

PAPER • OPEN ACCESS

## Encapsulation of *C. odorata* extracts for antimicrobial activity

To cite this article: Siti Nur Amylina Azmi *et al* 2019 *J. Phys.: Conf. Ser.* **1372** 012046

View the [article online](#) for updates and enhancements.

### Recent citations

- [Growth inhibition of foodborne pathogens by comicroencapsulation of lactobacilli cell free and propolis extracts](#)  
Aykut Burgut



**IOP | ebooks™**

Bringing together innovative digital publishing with leading authors from the global scientific community.

Start exploring the collection—download the first chapter of every title for free.

## Encapsulation of *C. odorata* extracts for antimicrobial activity

Siti Nur Amylina Azmi<sup>1</sup>, Che Wan Sharifah Robiah Mohamad<sup>1\*</sup>, Khairul Farihan Kasim<sup>2</sup>

<sup>1</sup>Biomedical Electronic Engineering Program, School of Mechatronic Engineering, Universiti Malaysia Perlis, 02600 Arau, Perlis

<sup>2</sup>School of Bioprocess Engineering, Universiti Malaysia Perlis, 01000 Jejawi, Perlis

\*robiah@unimap.edu.my

**Abstract.** The purpose of this study is to investigate the relationship between the methylcellulose (MC) and ethylcellulose (EC) of encapsulated *C. odorata* extracts (methanol and ethanol) and their antimicrobial properties against *E. coli* and *Staphylococcus aureus*. The encapsulated of *C. odorata* extract was prepared by solvent displacement method through dialysis tube cellulose membrane and it was freeze-dried to obtain the suspension of encapsulated *C. odorata* extract. Then, antimicrobial properties of encapsulated *C. odorata* extracts was performed by broth dilution method on target bacteria. A few drops of hydrochloric acid (HCl) were added into growth media until pH 4 to mimic the acidic conditions in human stomach. The results showed the encapsulated methylcellulose of methanol extract (MC-M) inhibit both bacteria types.

### 1. Introduction

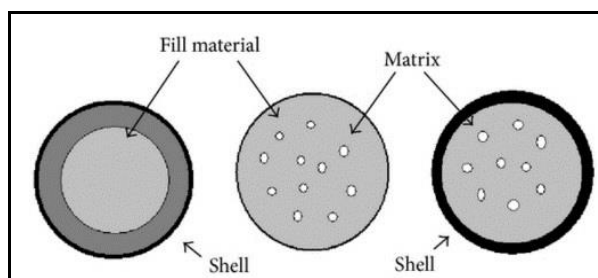
Nowadays, natural antimicrobial has become very important in global health because of their potential as antibiotics for many diseases [1]. Many types of antimicrobials have been created in order to destroy and inhibit the growth of bacteria since last half of the previous century. But now it is become more complicated because of resistance of bacteria for certain vaccination or antibiotic are developing increasingly. A study shown the nano-capsule represent a target of new compositions for inhibiting the growth of bacteria in medical field [2].

*Chromolaena odorata* (*C. odorata*) belongs to the family Asteraceae and is known locally in Nigeria through many common names as siam weed, Elizabeth weed, obirakara, olorohuru and independence weed [3]. The anti-microbial properties have made it a popular choice in disinfecting and treating open wounds [4]. The wider use as an effective therapy against diarrhoea, malaria fever, tooth ache, diabetes, skin diseases, dysentery and colitis has been severally documented [4-5]. The leaves could be ground and the extracted juice taken to alleviate fever or the treatment of diabetes [6]. This study is aimed at providing preliminary studies on *C. odorata* leaves and its potential as antimicrobial activity to combat infectious disease.

Cellulose have been famous in the food and pharmaceutical industries as a coating material due to a few characteristics that allows the increase of active ingredients ability and biodegradability. Other than that, cellulose does not react with the material to be encapsulated. Then, cellulose also help to seal and hold the active material within its structure during processing or storage. It also provided maximal protection of the active against environmental conditions. The encapsulation membrane/shell (wall material) around active compound/phytochemical were functioning as a barrier or main protective role [7]. Figure1 shows the schematic representative of matrix encapsulation system.



However, there are lack of information about the potential encapsulated of *C. odorata* as antimicrobial activity since its behaviour has not yet been scientifically investigated. This study was to examined the capability of encapsulated of *C. odorata* extract by used cellulose for microbial activity using broth dilution method.



**Figure 1:** Schematic representative of matrix encapsulation [7].

## 2. Methodology

### 2.1. Materials

*C. odorata* leave were collected from Changlun, Kedah. Fresh *C. odorata* leave was washed and dried at 40°C for 24 hours. After dried, the leaves are grinded into powder using mechanical blender.

### 2.2. *C. odorata* extraction

The Soxhlet extractor apparatus have been used to extract *C. odorata* leave. 30 g of fine *C. odorata* sample added into 250 ml of ethanol was used for minimum 6 hours to ensure optimum extraction of bioactive compound in the leave. The solvent then evaporated using EYELA rotary evaporator for 1 hour and the ethanol crude extracts obtained were kept in 4°C until further use. The extraction process was repeated using methanol as solvent

### 2.3. Encapsulation of *C. odorata* extract

Encapsulation of *C. odorata* extract using EC and MC were prepared by solvent displacement method through dialysis tube cellulose membrane. 0.1 g of *C. odorata* leave extract and 0.1 g cellulose (EC or MC) at ratio of 1:1 (w/w) was co-dissolved in 25 ml of solvent (ethanol or methanol) and 2.5 ml tween 20 (a polysorbate-type non-ionic surfactant). The solution was transferred into dialysis tube cellulose membrane and dialyzed against 250 ml distilled water. After 2 hours dialyzes process, 50 mL encapsulated sample was transferred into centrifuge tube follow with 4000 rpm for 30 minutes centrifugation. The obtained aqueous suspension was freeze dry and stored in -20°C.

### 2.4. Broth dilution methods

Typical Soy Broth (TSB) is used as a medium in broth dilution methods. Nine grams of TSB is dissolved in 300 ml distilled water until completely dissolve. The TSB medium was sterilized by autoclaving for 15 minutes at 121°C. The inoculums bacteria were prepared by used 0.5 McFarland bacterial. Overnight culture bacteria were transferred into 5 ml sterile distilled water and vortex for 30 seconds to dissolve the bacteria. Then, the turbidity of bacterial suspension was adjusted according to 0.5 McFarland or  $(1-2) \times 10^8$  CFU/ml. After that, 10 mL of  $2 \times 10^8$  CFU/ml microbial suspension was transferred into 30 ml of TSB. Then, 9 mL of bacterial broth was transferred into 1 mL of *C. odorata* leave extract. The set of test tubes containing a mixture of bacteria and the extracts were incubated at 37 °C for 24 hours. After 24 hours, both bacteria and extract solution were measured at 600 nm respectively.

### 2.5. Antioxidant activity

The antioxidant activity was determined using DPPH method. 2.0 ml of extract was mixed with 2.5 ml of 0.1 mM of DPPH solution. The mixture was incubated in the dark for 40 min. after that, the solution was measured using spectrophotometer at 517 nm. The percentage inhibition was calculated using equation below:

$$DPPH \text{ activity}(\%) = \left[ 1 - \frac{\text{absorbance of sample}}{\text{absorbance of DPPH}} \right] \times 100$$

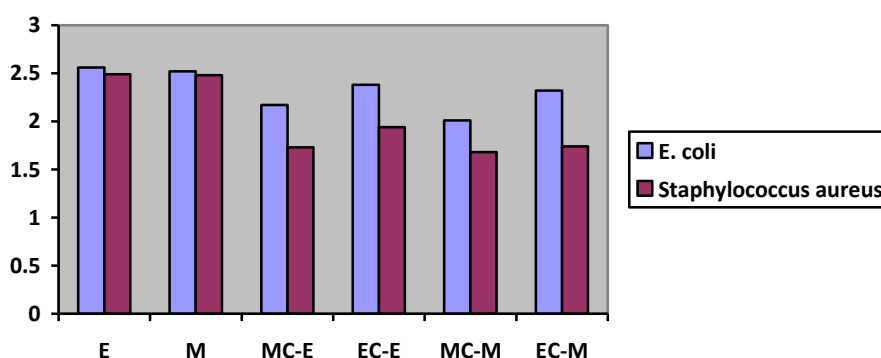
## 3. Result and Discussion

### 3.1. Antimicrobial activity of *C. odorata* leave extract

4 mg/ml of encapsulation sample with is methanol-methylcellulose (MC-M), methanol-ethylcellulose (EC-M), ethanol-methylcellulose (MC-E) and ethanol-ethylcellulose (EC-E) respectively.

**Table 1:** Antimicrobial activity of the *C. odorata* extract

Sample (4.0 mg/ml)	Concentration of bacteria (CFU/ml)	
	<i>E. coli</i> (G <sup>-</sup> )	<i>Staphy</i> (G <sup>+</sup> )
Crude <i>C. odorata</i> _ethanol (E)	2.56 X 10 <sup>8</sup>	2.49 X 10 <sup>8</sup>
Crude <i>C. odorata</i> _methanol (M)	2.52 X 10 <sup>8</sup>	2.48 X 10 <sup>8</sup>
MC-E	2.17 X 10 <sup>8</sup>	1.73 X 10 <sup>8</sup>
EC-E	2.38 X 10 <sup>8</sup>	1.94 X 10 <sup>8</sup>
MC-M	2.01 X 10 <sup>8</sup>	1.68 X 10 <sup>8</sup>
EC-M	2.32 X 10 <sup>8</sup>	1.74 X 10 <sup>8</sup>



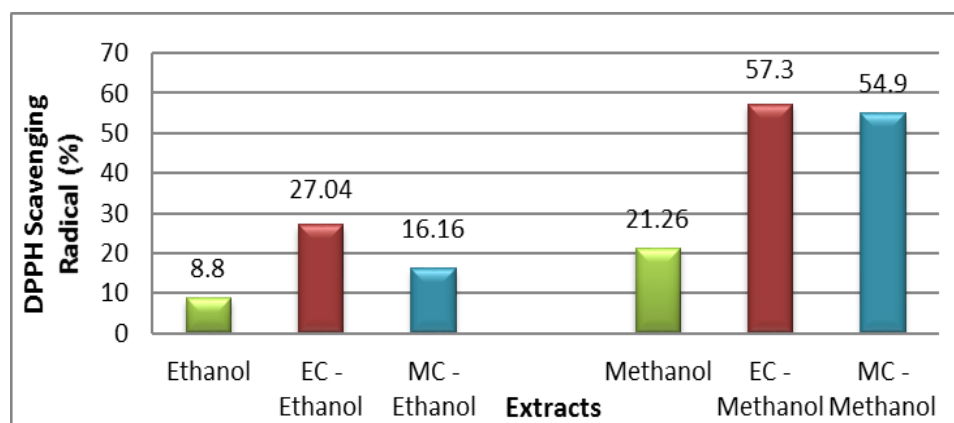
**Figure 2:** Antimicrobial value for *E. coli* and *Staphylococcus aureus*.

Table 1 and Figure 2 was shown that MC-M more effective in antimicrobial activity towards to *E. coli* (gram-negative) and *staphylococcus aureus* (gram-positive) with the inhibition of bacteria growth  $2.01 \times 10^8$  and  $1.68 \times 10^8$ . In addition, for EC-E showed least antimicrobial activity against *E. coli* ( $2.38 \times 10^8$ ) and *staphylococcus aureus* ( $1.94 \times 10^8$ ).

### 3.2. Antioxidant (AOA)

The free radical scavenging activity of the extracts was evaluated based on the ability to scavenge the DPPH. This assay is highly important to provide information about the reactivity of organic compounds with stable free radicals, because of the odd number of electrons.

The result of the study reveals that the extract of the leaves of *C. odorata* scavenged the free radicals by DPPH method. EC-methanol it shows a strong absorption band at 517 nm in visible spectrum (deep violet color). Figure 3 shows the bleaching of DPPH absorption indicates the capacity of the test drugs to scavenge the free radicals. Where, the ethanol (8.8%), EC-ethanol (27.04%), MC-ethanol (16.16%), methanol (21.26%) and MC-methanol (54.9%) have lowest antioxidant activity. Further studies were carried out to find out the correlation between *C. odorata* extract and encapsulated *C. odorata* extract with EC and MC in antioxidant and antimicrobial activity. It shows, methanol has interaction with antioxidant which it contributed to antimicrobial properties [8,10-12]. In another studies, *C. odorata* extract had a positive correlation between the antioxidant activity and total phenolic content. It could be capable in antimicrobial and anti-inflammatory activity of infectious disease [9, 13-14].



**Figure 3:** Percentage of DPPH scavenging radical

### 4. Conclusion

As a conclusion, *C. odorata* leave methanol extracts was shown high activity against antibacterial activity compared *C. odorata* leave ethanol extracts. Ethanol and methanol solvent were used to *C. odorata* leave extract then followed with encapsulated of MC and EC by solvent displacement method through dialysis tube cellulose membrane. The antimicrobial activity of both encapsulated extracts was tested using broth dilution method at concentration  $2 \times 10^7$  CFU/mL with slightly modification by adding a few drops of HCl into growth media. This modification was representing the acidic environment in human stomach at pH 4. After 24 hours of incubation, MC-M showed the positive result against *E. coli* and *Staphylococcus aureus* with the highest inhibition towards bacteria *S. aureus* as the concentration of bacteria observed is the lowest compared *E. coli* bacteria. Meanwhile, both crude *C. odorata* extract showed the lowest inhibition efficiency by showing the highest reading of target bacteria concentration compared to encapsulated sample.

### Acknowledgments

This work was supported by School of Mechatronic and School of Bioprocess, Universiti Malaysia Perlis, Malaysia.

### References

- [1] Manaia C M 2013 A brief multi-disciplinary review on antimicrobial resistance in medicine and its linkage to the global environmental microbiota *Front. Microbiol.* **4** 1–14
- [2] Bates B L, Hiles M C, Johnson C E 2013 Biofilm-Inhibiting Medical Product US Patent 8, 343, 536

- [3] Stanley M C, Ifeanyi O E, Nwakaego C C and Esther I O 2014 Antimicrobial effects of *Chromolaena odorata* on some human pathogens *International Journal of Current Microbiology and Applied Sciences*. vol.3, no.3 1006-1012
- [4] Omokhua A G, McGaw L J, Finnie J F and Van Staden J 2016 *Chromolaena odorata* (L.) RM King & H. Rob. (Asteraceae) in sub-Saharan Africa: A synthesis and review of its medicinal potential *Journal of ethnopharmacology* vol.183112-122
- [5] Nath L R, Gorantla J N, Joseph S M, Antony J, Thankachan S, Menon D B and Anto R J 2015 Kaempferide, the most active among the four flavonoids isolated and characterized from *Chromolaena odorata* induces apoptosis in cervical cancer cells while being pharmacologically safe *RSC Advances*. vol.5, no.122 100912-100922
- [6] Atindehou M, Lagnika L, Guérolde B, Strub J M, Zhao M., Van Dorsselaer and Sanni A 2013 Isolation and identification of two antibacterial agents from *Chromolaena odorata* L. active against four diarrheal strains *Advances in Microbiology*. vol.3, no.01, 115
- [7] Lauryna P, Mindaugas M, Valdas J, Liudas I, Dalia M K and Jurga B 2019 Microencapsulation of *Elsholtzia ciliata* Herb Ethanolic Extract by Spray-Drying: Impact of Resistant-Maltodextrin complemented with Sodium Caseinate, Skim Milk, and Beta-Cyclodextrin on the Quality of Spray-Dried Powders *Molecules*. vol 24, 1461-1483
- [8] Thophon S H S, Waranusantigul P, Kangwanransan N and Krajangsang S 2016 Antimicrobial activity of *Chromolaena odorata* extracts against bacterial human skin infections *Modern Applied Science*, vol.10 no. 2
- [9] Vinceković M, Viskić M, Jurić S, Giacometti J, Kovačević D B, Putnik and Jambrak A R 2017 Innovative technologies for encapsulation of Mediterranean plants extracts *Trends in Food Science & Technology*. vol.69, 1-12
- [10] Usunomena U and Efosa E G 2015 Phytochemical Analysis, Mineral Composition and In Vitro Antioxidant Activities of *Chromolaena Odorata* Leaves *Journal of Pharmaceutical Sciences*. vol.2, no.2, 16-20
- [11] Goy R C, Morais S T and Assis O B 2016 Evaluation of the antimicrobial activity of chitosan and its quaternized derivative on *E. coli* and *S. aureus* growth *Revista Brasileira de Farmacognosia*. vol.26, no.1, 122-127
- [12] Khan I, Miskeen S, Khalil A T, Phull A R, Kim J and Oh D H 2016 Foodborne Pathogens: *Staphylococcus aureus* and *Listeria monocytogenes* An Unsolved Problem of the Food Industry *Pakistan J. Nutr.* vol. 15, no. 6, 505–514
- [13] Sadgrove N, Greatrex B and Jones G L 2015  $\alpha$  - Cyclodextrin Encapsulation Enhances Antimicrobial Activity of Cineole Rich Essential Oils From Australian *Species of Prostanthera* ( *Lamiaceae* ). vol.2, no.2, 30–38
- [14] Trivedi M K, Branton A, Trivedi D, Nayak G, Mishra R and Jana S 2015 Characterization of Physicochemical and Thermal Properties of Biofield Treated Ethyl Cellulose and Methyl Cellulose *International Journal of Biomedical Materials Research*. vol.3, no.6, 83-91