed the water solubility of individual compounds, not of essential oils. Thanks to correct. He also included in his series parabens and sorbic acid. Please correct 2460.6 into 1282.2 mg/L (the most soluble VOC, eugenol) and remove benzyl paraben (synthesis compound) from your enumeration (and maybe also check for propyl benzoate?).

The sentences:

'Samperio et al. analyzed the aqueous solubility of over twenty essential oils at 25 °C for 24 hours, shaking at 250 rpm, and found that their solubility ranged from 1.6 mg/L to 2460.6 mg/L. In particular, nonanoic lactone, β -pinene, benzyl cinnamate, (R)-limonene, cyclohexane butyric acid, methyl nonanoate, benzyl paraben, propyl benzoate, and trans,trans-2,4-decadienal were almost insoluble in distilled water; less than 0.01% (v/v) in water [101].'

Were changed to:

'Samperio et al. analysed the aqueous solubility of over twenty plant VOCs at 25 °C for 24 hours, shaking at 250 rpm, and found that their solubility ranged from 1.6 mg/L to 1282.2 mg/L (eugenol). In particular, nonanoic lactone, β -pinene, benzyl cinnamate, (R)-limonene, cyclohexane butyric acid, methyl nonanoate, propyl benzoate, and trans,trans-2,4-decadienal were almost insoluble in distilled water; less than 0.01% (v/v) in water [103].'

23. Page 12, line 21: thanks to relativize in the text : the essential oil by itself is not very active (at 24h, 22.5 mg/mL !!!!) and the original publication indicates that the improvement from the nanocarrier is in fact null at 24h; the 20 mg/mL that you state has been measured at 48h.

The sentence:

'PFEO laden nanoparticles demonstrated improved antimicrobial activity against food-borne bacteria compared to PFEO, e.g. the total inhibitory concentrations of the nanoformulation and free compounds against Staphylococcus aureus were 20.0 mg/mL and 22.5 mg/mL, each [107].'

Was altered to read:

'PFEO laden nanoparticles demonstrated improved antimicrobial activity against food-borne bacteria compared to PFEO, e.g. the total inhibitory concentrations of the nanoformulation and free compounds against Staphylococcus aureus were 20.0 mg/mL and 22.5 mg/mL upon 48 h treatment, respectively [113].'

24. Page 12, line 53: this is the conclusion of Shenyi et al, but it's very surprising to see tertiary alcohols get oxidized to carboxylic acids in the presence of acetone and Au³⁺? Please state that no mechanism has been proposed for this (curious) explanation.

The sentence:

'Sheny et al. suggested that terpenoids including spathulenol, globulol, muurolol and cadinol can utilize their tertiary hydroxyl groups to reduce gold ions in the existence of acetone at high temperature and the oxidized terpenoids attach around the gold nanoparticles through the carboxylate ions [112].'

Was amended to read:

'Sheny et al. suggested that terpenoids including spathulenol, globulol, muurolol and cadinol can utilize their tertiary hydroxyl groups to reduce gold ions in the existence of acetone at high temperature and the oxidized terpenoids attach around the gold nanoparticles through the carboxylate ions, although further investigations are needed to elucidate the exact mechanism [118].'

25. Page 13, lines 22-27: with a boiling point of 449°C, resveratrol can hardly be considered as a VOC.

The following sentence was deleted:

'Resveratrol, an unstable VOC that can easily be converted to its cis form under illumination, was loaded into nanoliposomes to improve its photostability.'

Alternative examples were included:

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'Nootkatone, a volatile sesquiterpenoid compound, was encapsulated in cyclodextrins complexes to improve its photostability. A photodegradation assay showed that free nootkatone was subject to almost complete degradation (96%) after 60 min of UV irradiation, compared to 63% after encapsulation [125]. Kumar et al. found out that cyclodextrin-based nanosponges carrying Babchi (Psoralea corylifolia) essential oil protected cargo compounds from photodegradation such that the photodegradation rate constant exhibited by pure and encapsulated compounds were $6.909 \times 10^{-3} \text{ min}^{-1}$ and $2.303 \times 10^{-3} \text{ min}^{-1}$ in pseudo-first order kinetic model upon UV exposure, respectively [126].'

26. Page 13, lines 39-40: same remark for indole-3-carbinol (boiling point, 361°C).

The following sentence has been deleted:

'Gehrcke et al. reported indole-3-carbinol loaded PLC nanocapsules had improved the photostability such that over 77% of encapsulated volatiles remained after 300 min exposure of UV light compared to 20% for unprotected compounds [121].'

27. Page 15, line 42: please specify the model 28.

The sentence:

'The antibacterial activities of NLCs and NEs carrying citral have been tested against Staphylococcus aureus, Bacillus cereus and Escherichia coli. Average MICs of citral loaded NLCs were significantly lower than those of citral emulsion, suggesting that the NLCs are likely to be the better choice to enhance the antimicrobial activity of cargo compounds than NEs [143].'

Was amended to:

'The antibacterial activities of NLCs and NEs carrying citral have been tested against Staphylococcus aureus, Bacillus cereus and Escherichia coli. Average MICs of citral loaded NLCs were significantly lower than those of citral emulsion (e.g. MIC of emulsions against S. aureus was $500 \mu\text{g/mL}$, whereas that of NLCs was $125 \mu\text{g/mL}$), suggesting that the NLCs are likely to be the better choice to enhance the antimicrobial activity of cargo compounds than NEs [140].'

28. Page 16, line 5 and 9: reduce decimals 29.

The original:

'Chan et al. reported that MSNPs carrying isothiocyanate could maximize antimicrobial potential of the cargo compound against Pseudomonas aeruginosa biofilm by decreasing the biofilm thickness from $100.25 \pm 13.13 \mu\text{m}$ to $8.125 \pm 1.44 \mu\text{m}$ for 24 hours at concentration of 2 mg/L. By comparison, 350 mg/L free allyl isothiocyanate only resulted in a reduction of biofilm thickness to $31.0 \pm 4.42 \mu\text{m}$ under the same conditions [162].'

Has been changed to read:

'Chan et al. reported that MSNPs carrying allyl isothiocyanate could maximize antimicrobial potential of the cargo compound against Pseudomonas aeruginosa biofilm by decreasing the biofilm thickness from $100 \pm 13 \mu\text{m}$ to $8 \pm 1 \mu\text{m}$ for 24 hours at concentration of 2 mg/L. By comparison, 350 mg/L free allyl isothiocyanate only resulted in a reduction of biofilm thickness to $31 \pm 4 \mu\text{m}$ under the same conditions [142].'

29. Page 17, lines 43-44: the difference is not impressive. Looking at original data, I see that the authors have not tested for a difference between the 2 treatments; given the experimental variability, the difference may not be significant.

The sentence:

'After 48 h incubation the results revealed that 100 µg/mL of free VOCs decreased the viability of cancer cells to 44.25%, compared to encapsulated compounds which had a greater effect decreasing cell viability to only 37.44% of control [153].'

Was replaced by the results with clearer comparison:

'After 48 h incubation, the viability of MDA-MB-231 cells was reduced into approximately 40% in response to treatment of 50 µg/mL of volatiles laden Ch-NPs, which exhibited more cytotoxicity against cancer cells than free volatiles causing about 70% cell viability at the same concentration [152].'

30. Page 17, lines 45-48: this quite vague sentence supposedly explains the findings with Ocimum capsules of the former sentence. However, reading reference 164 indicates absolutely NO mention of Ocimum or Ch-NPs!!!!

We apologise that the incorrect reference was associated with this statement. This has now been corrected.

Also, the statement:

'After 48 h incubation the results revealed that 100 µg/mL of free VOCs decreased the viability of cancer cells to 44.25%, compared to encapsulated compounds which had a greater effect decreasing cell viability to only 37.44% of control [153]. It is thought that the Ch-NPs could penetrate the cancer cell membranes and cause DNA damage, leading to defects in the cellular genes [164]. It is thought that the Ch-NPs could penetrate the cancer cell membranes and cause DNA damage, leading to defects in the cellular genes [164].'

Has been changed to read:

'After 48 h incubation, the viability of MDA-MB-231 cells was reduced into approximately 40% in response to treatment of 50 µg/mL of volatiles laden Ch-NPs, which exhibited more cytotoxicity against cancer cells than free volatiles causing about 70% cell viability at the same concentration [152]. It is thought that the Ch-NPs could penetrate the cancer cell membranes and cause DNA damage, leading to defects in the cellular genes [153].'

31. Page 17, lines 53 to 58: this § as written seems to indicate a clinical trial to "prevent locoregional recurrence of cancer". Looking at the original publication, it appears that it presents in vitro tests only leading to a proposal: "We therefore propose the use of the citral-nanoparticle-polymer wafers for implantation into the tumor bed after surgical removal of a sarcoma as a means to control locoregional spread due to any remaining cancerous cells." Please rewrite accordingly.

The sentence:

'The wafer system exhibited 50% degradation over 25 days therefore releasing citral to prevent locoregional recurrence of the cancer [154].'

Was clarified:

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3 *'The wafer system was tested in vitro and exhibited 50% degradation over 25 days therefore*
4 *releasing citral. This could ultimately be employed to prevent locoregional recurrence of the*
5 *cancer in the medical practice [154].'*
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8 **32. Page 18, line 50: remove "plant"**

9 The sentence:

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11 *'Nevertheless, their usage in therapeutics, food, and agricultural settings are being inevitably*
12 *restricted by the volatile, unstable and hydrophobic attributes plant with which VOCs are*
13 *endowed.'*
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15 Was changed to:

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17 *'Nevertheless, their usage in therapeutics, food, and agricultural settings are being inevitably*
18 *restricted by the volatile, unstable and hydrophobic attributes with which VOCs are endowed.'*
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21 **33. Page 18, line 57: replace "adsorption" by "absorption"**

22 The sentence:

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24 *'The encapsulation of these compounds in nanostructured formulations, i.e. polymer- or lipid-*
25 *based nanoparticles and inorganic nanoparticles, has been shown to increase their biological*
26 *activities by improving the physicochemical properties, bioavailability, sustained release, and*
27 *cellular adsorption of plant derived VOCs.'*
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29 Has been changed to read:

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31 *'The encapsulation of these compounds in nanostructured formulations, i.e. polymer- or lipid-*
32 *based nanoparticles and inorganic nanoparticles, has been shown to increase their biological*
33 *activities by improving the physicochemical properties, bioavailability, sustained release, and*
34 *cellular absorption of plant derived VOCs.'*
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38 **34. Figure 1 This figure implies that sesquiterpenoids are only obtained via the mevalonate pathway**
39 **and that mono- and diterpenoids only by the MEP pathway, which is not so clear-cut. Depending on**
40 **the plant and organ, there are most probably crossings between these pathways.**

41 The final products of MVA pathway were denoted as sesquiterpenoids, diterpenoids, and
42 triterpenoids and those of MEP pathway were denoted as monoterpenoids, sesquiterpenoids
43 and diterpenoids.
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46 **35. Polyketides are not all derived through linolenic acids; where do you put compounds such as**
47 **cannabinoids or coniine?**

48 Figure 1 was intended to be a simple illustration indicating only the main pathways of plant
49 VOC synthesis.
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52 **36. Coumarins are missing in the blue part**

53 While that coumarin is an important phenylpropanoid, we just showed one of the typical
54 pathways, whose final product is eugenol, instead of exhibiting all the pathways such as
55 coumarin pathway, stilbene pathway, monolignol pathway and so on, since there is limited
56 space in the figure.
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37. Correct "carotinoids" and "gerayl"; and the structure of cinnamoyl-CoA; I don't think carotenoids qualify as VOCs?

The chemical name '*Geraylgeranyl pyrophosphate*' was corrected to '*Geranylgeranyl pyrophosphate*'.

'*Carotenoids*' were not considered as VOCs, so the term has been removed.

The structure of cinnamoyl-CoA was corrected.

For Peer Review

Nanoencapsulation of Plant Volatile Organic Compounds to Improve Their Biological Activities

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Abstract

Plant volatile organic compounds (VOCs, volatiles) are secondary plant metabolites that play crucial roles in the reproduction, defence, and interactions with other vegetation. They have been ~~shown~~proven to exhibit a broad range of biological properties and have been investigated for such as antimicrobial and anticancer activities. In addition, they are thought be more environmentally friendly than many other synthetic chemicals [1]. ~~and not to cause serious toxicity to environment.~~ Despite these facts, their applications in the medical, food and agricultural fields are considerably restricted due to their volatilities, instabilities, and aqueous insolubilities. Nanoparticle encapsulation of plant VOCs is regarded as one of the best strategies that could lead to the enhancement of the bioavailability and biological activity of the volatile compounds by overcoming their physical limitations and promoting their controlled release and cellular absorption. In this review, we will discuss the biosynthesis and analysis of plant VOCs, their biological activities and limitations. Furthermore, different types of nanoparticle platforms used to encapsulate the volatiles and the biological efficacies of nanoencapsulated VOCs will be covered.

Keywords: plant volatile organic compounds, essential oil, nanoencapsulation, nanoparticle, antimicrobial activity, anticancer activity

Abbreviations:

CEO clove essential oil
Ch-NPs chitosan nanoparticles
DEN-2 dengue virus type 2
EAFUS Everything Added to Foods in the United States
FMO frankincense and myrrh essential oils
GC gas chromatography
GRAS Generally Regarded as Safe
HSV-1 herpes simplex virus type 1
HSV-2 herpes simplex virus type 2
JUNV Junin virus
LOX Lipoxygenase
MDGC multidimensional gas chromatography
MEP Methylerythritol phosphate
MFCs minimum fungicidal concentrations
MICs minimum inhibitory concentrations
MLVs multilamellar nanoliposomes
MNPs metal nanoparticles
MRSA methicillin-resistant *Staphylococcus aureus*
MSNPs mesoporous silica nanoparticles
MVA mevalonic acid
NEs nanoemulsions
NLCs nanostructured lipid carriers
 $O_2(^1\Delta_g)$ singlet oxygen
 $O_2(^3\Sigma_g^-)$ triplet oxygen
PCL poly-ε-caprolactone
PFE0 pepper fragrant essential oil
ROS reactive oxygen species
SLNs solid lipid nanoparticles
SUVs unilamellar nanoliposomes
TGA thermogravimetric analysis
TPF thermoplastic flour
VOCs volatile organic compounds
~~VOCs—volatile organic compounds—~~

GRAS—Generally Regarded as Safe-
 EAFUS—Everything Added to Foods in the United States
 MVA—Mevalonic acid-
 MEP—Methylerythritol phosphate-
 LOX—Lipoxygenase-
 GC—gas chromatography-
 MDGC—multidimensional gas chromatography
 MICs—minimum inhibitory concentrations
 IZs—inhibition zones
 MFCs—minimum fungicidal concentrations-
 HSV-1—herpes simplex virus type 1-
 HSV-2—herpes simplex virus type 2-
 JUNV—Junin virus
 DEN-2—dengue virus type 2-
 GST—glutathione transferase
 DPPH—1,1-diphenyl-2-picrylhydrazyl
 ROS—reactive oxygen species
 $O_2(^3\Sigma_g^-)$ triplet oxygen
 $O_2(^1\Delta_g)$ singlet oxygen
 NEs—nanoemulsions
 SLNs—solid lipid nanoparticles
 NLCs—nanostructured lipid carriers-
 Ch-NPs—chitosan nanoparticles
 MSNPs—mesoporous silica nanoparticles
 MNPs—metal nanoparticles-
 PFFO—pepper fragrant essential oil-
 PCL—poly-ε-caprolactone-
 TGA—thermogravimetric analysis
 TPF—thermoplastic flour
 FMO—frankincense and myrrh essential oils
 MRSA—methicillin-resistant *Staphylococcus aureus*
 CEO—clove essential oil
 MLVs—multilamellar nanoliposomes

~~SUVs—unilamellar nanoliposomes-~~

1. Introduction

VOCs comprise a chemically diverse group of organic compounds. They have high vapour pressure under ambient conditions as a consequence of low boiling points. This causes large numbers of molecules to evaporate or sublime from the liquid or solid form of the compound into the air [2]. Many plants release diverse blends of VOCs from nearly all organs to attract pollinators, ~~and~~ to prevent attacks from pathogens and herbivores, and to communicate with the surrounding environment [3–5]. Plant VOCs account for about 1% of plant secondary metabolites currently known and typically comprise low molecular weight metabolites (<300 Da) with fairly low boiling points (e.g. 236.8 °C for carvacrol, 225 °C for citral and 198 °C for linalool) ~~(<260 °C)~~ [6]. The lipophilic nature of plant VOCs enables them to travel freely across cellular membranes and disperse into the surrounding atmosphere. Since most plant VOCs do not cause the serious toxicity to environment, they have been classified as GRAS (Generally Regarded as Safe) and listed in EAFUS (Everything Added to Foods in the United States) [7].

Plant VOCs are known to have a diverse array of biological properties including antibacterial [8,9], antifungal [10], antiviral [11], anticancer [12,13], insecticidal [14], anti-inflammatory [15], antidiabetic [16], neuroprotective [17] and antihepatotoxic activities ~~–~~ [18] [7–9]. Such properties can be applied in medical, food and agricultural fields. However, many of their biological activities are prevented from wide scale use because of the short lifetimes of the active components due to volatility. Protection and controlled release of VOCs *via* nanoencapsulation could increase the utility of these compounds [19]. In this study, the comprehensive literature survey was implemented utilising the Scopus and Pubmed (MEDLINE) databases from inception to 10 June 2020. English-language papers published as primary documents, meta-analyses, reviews and systematic reviews were included for the investigation. The title and abstract keywords employed during the search are as follows: plant volatile organic compounds; essential oil; synthesis; analysis; antimicrobial activity; anticancer activity; applicational limitation; nanoparticle encapsulation; enhanced bioavailability; enhanced biological activity. In terms of the enhanced biological activity of encapsulated plant VOCs, we focused on the improved antibacterial, antifungal, antiviral and anticancer activity of essential oils upon nanoparticle encapsulation.

1.1. Biosynthesis of plant volatile organic compounds (VOCs)

Plant VOCs are synthesized from primary metabolites through enzymatic modifications including oxidation, hydroxylation, acetylation and methylation. The three main groups of plant VOCs

(terpenoids, phenylpropanoids/benzenoids and fatty acid derivatives) are generated *via* several metabolic pathways such as the mevalonic acid (MVA), the methylerythritol phosphate (MEP), the shikimate/phenylalanine and lipoxygenase (LOX) pathways (**Fig. 1**) [20].

Terpenoids originate from the universal five carbon building components isopentenyl diphosphate and its allylic isomer dimethylallyl diphosphate [2]. Two metabolic pathways such as the MVA (**Fig. 1**; green-highlighted box) and MEP pathways (**Fig. 1**; pink-outlined box) are involved in the synthesis of hemiterpenes (C₅), monoterpenes (C₁₀), sesquiterpenes (C₁₅), and diterpenes (C₂₀). The MVA pathway is thought to take place in the cytosol, endoplasmic reticulum and peroxisomes to generate sesquiterpenes, while the MEP pathway occurs only in plastids and synthesizes the terpenoids having C₅, C₁₀ and C₂₀ [21].

The biosynthesis of phenylpropanoid and benzenoid compounds is initiated by the condensation of phosphoenolpyruvate and erythrose 4-phosphate and the subsequent shikimate pathway mediates the generation of phenylalanine (**Fig. 1**; blue-highlighted box) [22]. Cinnamic acid converted from phenylalanine undergoes many enzymatic reactions such as methylation and acetylation to generate cyclic volatiles including eugenol and benzyl benzoate [23,24].

Volatile fatty acid derivatives are generated from C₁₈ fatty acids, i.e. linoleic acid or linolenic acid, which are subjected to ~~peroxidation~~the oxygenation at C₉ or C₁₃ position by lipoxygenases, leading to the production of two kinds of compounds, the 9-hydroperoxy and 13-hydroperoxy derivatives of polyenoic fatty acids (**Fig. 1**; yellow-highlighted box) [25]. These intermediates undergo metabolic modifications *via* two branches of the LOX pathway, which are the oxide synthase pathway and hydroperoxide lyase pathway, and they are converted to fatty acid derivatives such as methyl jasmonate and green leaf volatiles [26].

1.2 Analysis of plant VOCs

Quantification or qualification of plant VOCs is difficult due to their presence in small amounts and close similarity between molecules. ~~The analytical techniques used to identify the plant VOCs are based on their physical and chemical properties such as polarities, melting and freezing points, relative densities, optical rotations, and refractive indices [18].~~ Although a number of analytical methods including organoleptic, physical, chemical and spectroscopic techniques are available for the characterization of the plant VOCs [27], the chromatographic techniques have been extensively used due to the ability to mediate comprehensive separation and identification of plant VOCs [28,29]. Gas chromatography (GC) is thought to be the most suitable platform for analysing plant VOCs due to their volatility and~~because they are~~ low-molecular weight ~~compounds~~ [30]. In particular, multidimensional GC (MDGC), which ~~combines~~adopts several columns with different stationary

phases, e.g. nonpolar and polar phases, can improve the efficiency of separation and authentication of enantiomeric plant VOCs [31]. Liquid chromatography is also employed as an alternative platform of GC for analysing thermally labile or less volatile compounds [32].

2. Biological activities and limitation of plant VOCs

2.1. Biological activities

2.1.1. Antimicrobial activities

Plant VOCs have a broad range of antimicrobial activities because plants have evolved their volatiles to defend themselves against deleterious bacteria, fungi and viruses. The volatile compounds could be used to replace many synthetic drugs which are hindered by high toxicities and low efficacies. However, this is dependent upon the bioavailabilities of the VOCs being improved [33].

- Antibacterial activity

Antibacterial properties of plant VOCs against both gram-positive and gram-negative bacteria have been demonstrated through a number of studies [34–39]. Essential oils extracted from several plants such as *Origanum* species and *Melaleuca alternifolia* have highly active VOCs that exert activity against multidrug resistant bacteria including *Acinetobacter baumannii* and *Pseudomonas aeruginosa* with minimum inhibitory concentrations (MICs) of 0.08 – 0.64 mg/mL ~~gram-positive and gram-negative bacteria with minimum inhibitory concentrations (MICs) of approximately 10 mg/mL~~ [40][31]. Soković et al. reported that carvacrol possessed higher antibacterial activity towards gram-negative and gram-positive bacteria with a MIC of 0.125 – 1.0 µg/mL than streptomycin (MIC = 1.0 – 3.0 µg/mL) ~~the inhibition zones (IZs) of carvacrol against gram-positive bacteria (27–36 mm) were much bigger than those of streptomycin (12–20 mm)~~. Gram-negative bacteria appeared to be resistant to the volatiles that they had examined, e.g. the inhibition zone of carvacrol against *Pseudomonas aeruginosa* (22 mm) was smaller than those against gram-positive bacteria [36]. In a study that assessed the antibacterial potentials of citral, carvacrol, geraniol, terpineol, perillaldehyde, eugenol, linalool and citronellal against foodborne pathogens, citral and carvacrol exhibited the highest inhibitory activities against *Escherichia coli*, *Salmonella typhimurium*, *Listeria monocytogenes*, and *Vibrio vulnificus* among all the volatiles, e.g. MICs of citral and carvacrol against *Vibrio vulnificus* were 100 µg/mL and 250 µg/mL, respectively.[41]. Plant VOCs with long complex chain alcohols, and aldehydes such as citral, linalool were shown to be effective in inhibiting the growth of gram-positive bacteria including *Listeria monocytogenes* [42].

It is thought that VOCs exert their activities by means of their hydrophilic or lipophilic properties. Many studies have demonstrated that the functional groups including phenols, ~~terpenes~~, ketones, aldehydes and oxides of volatile compounds are related with the antimicrobial activities of plant VOCs [43]. Phenolic alcohol groups of terpenoids, for instance, may block the activity of membrane related enzymes [44]. Another study revealed that monoterpenoids possessing phenol groups could prevent the synthesis of flagellin, which is the component of the flagella used for bacterial movement [45]. VOCs have also been shown to cause membrane protein denaturation, subsequent potassium efflux and cell lysis [46]. Furthermore, many plant VOCs might directly affect the synthesis of DNA, RNA and proteins in microbes [47,48]. Vulgarone B, for example, is a constituent VOC produced in *Artemisia iwayomogi*, which has been proven to introduce single nicks in DNA strands of *Staphylococcus aureus* [49].

- Antifungal activity

VOCs originating from plants such as oregano, rosemary, thyme basil, citrus and fennel have been proven to have antifungal activities against *Candida albicans*, *Aspergillus niger*, *Cryptococcus neoformans* and *Fusarium oxysporum* [50–53]. Moghadam et al. reported *Ziziphora clinopodioides* VOCs inhibited the growth of *Aspergillus flavus* and *Aspergillus parasiticus* with minimum fungicidal concentrations (MFCs) of 0.781.25 µg/mL and 0.390.625 µg/mL within 2 days on Muller-Hinton agar plates each, and also substantially hindered aflatoxin B₁ production from those fungi at a concentration of 6.250 µg/mL and 3.12 mg/mL after the 29 days of incubation in maize respectively [54]. Plant VOCs from *Mentha x piperita* displayed significant fungicidal activities, in which MICs of volatiles against *Candida* species ranged from 0.25 - 1% (v/v). Fungistatic activities of the compounds against dermatophytes (MIC= 0.125 - 0.5%, (v/v)) was evaluated to be higher than those of azole drugs with MICs up to 4% (v/v) [55].

Bona et al. investigated the antifungal activities of 12 essential oils against *Candida albicans* while comparing to synthetic antifungal drugs such as clotrimazole, fluconazole and itraconazole. This showed that pathogenic yeasts were more susceptible to plant VOCs compared to the conventional treatments, and in particular plant VOCs from oregano and winter savoury showed fungal inhibition rates that were more than 200% that of clotrimazole. It was suggested that the VOCs mostly affected the cell wall and membranes of the fungi [56]. Indeed, plant VOCs might demolish the fungal cell wall and cytoplasmic membranes, leading to a leakage of the cytoplasm and its coagulation [57]. Eugenol and carvacrol were observed to cause mycelial deformation of *Cladosporium herbarum* when they inhibited the growth of the fungi. The morphological deformations might be correlated with the action of the compounds on cell wall enzymes involving chitinases and glucanases [58].

- Antiviral activity

Many plant VOCs have demonstrated antiviral properties against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), poliovirus, coxsackievirus B1, avian influenza virus, rhinovirus, and dengue virus type 2 [59–63]. HSV-1 and HSV-2 are very common viruses that are estimated to infect 60 – 95% of adult human population and have been extensively studied for the susceptibility to plant VOCs. Plant VOCs of *Hyptis mutabilis* [64], *Glechhonia marifolia* [65] and *Artemisia arborescens* [66] suppressed the replication of HSV-1. *Mentha piperita* VOCs exhibited high levels of virucidal activity against HSV-1 and HSV-2, and against an acyclovir- (an antiviral drug) resistant strain of HSV-1 [67]. Anti-influenza viral properties of *Pogostemon cablin* VOCs against H1N1 and H2N2 were elucidated by Li et al. [68] and Wu et al. [69]. García et al. screened essential oils obtained from several aromatic plants for the virucidal activity against HSV-1, Junin virus (JUNV) and dengue virus type 2 (DEN-2) and concluded that *Lippia junelliana* and *Lippia turbinata* essential oils possessed potent inhibitory activities against JUNV, while *Artemisia douglasiana* and *Eupatorium patens* essential oils had distinguishable effects on DEN-2 infectivity [70]. Hammami et al. also claimed that *Teucrium pseudochamaepitys* VOCs can give rise to the significant reduction of Coxsackievirus B virus infectivity of human endothelial type 2 (Hep-2) cells grown *in vitro* [60].

2.1.2. Anticancer activity

The preventive and suppressive effects of plant VOCs on various cancers such as brain, breast, colon, liver, lung, mouth and prostate cancers, and leukaemia have been proven in a number of studies [71,72]. Plant VOCs are able to prevent carcinogenesis through their antimutagenic and antioxidant capacities-activities. ~~These antimutagenic properties may originate from the change of cell membrane permeability that leads to the prevention of mutagen penetration into cells, and inhibition of DNA damage in mammalian cells [31].~~ Turmeric essential oil has been shown to exhibit significant antimutagenic activities against mutagens including sodium azide [73]. β -Caryophyllene was also evaluated to possess strong antimutagenic activity against 2-nitrofluorene, because the compound might inactivate mutagens and block DNA damages *in vitro* [74]. ~~The antioxidant characteristics of plant VOCs also contribute to the cancer prevention. Plant VOCs can scavenge intracellular radicals or increase the detoxifying activity of enzymes such as glutathione reductase and glutathione transferase (GST) [66]. Twenty-five essential oils were assessed for antioxidant activities using the 1,1-diphenyl 2-picrylhydrazyl (DPPH) assay, with the greatest antioxidant activities shown by thyme VOCs, followed by clove leaf and cinnamon leaf VOCs [67]. Teneva et al. reported that *Citrus aurantium* L. zest VOCs manifested over 85% inhibition of DPPH activity at~~

~~a low concentration, i.e. 1 mg/mL [68]. In addition, the essential oil mixture whose active ingredients were 13.5% thymol and 4.5% cinnamyl aldehyde was found to increase the activity of GST noticeably in the spleen of weaned pigs [69].~~

Plant VOCs also can induce apoptosis and cell cycle arrest in cancer cells [75]. Apoptotic cell death may arise from the increased levels of reactive oxygen species (ROS) as a result of exposure to plant VOCs. Schisandrae semen VOCs demonstrated an immediate generation of ROS in human leukaemia U937 cells, and triggered the mitochondria-dependent apoptotic signalling pathways [76]. Pavithra et al. suggested that *Pamburus missionis* VOCs increased the intracellular ROS level in epidermoid cancer cells and that inhibition of the VOC induced-ROS production resulted in a decrease in apoptosis [77]. Plant VOCs can be involved in the regulation of signalling pathways such as the NF- κ B, PIK/AKT/mTOR, ERK1/2-Bcl-2/survivin, and MAPK pathways in cancer cells to induce cell death (**Fig. 2**). Zito et al. reported that *Cyphostemma juttae* VOC can cause a substantial reduction of NF- κ B activation and downregulate the NF- κ B target genes in breast cancer cells [78]. It had been determined that VOCs of three *Salvia* species, i.e. *S. aurea*, *S. judaica* and *S. viscosa* increased Bax/Bcl-2 expression ratio in prostate cancer cells [79], *Litsea cubeba* VOCs dephosphorylated Ser473 and Thr308 of Akt through the suppression of mTOR and pPDK1 in lung cancer cells [80], and curcuminol inhibited the expression of phosphorylated STAT3 *via* the JAK1/2 and Src pathways and blocked the synthesis of HIF-1 α *via* the mTOR and MAPK pathways to suppress the growth of hepatic cancer cells [81]. Cell cycle arrest in the cancer cells by plant VOCs is also believed to be one line of therapeutic strategies where many genes involved in cancer cell cycles can be hampered. β -caryophyllene, for example, has been proven to regulate G1 cell cycle progression by decreasing the expression of CDK2, CDK4, CDK6, cyclin D1 and cyclin E and increasing the levels of p21 and p27 in lung cancer cells [82]. Furthermore, the metastasis and angiogenesis processes of cancer cells were constrained by plant VOCs such as D-limonene [83] and *Tridax procumbens* VOCs [84].

2.2. Limitations

Plant VOCs can be easily dissipated through evaporation [85], and degraded (oxidized [86], isomerized [87], dehydrogenized [86], or polymerized [88]) by light, heat or air, unless they are protected by external factors [89]. The insolubility of plant VOCs in the aqueous phase may also restrict their applications [90].

2.2.1 Deactivation by light

Ultraviolet light and visible light are able to induce the excitation of quantum state of sensitizers such as riboflavin and chlorophylls and the transition energy from an excited state to ground state causes the autoxidation of plant VOCs [91]. During the autoxidation process, molecules may accept a proton or an electron from other molecules and as a result radicals can be produced in volatile molecules [88]. Maria Beltrame et al. reported that essential oils of Marjoram (*Origanum majorana*) were likely to undergo the rapid photodegradation and exhibit the dramatic change in their chemical profiles even in 5 min under UV light. The main components of the essential oils degraded by the light were *p*-diisopropyl-benzene, 2-undecanone and *m*-diisopropyl-benzene [92]. ~~Plant VOCs of fennel seed (*Foeniculum vulgare*) that had been stored under illumination for 4 months underwent a noticeable compositional change due to the dramatic degradation of some of the VOCs. The major VOCs including α -pinene, β -myrcene, limonene, γ -terpinene, and *trans*-anethol could no longer be detected, whilst eugenol, estragol and fenchone remained in considerable amounts. *Trans*-anethol, for example, can be oxidized to anisaldehyde or undergo isomerization to *cis*-anethol [86].~~ The extent of degradation of individual VOCs is likely to depend on the antioxidant level in the surrounding environment. According to the work done by Turek et al., the α -terpinene component of rosemary oil was reduced to 16.4% upon light exposure for 12 weeks, while there was no decrease of the volatile compound in the dark condition. However, under the same conditions α -terpinene in thyme oil had been reduced from 91.5% to 73.3%, indicating that there is a lower degree of photodegradation in thyme oil than in rosemary oil. Thus, it could be considered that the antioxidant components of thyme oil such as thymol and carvacrol could prevent photo-autoxidation of α -terpinene more effectively than those in rosemary oil [93]. The involvement of triplet oxygen ($O_2(^3\Sigma_g^-)$) in the photooxidation was recently identified by Dimarco Palencia et al. Oregano essential oil, whose major ingredients are carvacrol and thymol, was shown to be photodegraded through the excitement of endogenous sensitizers such as flavins, NADH/NADPH, and urocanic acid and the formation of singlet oxygen ($O_2(^1\Delta_g)$) followed by the oxidation of VOCs by $O_2(^1\Delta_g)$ [94].

2.2.2. Deactivation by heat and air

It is predictable that the degradation of essential oils could be hastened with an increase in temperature, since chemical reactions such as oxidation and decomposition accelerate with heat. Many studies found that plant VOCs change their compositions under high temperature conditions during storage. Analysis of the thermal stability of laurel, oregano and rosemary VOCs showed that all terpenes indicated a likelihood of thermo-degradation and monocarbonyl compounds, and

hexanal, 2-heptanal, and 2,4-decadienal appeared as a result of autoxidation [95]. Hădărugă et al. also reported that terpenes including sesquiterpenes had a greater susceptibility to degradation than the corresponding alcohols, and as such epoxidated sesquiterpenes and monoterpenes were found to be abundant after high temperature treatment [96]. The degradation of VOCs can result in a reduction in their bioactivities, ~~for example, their such as~~ antioxidant ~~capacity~~activities. Indeed plant VOCs used in food preservations have been shown to have decreased antioxidant ~~capacity~~activities after heating [97–99].

Since oxidation reactions account for the main degradation of plant VOCs, their access to oxygen has a crucial impact on stability. As a consequence, plant VOCs are less likely to be degraded in an environment with low oxygen levels [88]. It has been reported that lavender and thyme oil that had been stored for 72 weeks at low temperature were degraded as a result of large amounts of dissolved oxygen that contributed to peroxide generation. This is in agreement with Henry's law which shows that oxygen solubility in liquid is high at low temperature [93]. In order to prevent the oxidation of VOCs by oxygen, inert gases such as argon might be employed in the storage of essential oils. With regards to temperature-dependent degradation, alkyl or hydroxy radicals within plant VOCs are responsible for oxidation at high temperature due to limited amount of oxygen [100].

2.2.3. Insolubility in water

Plant VOCs, which are mostly soluble in organic solvents, have poor solubilities in water and body fluids such as blood. This is the one of main reasons why the plant VOCs have not been widely applied in medical, food and agricultural fields [43,101,102]. Samperio et al. analysed the aqueous solubility of over twenty ~~plant VOCs essential oils~~ at 25 °C for 24 hours, shaking at 250 rpm, and found that their solubility ranged from 1.6 mg/L to ~~1282.22460.6~~ mg/L (eugenol). In particular, nonanoic lactone, β -pinene, benzyl cinnamate, (R)-limonene, cyclohexane butyric acid, methyl nonanoate, ~~benzyl paraben~~, propyl benzoate, and trans,trans-2,4-decadienal were almost insoluble in distilled water; less than 0.01% (v/v) in water [103]. The insolubility of VOCs in water could be improved; the limitation of thyme white essential oil, for example, has been overcome through encapsulation into cellulose nanocrystals generating a natural antimicrobial agent [104].

3. Nanoencapsulation of plant VOCs and their improved biological activities

Encapsulation of bioactive compounds can be defined as a process of surrounding droplets of the compounds with coatings, or immersing them in heterogeneous or homogeneous matrices [105]. Nanoencapsulation of plant VOCs can decrease the volatility of bioactive compounds, protect them from external factors such as oxygen, light, moisture and pH, and increase their solubilities.

Encapsulated plant VOCs are likely to possess higher biological activities than free compounds, as the nanoparticles can mediate the controlled release and increased cellular accessibility of the volatiles [106].

3.1. Nanoencapsulation systems for carrying plant VOCs

Several types of nanoencapsulation platforms have been used to increase the availability of plant VOCs in pharmaceutical, food, and agricultural areas, including polymer-based, lipid-based and inorganic nanoparticle systems. It is thought that plant VOCs form hydrophobic interactions with the hydrophobic cavities of nanocapsules, or the polymers of nanocapsules and nanospheres. They may also interact with the surfactants of nanoliposomes or lipid phases of nanoemulsions (NEs), solid lipid nanoparticles (SLNs), and nanostructured lipid carriers (NLCs). The interaction of VOCs with inorganic nanoparticle systems is likely to be *via* van der Waals bonds, or electrostatic interactions in the case of metal nanoparticles (**Fig. 3**).

The encapsulation of plant VOCs in organic nanostructured systems, i.e. polymer- or lipid- based nanoparticles, has been extensively discussed by de Matos et al. [107]. Among those platforms, chitosan nanoparticles (Ch-NPs) have been most widely applied mainly due to their good physicochemical properties and intrinsic antimicrobial potentials. Ch-NPs have drawn attention as a non-parenteral delivery formulation of plant VOCs to treat various diseases including cancers. Initial drawbacks such as low encapsulation efficiency of Ch-NPs can be improved by employing inclusion complexes such as β -cyclodextrin (**Fig. 4**) [102]. Other commonly used systems for the encapsulation of volatiles are mesoporous silica nanoparticles (MSNPs) and metal nanoparticles (MNPs).

MSNPs possess honeycomb-like structures with a large number of pores, whose diameters range between 2 - 50 nm and are characterized by a high specific surface area, adjustable particle diameter and pore size, and the feasibility of the surface functionalization [109]. The fabrication of MSNPs is principally based on the hydrolysis and condensation of alkoxysilanes. Surfactants or block copolymers can formulate the meso-structures above the critical micelle concentration, which is followed by condensation of silica precursors on the surface of the micelles, and the micelles are removed during the fabrication process [110,111]. Among several types of MSNPs, MCM-41 nanoparticles with a hexagonal structure and mesopores with a diameter of 2.0 - 6.5 nm have often been employed to encapsulate plant VOCs [112]. Jin et al. synthesized MCM-41 nanoparticles with an average size of 717 nm and a zeta potential of -43.90 mV and loaded Pepper fragrant essential oil (PFEO) into the hollow MSNPs by stirring n-hexane solution containing the volatiles and nanoparticles for 24 h. PFEO laden nanoparticles demonstrated improved antimicrobial activity

against food-borne bacteria compared to PFEO, e.g. the total inhibitory concentrations of the nanoformulation and free compounds against *Staphylococcus aureus* were 20.0 mg/mL and 22.5 mg/mL upon 48 h treatment, respectively each [113]. Solvent immersion appeared to be the best way to load volatiles into MSNPs, as the other methods such as the melting process might result in loss of VOCs during the loading process. *Thymus eriocalyx* and *Thymus kotschyanus* VOCs, for example, were loaded into MCM-41 nanoparticles by submerging the MSNPs in an acetone solution containing the cargo VOCs. The MSNPs encapsulation increased the mite mortality rates of *Thymus eriocalyx* and *Thymus kotschyanus* VOCs up to 2.5 and 3.2 times, respectively [114].

MNPs are nanoscale entities composed of pure metals such as gold, silver, titanium, zinc and platinum or their compounds, e.g. oxides, hydroxides, sulphides and chlorides [115]. MNPs can be formed from “magic clusters”, where the cluster consists of definitive number of atoms resulting in exceptional stability, but they might be easily coalesced due to the deficiency of repellent forces between metal nanoclusters unless the nanoparticles are stabilized. The stabilization of MNPs could be accomplished by applying capping agents that are able to generate an electrostatic repulsion or a steric hindrance between nanoparticles [116]. Plant VOCs have been exploited as the capping or reducing agents in the formation of MNPs and increased the innate biological activities of the MNPs. *Nigella sativa* VOCs coated gold nanoparticles were fabricated by using the VOCs as the capping agent; these were spherical and the particle size ranged between 15.6 - 28.4 nm. To do so, the volatile compounds were added to chloroauric acid ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) and heated at 100 °C under agitation, leading to the rapid reduction of Au^{3+} to Au^0 [117]. Shen et al. suggested that terpenoids including spathulenol, globulol, muurolol and cadinol can utilize their tertiary hydroxyl groups to reduce gold ions in the existence of acetone at high temperature and the oxidized terpenoids attach around the gold nanoparticles through the carboxylate ions, although further investigations are needed to elucidate the exact mechanism [118]. Gold reduction by plant VOCs originated from *Artemisia vulgaris* and *Ferula persica* has been achieved after incubation at ambient temperature for 24 h [119,120]. Green synthesis of silver nanoparticles using plant VOCs has also been reported in several studies using plant VOCs as reducing agents [121,122].

3.2. Bioavailability and biological activity of nanoencapsulated plant VOCs

3.2.1. Bioavailability of nanoencapsulated plant VOCs

- Improvement of photostability

The autoxidation of plant VOCs, which is triggered by UV or visible light, can be prevented through nanoencapsulation. Sebaaly et al. reported that eugenol laden liposomes showed a significant

photostability compared to free eugenol. After exposing samples to UV light for 10 hours, 95% of eugenol remained in lipoid S100 liposomes, whereas more than 40% of free eugenol had been degraded under the same conditions [123]. Resveratrol, an unstable VOC that can easily be converted to its *cis* form under illumination, was loaded into nanoliposomes to improve its photostability. Nanoliposomes maintained 70% of the active (trans-) resveratrol after 16 min of UV exposure whereas only 10% volatiles in the free form remained [124]. Nootkatone, a volatile sesquiterpenoid compound, was encapsulated in cyclodextrins complexes to improve its photostability. A photodegradation assay showed that free nootkatone was subject to almost complete degradation (96%) after 60 min of UV irradiation, compared to 63% after encapsulation [125]. Kumar et al. found out that cyclodextrin-based nanosponges carrying Babchi (*Psoralea corylifolia*) essential oil protected cargo compounds from photodegradation such that the photodegradation rate constant exhibited by pure and encapsulated compounds were $6.909 \times 10^{-3} \text{ min}^{-1}$ and $2.303 \times 10^{-3} \text{ min}^{-1}$ in pseudo-first order kinetic model upon UV exposure, respectively [126].

Photodegradation of *Zanthoxylum riedelianum* VOCs by UV radiation was significantly reduced after their encapsulation into poly-ε-caprolactone (PCL) nanospheres. Pereira et al. indicated that only 43% of the encapsulated VOCs were degraded upon 9 h of UV radiation exposure, while free VOCs were degraded by 76% [127]. Similarly, PCL nanospheres protected *Zanthoxylum rhoifolium* VOCs from photodegradation, so that the encapsulated volatiles showed only 44.76% photodegradation, whereas the free VOCs suffered 94.33% degradation after 7 h exposure to light [128]. ~~Gehreke et al. reported indole-3-carbinol loaded PLC nanocapsules had improved the photostability such that over 77% of encapsulated volatiles remained after 300 min exposure of UV light compared to 20% for unprotected compounds [121].~~

- Improvement of thermostability

Nanoencapsulation of plant VOCs into polymer-based nanoparticles such as chitosan, PLGA gelatin/gum arabic nanoparticles has been shown to increase thermostability. Essential oils of *Mentha piperita* and *Camellia sinensis* were loaded into Ch-NPs and the thermal stability of nanoencapsulated essential oils investigated *via* thermogravimetric analysis (TGA). Pure essential oils exhibited temperatures of maximum degradation rate (T_d) of 160 °C and 200 °C, while the nanoparticles had T_d of 350 °C, indicating an enhancement in thermostabilities of encapsulated compounds by 2.18 and 1.75 fold, respectively [129]. Upon chitosan nanoparticle encapsulation, the thermal stability of eugenol was investigated through its extrusion at 155 °C with thermoplastic flour

(TPF). TPF containing encapsulated eugenol showed an 8-fold higher remaining compound content than that containing free eugenol [130].

Almeida et al. optimized the PLGA nanoparticle fabrication process using the Box-Behnken design, so that they could increase the thermal stability of the cargo VOCs from the volatilization point of 102.4 °C to 133.0 °C [131]. Heat-resistant flavour nanocapsules carrying jasmine essential oil were also reported by Lv et al. Their results showed that the crosslinking of jasmine oil with gelatin/gum arabic nanoparticles by transglutaminase enabled the cargo essential oil to endure a water bath of 80 °C for several hours [132]. Zein nanoparticles have also been assessed with regard to increasing the thermal stability of thymol and carvacrol. TGA results indicated that the degradation of unloaded thymol and carvacrol took place at approximately 140 °C, whereas the major degradation of plant VOCs loaded into nanoparticles occurred at around 450 °C [133].

- Improvement of aqueous solubility

Poor aqueous solubility of plant VOCs has been improved through encapsulation into nanoparticles, especially lipid-based systems such as NEs and SLNs. Upon NE encapsulation, plant VOCs dissolve in hydrophobic phases stabilized by hydrophilic surfactants, increasing their solubility in the aqueous phase. Carvacrol, limonene and cinnamaldehyde were encapsulated in the sunflower oil droplets of NEs to overcome their own hydrophobic chemical nature. This resulted in an increase in the water solubility of carvacrol, cinnamaldehyde and limonene by up to 2-, 6-, and 20-fold, respectively [134].

SLNs have been used for loading frankincense and myrrh essential oils (FMO) and were effectively dispersed into the water phase, resulting in better bioavailability of FMO, and an increased *in vivo* anticancer efficacy against mouse hepatoma cells [135]. Polymer based particles have been investigated with, for example, menthone and citral encapsulation into starch nanoparticles. Menthone loaded nanoparticles, in particular, were completely dispersed into aqueous media, whereas free menthone was insoluble in water. Here the hydrophilic shell material of the nanoparticles played a role in improving the solubility of cargo VOCs [136].

3.2.2. Biological activity of nanoencapsulated plant VOCs

In addition to the role of nanoparticles in improving the stability of volatile compounds, nanoencapsulation may also improve the bioavailability and bioactivity of active compounds (see **Table 1** for summary). This may be due to enhanced cellular absorption, and/or controlled release of plant VOCs.

- Antibacterial activity

Various antibacterial, antifungal and antiviral activities of plant VOCs could be considerably increased upon nanoparticle encapsulation. Ch-NPs loaded with VOCs from *Carum copticum* showed increased antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Escherichia coli*, *Salmonella typhimurium* and *Proteus vulgaris*. ~~The loaded Ch-NPs showed a greater inhibition zone against the bacteria than free volatiles, e.g. in *Staphylococcus aureus* the average inhibition zone increased from 9.7 mm to 11.3 mm, after encapsulation [133].~~ Polycaprolactone nanocapsules loaded with *Origanum majorana* VOCs also exerted higher antibacterial activity against *Aeromonas hydrophila*, an infectious bacterium for silver catfish, than free compounds. Similar antimicrobial effects were obtained at a 50-fold lower dose when the compound was encapsulated within nanocapsules. The ability to treat the bacterial infection of catfish with very low concentrations (5 µ/L) of volatiles is more environmentally-safe and cost effective [137]. The anti-biofilm effects of free cinnamon oil and nanoliposomes carrying the oil were tested against methicillin-resistant *Staphylococcus aureus* (MRSA) by Cui et al. The logarithmic value of viable MRSA population in the biofilms treated with cinnamon oil was decreased by 1.49 times whereas those treated with cinnamon oil loaded nanoliposomes led to the reduction of MRSA cells by 2.45 times. The researchers claimed that the boosted anti-biofilm activity could be attributed to the improved stability of the plant VOCs by nanoencapsulation [138]. Rosemary essential oil loaded NLCs were reported to possess antibacterial activity against gram-positive bacteria including *Staphylococcus epidermidis* and reduce the rate of tissue bacterial colonization and wound size, leading to an acceleration in healing the infected wound [139]. The antibacterial activities of NLCs and NEs carrying citral have been tested against *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli*. Average MICs of citral loaded NLCs were significantly lower than those of citral emulsion (e.g. MIC of emulsions against *S. aureus* was 500 µg/mL, whereas that of NLCs was 125 µg/mL), suggesting that the NLCs are likely to be the better choice to enhance the antimicrobial activity of cargo compounds than NEs [140]. Bravo Cadena et al. investigated the encapsulation of cinnamaldehyde, allyl isothiocyanate and Ajwain essential oil into MSNPs to enhance their antibacterial activities. They revealed that cinnamaldehyde loaded MSNPs could increase its antibacterial activity by 10-fold compared to the free compound, and eliminate over 95% of bacterial growth of five different bacterial species including *Pseudomonas syringae* [7]. Cinnamaldehyde load MSNPs were further incorporated into pea seed coatings, which increased the number of symptomless plants against pea bacterial blight by 143% after a twenty-day cultivation compared to seed coatings without the nanoparticles [141]. Chan et al. reported that

MSNPs carrying allyl isothiocyanate could maximize antimicrobial potential of the cargo compound against *Pseudomonas aeruginosa* biofilm by decreasing the biofilm thickness from 100.25 ± 13.13 μm to 8.125 ± 1.44 μm for 24 hours at concentration of 2 mg/L. By comparison, 350 mg/L free allyl isothiocyanate only resulted in a reduction of biofilm thickness to 31.0 ± 4.42 μm under the same conditions [142].

- Antifungal activity

Ch-NP encapsulation was shown to improve the antifungal activity of plant VOCs by Hasheminejad et al. They reported that free clove essential oil (CEO) could not inhibit the growth of *Aspergillus niger* at a concentration of 3 mg/mL, yet CEO laden Ch-NPs demonstrated the complete inhibition of the fungus at a 2-fold lower concentration, i.e. 1.5 mg/mL [143]. The enhanced antifungal activity of CEO loaded Ch-NPs could be related to the fact that the nanoparticles mediate the controlled release of VOCs and possess their own antimicrobial activity as well [144]. Lipid-based nanometric systems, i.e. NEs and nanoliposomes, containing *Cymbopogon densiflorus* VOCs were investigated for improved antifungal activities against five fungi such as *Candida albicans* and *Candida parapsilosis*. Both formulations showed improved antifungal potential of up to 5 times that of free compounds and nanoliposomes carrying the plant VOCs seemed to be more effective in suppressing the growth of fungi than NEs loaded with the same volatiles. The MIC of free plant VOCs for *Candida albicans* was 0.5 mg/mL, while those of NEs and nanoliposomes were 0.2 mg/mL and 0.1 mg/mL, respectively [145].

Zataria multiflora VOC loaded SLNs were also demonstrated to improve the efficiencies of plant VOCs in controlling several fungal pathogens including *Aspergillus ochraceus* and *Aspergillus niger*. Their results demonstrated a 79% inhibition on the growth of fungal pathogens with the volatiles loaded SLNs, while free plant VOCs exhibited only a 54% inhibition [146]. The antifungal activity of SLNs loaded with *Eugenia caryophyllata* VOCs against *Candida albicans* (MIC = 0.10 $\mu\text{g/mL}$) have been proven to be much higher than that of free volatiles (MIC = 0.25 $\mu\text{g/mL}$). The encapsulation of volatile compounds into SLNs was proposed to enhance the antimicrobial activity through promoting the passive cellular absorption of plant VOCs into pathogens [147].

- Antivirus activity

The elevated antiviral activities of plant VOCs by nanoencapsulation have been confirmed through many studies. Poly (D, L-lactide-co-glycolide) nanoparticles carrying *Cymbopogon citratus* VOCs showed a strong inhibition of HSV-1 and HSV-2 at a non-cytotoxic concentration which was 42 times lower than that of free compounds. The incorporation of the volatile compounds in the

nanoparticles provided greater contact area resulting in a better interaction with viral membranes, and also sustained drug release from the formulation and protection of the compounds from volatilization [148]. Antiherpetic activity was also investigated in multilamellar (MLV) and unilamellar (SUV) nanoliposomes loaded with *Artemisia arborescens* VOCs. *In vitro* antiviral assays based on the cytopathic effect inhibition method demonstrated that free VOCs and SUVs induced approximately 20% inhibition against HSV-1 at 100 µg/mL, while MLVs encompassing VOCs caused more than 60% viral inhibition at the same concentration [149]. However, liposome incorporated plant VOCs do not always show higher activity than free compounds. Valenti et al. reported that *in vitro* antiherpetic activity of free *Santolina insularis* VOCs was worse in liposomal VOCs, i.e. free VOCs and MLVs exhibited 50% HSV-1 inhibition at 0.88 µg/mL and 4.6 µg/mL, respectively. Although the liposomal incorporation of the VOCs did not contribute to an improved antiviral activity, the vesicular inclusion substantially improved the stability of the cargo compounds, leading to efficacy after one year of storage [150].

- Anticancer activity

~~*In vitro* antitumour~~ properties of plant VOCs could also be enhanced *via* nanoencapsulation ~~by due to~~ improved stability and cellular absorption. *In vitro* experiments on human lung adenocarcinoma epithelial (A549) cells showed that ~~t~~urmeric oil and lemongrass oil loaded alginate and chitosan nanocapsules ~~were proven to~~ have higher antiproliferative activity ~~against human lung adenocarcinoma epithelial (A549) cells~~ than free compounds. The viabilities of cancer cells were decreased by 40% and 20% upon 24 h treatment of 0.4 mg/mL turmeric oil and lemongrass oil loaded nanocapsules, respectively, while the same concentration of free oils showed less than 10% inhibitory activity [151]. Trimethyl Ch-NPs carrying *Ocimum gratissimum* VOCs, and free volatile compounds, were tested for impact on cell viability and antiproliferation of breast cancer (MDA-MB-231) cells. After 48 h incubation, ~~the viability of MDA-MB-231 cells was reduced into approximately 40% in response to treatment of 50 µg/mL of volatiles laden Ch-NPs, which exhibited more cytotoxicity against cancer cells than free volatiles causing about 70% cell viability at the same concentration~~ the results revealed that 100 µg/mL of free VOCs decreased the viability of cancer cells to 44.25%, compared to encapsulated compounds which had a greater effect decreasing cell viability to only 37.44% of control [152]. It is thought that the Ch-NPs could penetrate the cancer cell membranes and cause DNA damage, leading to defects in the cellular genes- [153][164]. White et al. found that bovine serum albumin nanoparticle encapsulation could increase the cytotoxicity of citral against rhabdomyosarcoma cells. The viability of RH30 cells was decreased to approximately 40% of the control for the nanoparticle encapsulated citral, compared to an equivalent amount (500

μM) of free citral after which 70% of cells remained viable. The citral laden nanoparticles were also loaded into a biodegradable polyanhydride wafer for slow release at the tumour bed after a surgery. The wafer system was tested in vitro and exhibited 50% degradation over 25 days therefore releasing citral. This could ultimately be employed to prevent locoregional recurrence of the cancer [154]. Nanoliposomes were used to encapsulate VOCs from *Citrus bergamia* which have poor water solubility, low stability and limited bioavailability. The encapsulated compounds were tested against human neuroblastoma (SH-SY5Y) cells at 0.01% (v/v) for 72 h and showed a 40% cytotoxicity rate, compared to free volatiles, which caused the death of less than 10% of cells under the same conditions [155]. NEs carrying *Cuminum cyminum* VOCs [156] and ginger and frankincense essential oils [157] were noted to have *in vitro* antitumour activities against human tongue carcinoma (SAS) cells ($IC_{50} = 1.5 \mu\text{L/mL}$) and breast adenocarcinoma (MCF-7) cells ($IC_{50} = 20.548 \mu\text{g/mL}$), respectively. Ali et al. encapsulated *Origanum glandulosum* VOCs into sodium alginate nanocapsules and NEs and evaluated the cytotoxic effect of encapsulated volatiles and free compounds on human hepatocellular carcinoma (HepG2) and normal liver (THLE2) cells. Nanocapsules carrying the VOCs possessed higher cytotoxicity against liver cancer cells with an IC_{50} of $54.93 \mu\text{g/mL}$, compared to an IC_{50} of $73.13 \mu\text{g/mL}$ for free compounds, and $131.6 \mu\text{g/mL}$ for NEs. The low anticancer efficacy of the NEs might be correlated with their thermodynamic instability. In terms of the cytotoxicity against normal cells, the nanocapsules exhibited almost 2-fold higher IC_{50} than the others. This result indicates that *Origanum glandulosum* VOCs laden nanocapsules are able to target hepatic cancer cells without causing toxicity towards healthy liver cells [158]. Gold nanoparticles have also been investigated: *Nigella sativa* VOCs were coated onto the particles to investigate controlling human lung cancer (A549). *In vitro* antitumour assays showed that IC_{50} against A549 cells of bulk gold, free volatile compounds and gold nanoparticles coated with the VOCs were $87.20 \mu\text{g/mL}$, $64.15 \mu\text{g/mL}$ and $28.37 \mu\text{g/mL}$, respectively [117]. This leads to the hope that metal nanoparticles could be used as effective therapeutics to treat lung cancer in the future.

4. Concluding remarks

Volatile compounds derived from plants possess beneficial biological properties, including antimicrobial and anticancer activities, and do not cause the serious toxicity towards the human body or the environment. Nevertheless, their usage in therapeutics, food, and agricultural settings are being inevitably restricted by the volatile, unstable and hydrophobic attributes plant-with which VOCs are endowed. The encapsulation of these compounds in nanostructured formulations, i.e. polymer- or lipid- based nanoparticles and inorganic nanoparticles, has been shown to increase their

biological activities by improving the physicochemical properties, bioavailability, sustained release, and cellular **ab**sorption of plant derived VOCs. Commercial applications of plant derived VOCs are anticipated to achieve more wide scale in use as the field of nanotechnology develops further.

Author Contributions

Conceptualization: H.E.T.; writing-original draft preparation: H.M.; writing-review and editing: H.E.T., H.M.; supervision: H.E.T.

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Conflicts of Interest

The authors declare no conflict of interest.

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Legends for Figures

Fig. 1 Biosynthesis of plant VOCs. The biosynthesis pathways of terpenes are highlighted in blue and green, where the green background indicates the MVA pathway and the pink background one shows the MEP pathway. The blue- and yellow-highlighted boxes illustrate the phenylpropanoids/benzenoids and fatty acid derivatives biosynthesis, respectively. Acetyl-CoA, pyruvate, phosphoenolpyruvate and erythrose 4- phosphate, which are the fundamental materials for the generation of plant VOCs, are held in the middle rectangular box. Here, the stacked arrows indicate the involvement of multiple conversion reactions.

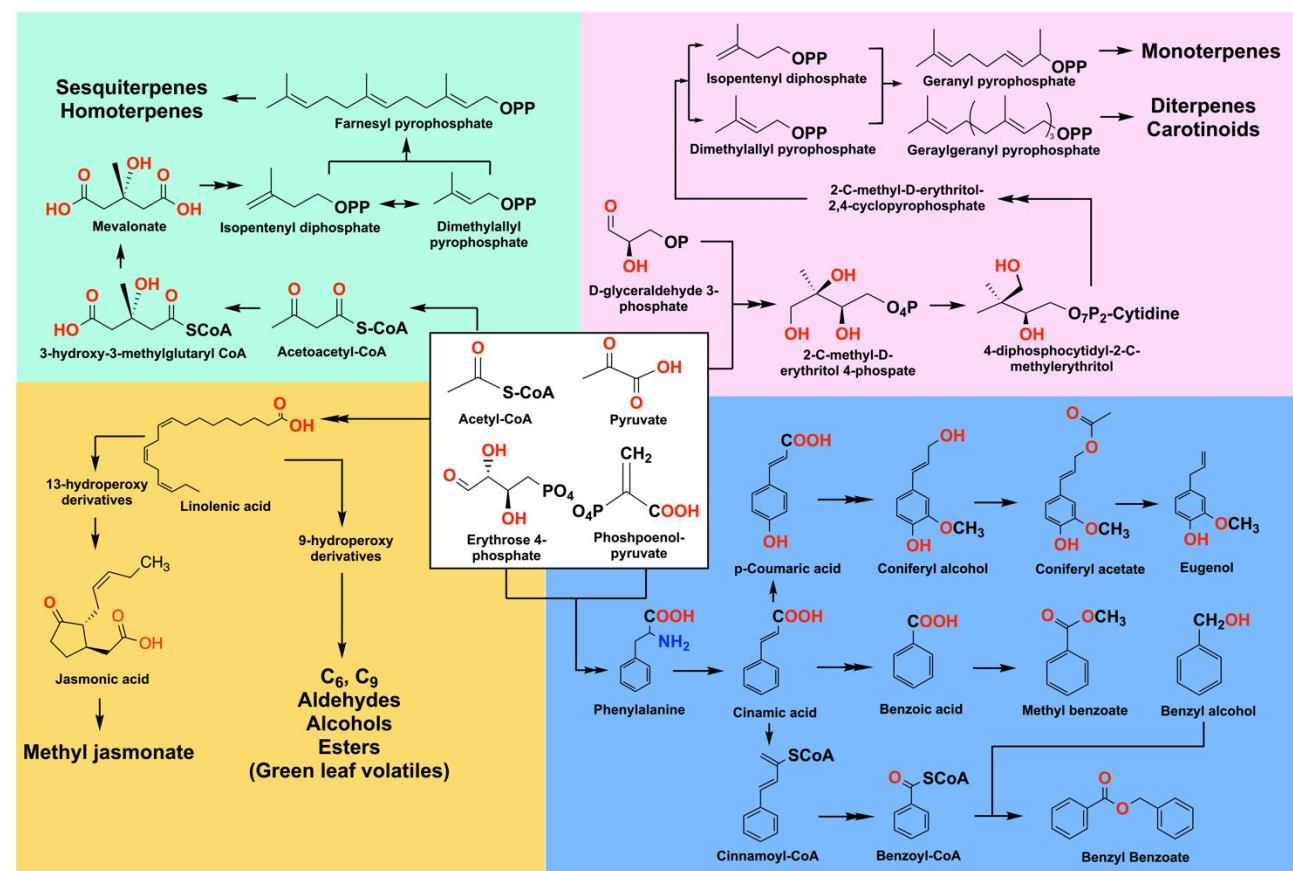
Fig. 2 Schematic illustration of action of plant VOCs on anti-apoptotic pathways in cancer cells.

Fig. 3 Structures of nanoparticle platforms carrying plant VOCs. Nanocapsule (a) and nanosphere (b) are the polymer-based nanoparticles, while nanoliposome (c), nanoemulsion (d), solid lipid nanoparticle (e) and nanostructured lipid carrier (f) are constructed from lipid to form the lipid-based nanoparticle system. The inorganic nanoparticle system includes mesoporous silica nanoparticles (g) and metal nanoparticles (h).

Fig. 4 Schematic representation of the formation of β -cyclodextrin/chitosan nanoparticles carrying plant VOCs.

Table 1 Biological activities of nanoparticles carrying plant VOCs

Bioactive compounds	Nanoparticles	Biological activities	References
Lemongrass essential oil	Poly(lactic acid) nanocapsule	Antibacterial	[159]
<i>Origanum majorana</i> VOCs	Polycaprolactone nanocapsule	Antibacterial	[131]
Carvacrol	Chitosan nanoparticle	Antibacterial	[161]
<i>Carum copticum</i> VOCs	Chitosan nanoparticle	Antibacterial	[162]
Cinnamon essential oil	Nanoliposome	Antibacterial	[138]
Tea tree essential oil	Nanoliposome	Antibacterial, antifungal	[163]
D-limonene	Nanoemulsion	Antibacterial	[164]
<i>Cuminum cyminum</i> VOCs	Nanoemulsion	Antibacterial, anticancer	[156]
Thyme essential oil	Nanoemulsion	Antibacterial	[165]
Carvacrol	Nanoemulsion	Antibacterial, antifungal	[166]
<i>Cymbopogon densiflorus</i> VOCs	Nanoliposome, nanoemulsion	Antibacterial, antifungal	[145]
<i>Eugenia caryophyllata</i> VOCs	Solid lipid nanoparticle	Antibacterial, antifungal	[147]
<i>Rosmarinus officinalis</i> VOCs	Nanostructured lipid carrier	Antibacterial	[139]
Citral	Nanostructured lipid carrier	Antibacterial, antifungal	[140]
Eucalyptus & rosemary VOCs	Solid lipid nanoparticle	Antibacterial	[167]
Cinnamaldehyde, allyl isothiocyanate, Ajwain essential oil	Mesoporous silica nanoparticle	Antibacterial	[7]
Pepper VOCs	Mesoporous silica nanoparticle	Antibacterial	[113]
<i>Coleus aromaticus</i> VOCs	Silver nanoparticle	Antibacterial	[168]
<i>Eugenia caryophyllata</i> VOCs	Chitosan nanoparticle	Antifungal	[143]
Carvacrol	Nanoemulsion	Antifungal	[169]
<i>Zataria multiflora</i> VOCs	Solid lipid nanoparticle	Antifungal	[146]
<i>Cymbopogon citratus</i> VOCs	Poly (D,L-lactide-co-glycolide) nanoparticles	Antiviral	[148]
<i>Artemisia arborescens</i> VOCs	Nanoliposome	Antiviral	[149]
<i>Santolina insularis</i> VOCs	Nanoliposome	Antiviral	[150]
<i>Curcuma longa</i> & <i>Cymbopogon citratus</i> VOCs	Chitosan-alginate nanocapsule	Anticancer	[151]
<i>Ocimum Gratissimum</i> VOCs	Chitosan nanoparticle	Anticancer	[152]
Citral	Trimethyl chitosan nanoparticle	Anticancer	[154]
<i>Citrus bergamia</i> VOCs	Bovine serum albumin nanoparticle	Anticancer	[155]
Ginger & frankincense VOCs	Nanoliposome	Anticancer	[157]
<i>Origanum glandulosum</i> VOCs	Nanoemulsion	Anticancer	[170]
<i>Mentha spicata</i> VOCs	Sodium alginate nanocapsule, nanoemulsion	Anticancer	[171]
Citral	Nanoemulsion	Anticancer	[172]
<i>Nigella sativa</i> VOCs	Solid lipid nanoparticle	Anticancer	[172]
	Gold nanoparticle	Anticancer, antibacterial	[117]

Fig. 1

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Fig. 2

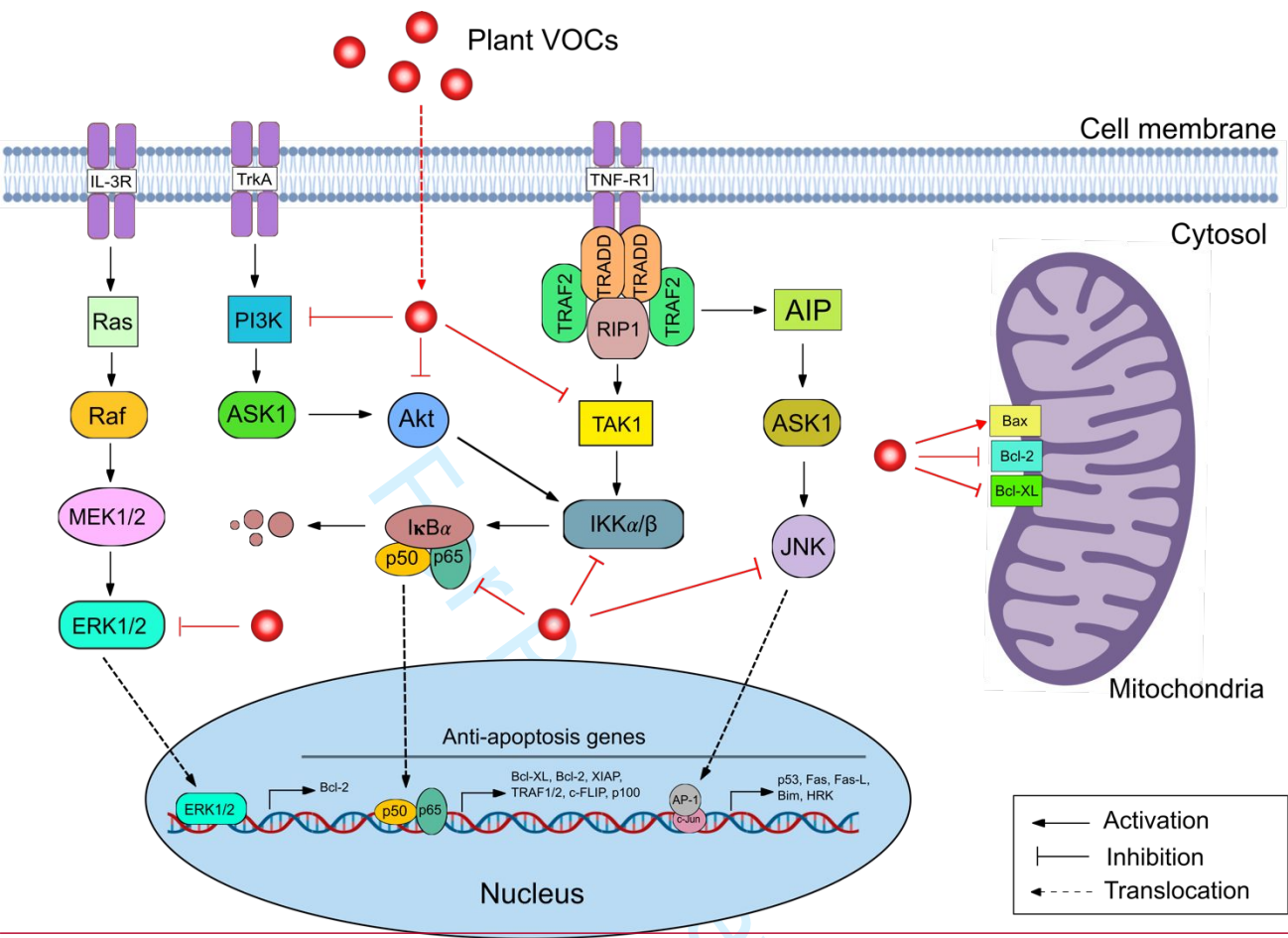


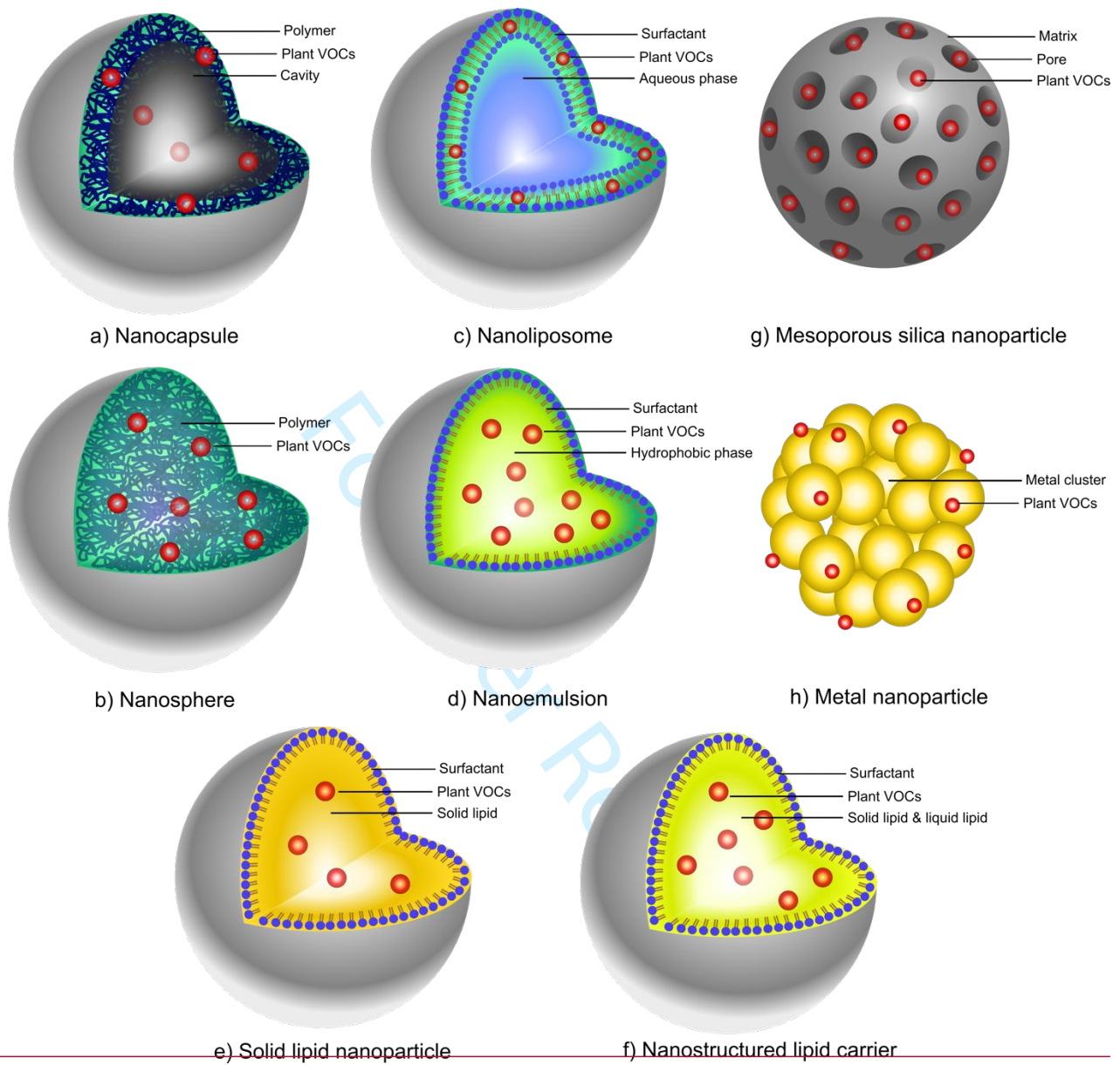
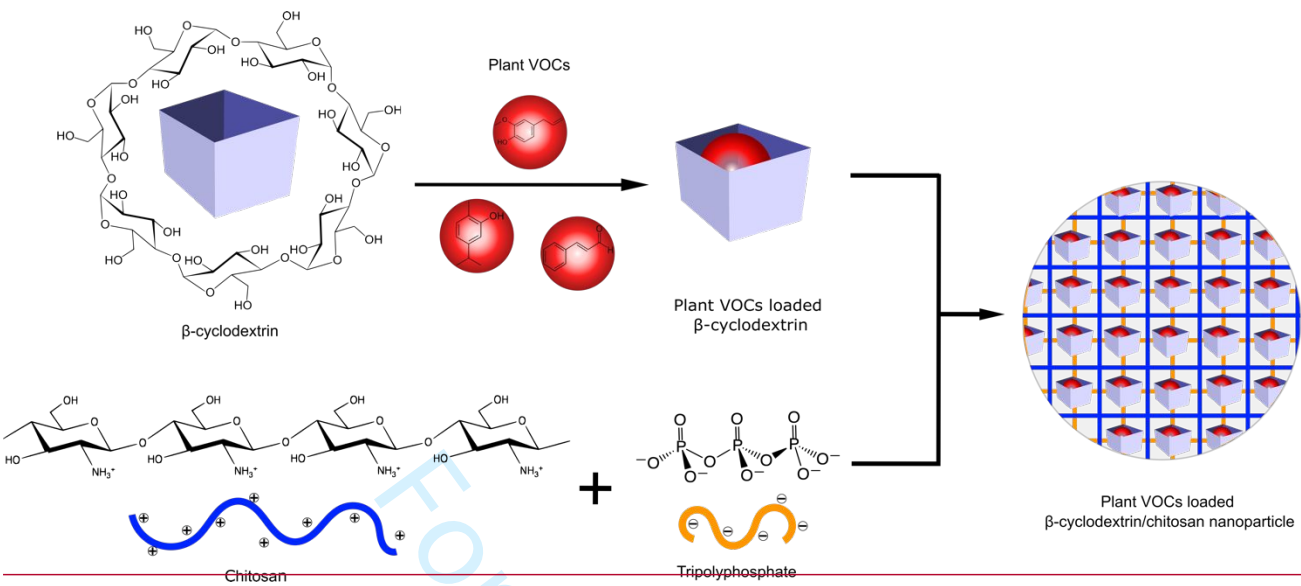
Fig. 3

Fig. 4



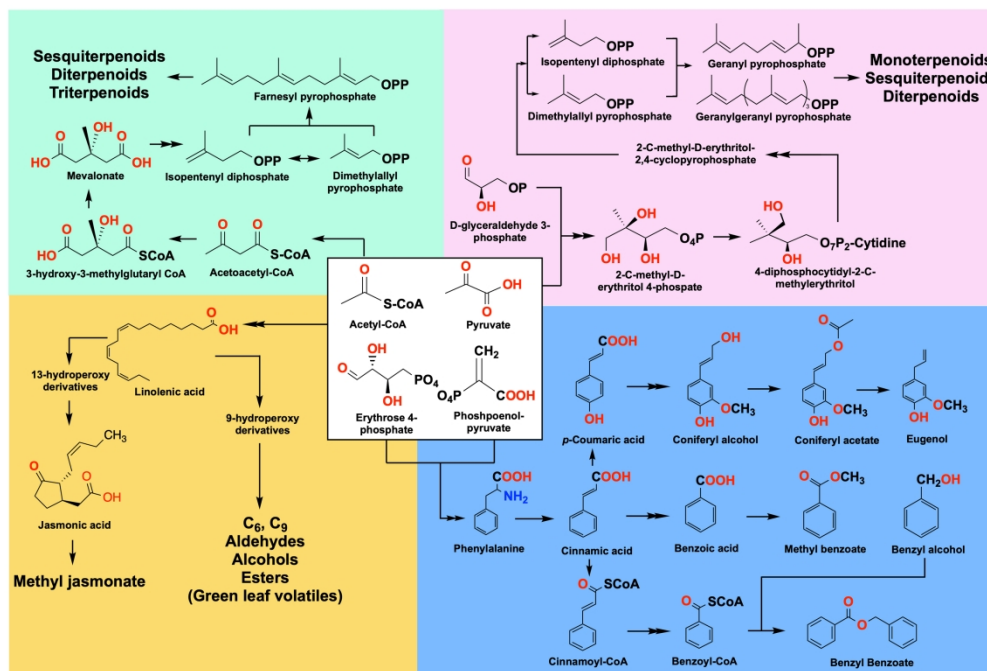


Figure 1

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Figure 2

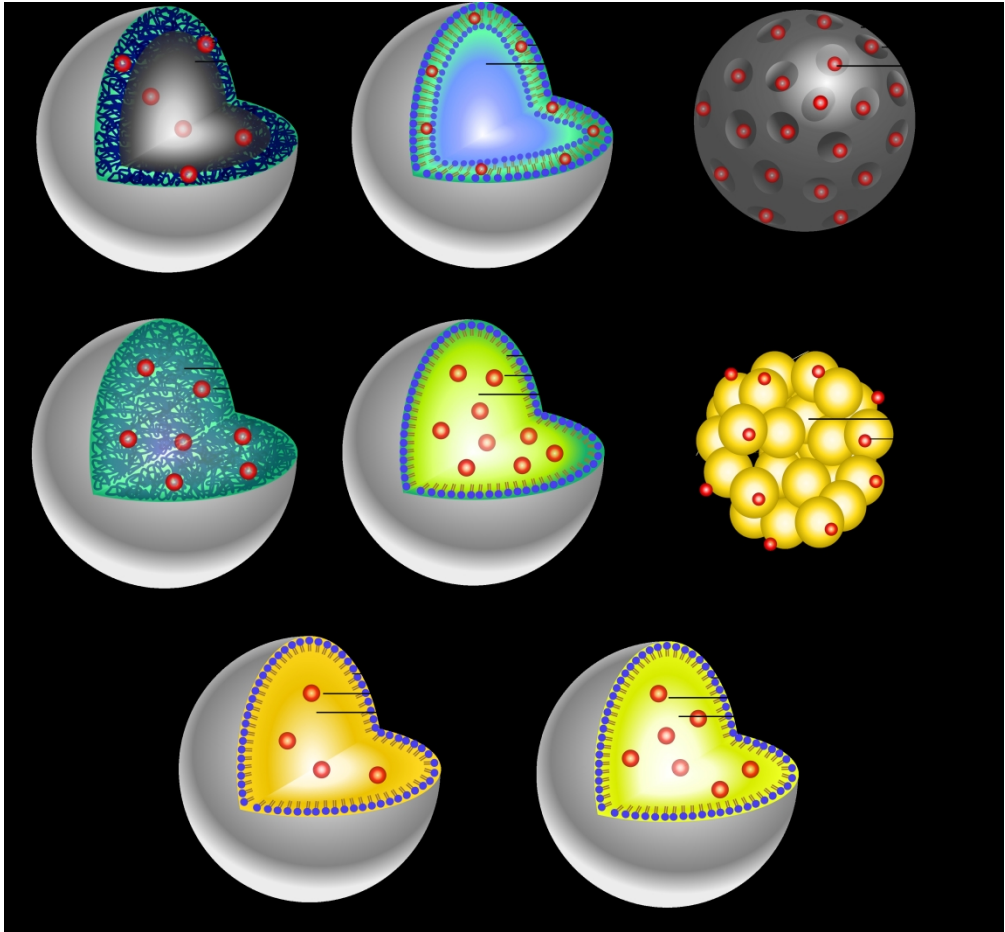


Figure 3

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Figure 4