Chapter 3

Stabilization of the antioxidant properties of *C. terminalis* and *M. fragrans* extracts by encapsulation

3.1. LITERATURE SURVEY OF ENCAPSULATION

Polyphenols from the plant kingdom represent a wide range of molecules (Robarts and Antolovich, 1997). The basic structure is composed of a benzene ring linked to one or more hydroxyl groups, free or attached with another chemical functional group (e.g. dimethyl ether, ester, sugar). Nowadays, consumer’s interest is focused on healthy and minimally processed foods. However, replacing synthetic antioxidants with natural products is not simple due to their lack of stability. Thus, protecting active compounds against adverse conditions becomes necessary.

Crude extracts of fruits, herbs, vegetables, cereals and other plant materials rich in phenolic compounds have attracted great interest in the food industry especially for their protection against lipid oxidation or degradation and thereby improving the quality and nutritional value of food.

Encapsulation has been defined as the technology of packaging solid, liquid and gaseous materials in small capsules that release their content at a controlled rate over prolonged periods of time (Champagne & Fustier, 2007). Encapsulation is a technique where a bioactive compound is entrapped into a polymer, thereby protecting it from oxygen, water or other conditions to improve its stability.

Microencapsulation is a process in which unstable compounds like polyphenols are entrapped in a protective polymer. Core materials can thereby be protected from degradation or oxidation in order to stabilize the molecule for a longer period of time and improve the range of applications. Milk proteins, such as casein, were mainly studied, owing to their excellent nutritional value and their numerous functional properties such as
barrier to oxygen transfer (Dalgleish, 1989) and their ability to form a complex due to intermolecular hydrogen, electrostatic and hydrophobic bonds as well as for their cross-linking ability via disulphide bonds or bityrosoine formation (Mchung & Krochata, 1994). Edible beads based on casein offer potential solutions to stabilize the molecules, by serving as a barrier to oxygen transfer in food system (Dehkharghanian, 2009, Taylor & Richardson, 1980). Casein is often used as soluble sodium caseinate (Na-caseinate) or as calcium caseinate (Ca-caseinate).

It has been reported that most of the polyphenolic compounds are highly unstable and rapidly transformed into various reactive products. They are generally prone to degradation on storage (Chandrasekhar et al., 2011). The objective of this study was to encapsulate the polyphenols from these C. terminalis and M. fragrans extracts into casein beads, with an aim to achieve the sustained release and improved stability over a period of 12 months of storage. To evaluate its stability of polyphenols and antioxidant activity both in the encapsulated and unencapsulated extracts of C. terminalis polyphenol extract (CTPE) and M. fragrans polyphenol extract (MFPE) were studied.

We also made an attempt to find out the effects of temperature and time on the release of polyphenols from casein encapsulated methanolic extracts of C. terminalis and M. fragrans.

3.1.1. CASEIN BEADS

Casein is a milk protein which is a biocompatible polymer, edible and easily degradable without any side effects (Lee et al., 2000). Casein is organized in micelles form and is designed by nature to stabilize, transport and concentrate mainly calcium and protein for neonates (De Kruif, and Holt, 2003). Stabilization of polyphenols could also be improved by casein encapsulation.

Caseins are often described as rheomorphic (from the greek rheos, meaning stream and morphe, form proteins which means that they have relatively little secondary
or tertiary structure under physiological conditions. All casein proteins are extremely flexible and essentially unfolded. They have an amphiphilic character arising from a separation between distinct hydrophobic and hydrophilic regions along the polypeptide chain. Due to its characteristics, the different caseins exhibit a strong tendency to associate through hydrophobic and electrostatic interactions (Dickinson, 2006). Emulsions consist of droplets of one liquid dispersed in another one, which is termed the continuous phase (Robins et al., 2002). The dispersed phase of oil in water emulsions is in general less dense than the continuous phase, causing the droplets to rise (cream) in a gravitational field. The larger the droplets the faster the creaming rate. To prevent phase separation, a stabilizer, which will be located at the interface of the droplets, is needed. Due to its amphiphilic character, sodium caseinate is a good stabilizer for oil in water emulsions. The caseins adsorb at the oil-water interface and stabilize the emulsion droplets through a combination of steric and electrostatic interactions. For this ability sodium caseinate finds a wide use in the food industry.

3.2. MATERIALS

The encapsulating agent used is 2% (w/v) sodium caseinate. 2, 2-Diphenyl-1-picyrl-hydrazyl (DPPH), gallic acid, ascorbic acid, and xanthane were purchased from Sigma-Aldrich (St. Louis, MO, USA). Folin–Ciocalteu reagent (FC), sodium carbonate, sodium phosphate, glycerol and trichloroacetic acid (TCA) reagents were obtained from Qualigens (Mumbai). All other chemicals used were of high quality analytical grade.

3.3. METHODS

3.3.1. ENCAPSULATION

There are several methods for the encapsulation in the food industry such as spray drying, spray chilling, coacervation, fluidized bed coating, extrusion and liposome entrapment (Risch, 1995). Because enzymes are very sensitive and easily denatured, freeze-drying is one of the best methods for preservation of biomolecules and should be
employed for their encapsulation (Yasui & Hashimoto, 1966). However, during freeze
drying some proteins in these enzymes could be denatured due to the crystallization of
the solvent water that loosens the hydrophobic bonds of protein resulting from broken
intra molecular hydrogen bonds and distribution of the protein shell (Hanafusa, 1969).
Thus, some stabilizers should be employed to decrease deactivation and destabilization of
freeze-dried enzyme (Arakawa., 2001).

3.3.1.1. Preparation of Casein beads encapsulated with *C. terminalis* and *M. fragrans*
extracts separately

The plant extracts were prepared as described in the section 2.3.2. Encapsulation
of the extracts in casein beads was prepared according to the modified method of
Dehkharghanian *et al.*, (2009). A solution of caseinate beads was prepared by mixing
sodium caseinate, glycerol, xanthane and distilled water with and without extract as
control. Total weight of the solution was 50 g. The solution was stirred for 1 h and
centrifuged at 1098 g for 10 min at 20°C. After the solution was homogenized, the beads
were obtained by dropping the mixture from a burette into 1L of 2N hydrochloric acid.
They were then kept in for 15 min and the beads were filtered through gauze cloth and
washed with distilled water and dried at room temperature in the dark. The flow chart of
bead preparation is shown in figure 3.1. The total weight and diameter of the beads were
measured. The beads were stored at room temperature.
FIGURE 3.1. The flow chart of Caseinate bead preparation of *C. terminalis* and *M. fragrans* extracts

Sodium caseinate, glycerol, xanthane and distilled water (50g)  
Stirred for 1 h and centrifuged at 1098 g for 10 min at 20°C.

Caseinate beads without extract

Caseinate beads with *C. terminalis* extract

Caseinate beads with *M. fragrans* extract

Magnetic agitator

2N HCl

Dried one week in shade at room temperature
3.3.2 BEADS DESTABILIZATION AND ACTIVE COMPONENT RELEASE

Beads destabilization was done according to the modified method of Dehkharghanian et al., 50 mg of beads were taken in 1ml of distilled water and boiled at 60°C for 7hr, added 20% of TCA (trichloro acetic acid) and grounded, filtered and centrifuged at 2000 rpm. This solution was used for estimation of polyphenols.

3.3.3. STORAGE STABILITY TEST

The storage stability test was carried out every one month for a period of 12 months to check the stability of polyphenols and antioxidant activites of both the plant extracts.

3.3.4. STABILITY OF POLYPHENOL CONTENT IN UNENCAPSULATED AND ENCAPSULATED METHANOLIC EXTRACT OF C. TERMINALIS AND M. FRAGRANS FOR A PERIOD OF 12 MONTHS.

All phenolic compounds are highly unstable and rapidly transformed into various reactive products. They are generally prone to degradation on storage. Thus we encapsulated polyphenols into caseine beads.

Encapsulation is a technique where a bioactive compound is entrapped into a polymer, thereby protecting it from oxygen, water or other conditions to improve its stability. The encapsulation process not only gives stability to the polyphenols, but also masks their bad taste (Chandrasekhar et al., 2011). To measure the content of total phenols in all the encapsulated samples singleton, Orthofer and Lamuela–Raventos, (1999) method was followed.
3.3.4.1. By Folin Ciocalteu’s Method

Total phenol content of the extracts was determined colorimetrically at 725 nm using the Folin-Ciocalteu’s reagent according to the modification of the Slinkard et al., (1977) method. The methanolic extracts of both the plants (0.1ml-0.3ml) were mixed with 0.1ml of Folin–Ciocalteu’s reagent followed by addition of 0.25ml of sodium carbonate (25%) and made up to 1ml using water. After 1 hour, the absorbance of the sample was measured at 725 nm against a blank using a double-beam ultraviolet-visible spectrophotometer Hitachi U-1100 (Hitachi, Ltd., Tokyo, Japan). Gallic acid was used as standard for preparing the calibration curve. The phenolic content was expressed as mg equivalence of Gallic acid per gram of the extract. The experiment was done in triplicate.

3.3.5. ANTIOXIDANT ACTIVITY OF UNENCAPSULATED AND ENCAPSULATED METHANOLIC EXTRACTS OF C. TERMINALIS AND M. FRAGRANS FOR A PERIOD OF 12 MONTHS

Polyphenols are susceptible to degradation on storage. All polyphenols possess antioxidant activity. When the polyphenol content decreases, the antioxidant activity also decreases. Thus we studied the antioxidant activity in the encapsulated casein beads and compared with the unencapsulated methanolic extracts of C. terminalis and M. fragrans for a period of 12 months.

Total antioxidant by phosphomolybedenum method and free radical scavenging capacity is a direct method for the measurement of the antioxidant property. Free radical scavenging capacities of the caseinate beads with or without C. terminalis and M. fragrans and unencapsulated C. terminalis and M. fragrans were evaluated by DPPH radical scavenging method.
3.3.5.1. By Phosphomolybdenum Method

The antioxidant activity of the extracts was evaluated by phosphomolybdenum method according to the procedure of Prieto, Pineda, and Aguilar (1999). The assay is based on the reduction of Mo (VI)-Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH. Absorbance was measured at 695nm. The antioxidant activity was expressed as the number of equivalents of ascorbic acid. Results are given in table. 2.12.

3.3.5.2. 2, 2-diphenyl-1-picryl-hydrazyl radical (DPPH) free Radical Scavenging Method

The antioxidant activity of the extracts was evaluated by using the stable 2, 2-diphenyl-1-picryl-hydrazyl radical (DPPH) according to the method of Bandoniene et al. (2002) with slight modification. The methanolic extracts of both the plants (0.1ml) was added to 0.5ml of DPPH of $2 \times 10^{-4}$M solution and made up to 1ml using methanol and the absorbance was read at 515 nm against methanol using a double-beam ultraviolet-visible spectrophotometer Hitachi U-1100 (Hitachi, Ltd., Tokyo, Japan). Simultaneously, the absorbance at 515 nm of the blank sample (0.1 ml methanol + 0.5 ml methanolic solution of DPPH) was measured against methanol.

The radical scavenging activities of the tested samples, expressed as percentage inhibition of DPPH, were calculated according to the following formula proposed by Yen et al. (1994).

\[
\% \text{ Inhibition} = 100 \times \left( \frac{A - A_0}{A_0} \right)
\]

Where $A_0$ is the absorbance at 515nm of the blank sample at time $t = 0$ min and $A$ is the final absorbance of the test sample at 515 nm. The extract concentration providing 50% inhibition ($EC_{50}$) was calculated from the graph of scavenging effect percentage against extract concentration. Results are given in table. 2.12.
3.3.6. EFFECTS OF TEMPERATURE AND TIME ON THE RELEASE OF POLYPHENOLS FROM CASEIN ENCAPSULATED CORDYLINE TERMINALIS METHANOLIC EXTRACT.

The experiment was carried out to standardize the temperature and time limit for the release of polyphenol content from the encapsulated beads of CTPE and MFPE. Beads weighing 50 mg were taken in 1 ml of water and heated at various temperatures (30°C, 40°C, 50°C and 60°C) and at different time intervals (1 h, 4 h and 7 h). The release of polyphenol content was estimated in each case by Folin-Ciocataeu’s method.

3.4. RESULTS AND DISCUSSION

For stability of polyphenols, encapsulation was done with sodium caseinate in a protective polymer such as casein. Casein, the milk protein possess excellent nutritional value and numerous functional properties such as barrier to oxygen transfer, stability to form a complex due to hydrophobic bonds as well as for the cross-linking ability via disulphide bonds or by tyrosine formation. In our work we achieved an encapsulation of 68.1 mg/g for C. terminalis extract CTE and 57.1 mg/g of casein for M. fragrans extract MFE. The average sizes of the beads of C. terminalis and M. fragrans extracts were 2.1±0.3 and 2.33±0.1 mm respectively. The beads without extracts the size was 1.8±01mm shown in (table 3.1).

Table 3.1. Diameter of casein beads in encapsulated and unencapsulated beads of C. terminalis and M. fragrans

<table>
<thead>
<tr>
<th>S. No</th>
<th>Diameter of beads</th>
<th>Total weight of the beads</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. terminalis extract</td>
<td>2.1± 0.3mm</td>
<td>7.021g</td>
</tr>
<tr>
<td>M. fragrans extract</td>
<td>2.33±0.1mm</td>
<td>6.768g</td>
</tr>
<tr>
<td>Beads without extract</td>
<td>1.8±0.18 mm</td>
<td>6.155g</td>
</tr>
</tbody>
</table>
3.4.1. POLYPHENOL CONTENT IN ENCAPSULATED CASEIN BEADS OF C. TERMINALIS AND M. FRAGRANS EXTRACTS DURING TWELVE MONTHS STORAGE

In the case of encapsulated beads of CTPE and MFPE, by heating 50 mg of beads with 1 ml of deionized water at 60°C for 7 h, the polyphenols were released. Heating the beads at 60°C in water for 7 hrs helped in the digestion of casein and released the contents. The results of polyphenol content are shown in Figure 3.1 and antioxidant activity is shown in Figure 3.2. We observed a continuous decrease of polyphenol content for a period of three months in the unencapsulated extracts of C. terminalis and M. fragrans. During the next two months the polyphenol content was stable, but on the sixth month decrease in the polyphenol content was noticed. This is in agreement with Montoro et al., (2006) who reported that flavonoids and anthocyanins are unstable and the extracts cannot be used over three months after their preparation.

![Graph showing stability of polyphenol content in unencapsulated and encapsulated methanolic extracts of C. terminalis & M. fragrans during a period of six months of storage. Values are expressed as mean ± SD of Gallic acid equivalents, n=3 (p< 0.001). Ca-Encapsulated CTPE- Casein Encapsulated C. terminalis methanolic extract, Ca-Encapsulated MFPE - Casein Encapsulated M. fragrans methanolic extract.](image)

**FIGURE 3.1:** Stability of polyphenol content in unencapsulated and encapsulated methanolic extract of C. terminalis and M. fragrans for a period of 12 months.
The encapsulated CTPE showed the polyphenol content of 72.94 mg/g polyphenols equivalent of gallic acid at day 1. Every month for a period of six months, the polyphenol content was analysed, it was stable with an average mean value of 72.35 mg/g of gallic acid equalance GAE. Even after one year the polyphenol content remained constant and was 72.38 mg/g of GAE. Whereas in the unencapsulated CTPE showed polyphenol content of 106.2 mg/g of GAE at day 1. The polyphenol content was analysed and found to be unstable every month for a period of six months. At the end of the six month the polyphenol content decreased to 60.02 mg/g of GAE. At one year the polyphenol content decreased further and to 52.18 mg/g of GAE.

The polyphenol content of MFPE was encapsulated and observed to be 41.90 mg/g polyphenols equivalent of gallic acid at day 1. Every month for a period of six months, the polyphenol content was analyzed and was stable with an average mean value of 41.39 mg/g of GAE. There was no further decrease in polyphenols content up to one year.

Whereas in the unencapsulated MFPE showed polyphenol content 72.48 mg/g of GAE at day 1. Subsequently every month for a period of six months, the polyphenol content was analyzed and found to be unstable. At the end of six months the polyphenol content decreased to 35.20 mg/g of GAE. The decreasing tendency continued even after one year to 30.65 mg/g of GAE.

3.4.3. ANTIOXIDANT STABILITY IN ENCAPSULATED CASEIN BEADS OF C. TERMINALIS AND M. FRAGRANS EXTRACTS DURING TWELVE MONTHS STORAGE:

Antioxidant activity was also were determined every month over a period of six months and subsequently after one year to evaluate whether the encapsulation technique could reduce or maintain the antioxidant power of the loaded extract. The concentration of antioxidant needed to decrease by 50% of the initial substrate concentration (E<sub>C50</sub>), is a parameter widely used to measure the antioxidant power. The lower the E<sub>C50</sub> value, the higher the antioxidant power (Sanchez-Moreno et al., 1998). The EC50 values of
antioxidant activity in the encapsulated CTPE and MFPE at 1 day were 13.66 and 11.13 mg/g of ascorbic acid equivalence (AAE). After six months, the antioxidant activity of CTPE and MFPE the extracts was 24.15 and 18.21 mg/g of AAE respectively, and after one year it was 27.38 and 20.98 mg/g of AAE. The polyphenol content and antioxidant activity values of the encapsulated extracts of *C. terminalis* and *M. fragrans* were stable for a period up to one year as shown in figure 3.2.

Encapsulated CTPE and MFPE showed significant antioxidant activity compared to the unencapsulated extracts (*Figure 3.2*). The encapsulated CTPE and MFPE showed good protection against oxidation, and its relatively strong protective effect in casein beads could be attributed to the amphiphilic properties of phenolic constituents. It is generally assumed that an increase in the number of hydroxyl groups in a phenol enhances the hydrogen donor ability and inhibition of oxidation (Rice-Evans & Miller, 1996). Thus, the encapsulation technique maintained the antioxidant power of CTPE and MFPE.

![FIGURE 3.2: Antioxidant activity of unencapsulated and encapsulated methanolic extracts of *C. terminalis* and *M. fragrans* for a period of 12 months](image)

EC50 values of unencapsulated and encapsulated methanolic extracts of *C. terminalis* & *M. fragrans* on Free radical scavenging activity during six months of storage. Values are expressed as mean ± SD of Ascorbic acid equivalents, n=3 (p≤ 0.001).
3.4.1 EFFECTS OF TEMPERATURE AND TIME ON THE RELEASE OF POLYPHENOLS FROM CASEIN ENCAPSULATED C. TERMINALIS METHANOLIC EXTRACT

To study the effect of temperature and time of incubation on the release of polyphenols from the encapsulated beads of CTPE and MFPE, the beads were heated at different temperatures and at different time intervals. The results are shown in Figures 3.3 and 3.4. We observed that the release of polyphenols from the encapsulated beads depends on the temperature and time of incubation. In the case of CTPE and MFPE, maximum amounts of polyphenols were released when the beads were incubated at 60°C for 7 h. At 40°C and 50°C respectively, CTPE and MFPE showed a degradation of their polyphenol content (Figures 3.3 and 3.4), suggesting that the correct selection of the temperature and time control is an important step in guaranteeing the stability and amount of the polyphenols in the beads.

The results also indicate that the temperature had a significant effect on the total polyphenol content of CTPE and MFPE. The wall material successfully used for encapsulation of polyphenol was protein (sodium caseinate) emulsion, which has been used in green tea polyphenol encapsulation (Dehkharghanian et al., 2009). This is a multipurpose encapsulation process, creating a novel nutraceutical product suitable for a variety of applications in functional food manufacturing. Further studies need to be carried out based on these conditions because time and temperature are the important factors to be considered when it is administrated to animal models for bioavailability studies.
Temperature and time dependence on the release of polyphenols from the encapsulated beads of *C. terminalis* extract. Values are expressed as mean ± SD of Gallic acid equivalents, n=3 (p≤ 0.001).

**FIGURE 3.3:** Effects of temperature and time on the release of polyphenols from casein encapsulated *C. terminalis* methanolic extract.

Temperature and time dependence on the release of polyphenols from the encapsulated beads of *M. fragrans* extract. Values are expressed as mean ± SD of Gallic acid equivalents, n=3 (p≤ 0.001).

**FIGURE 3.4:** Effects of temperature and time on the release of polyphenols from casein encapsulated *M. fragrans* methanolic extract.
GENERAL CONCLUSION AND FUTURE PERSPECTIVE

The interest in polyphenols has grown considerably because of their high capacity to entrap the free radicals associated with different diseases like cancer, inflammation, chronic diseases and metabolic disorders by balancing the Reactive Oxygen Species (ROS). Recently, there has been an increasing interest in determining relevant dietary sources of antioxidant phenolics. Assessment of biological properties of plant extracts remains an interesting and useful task in identifying new sources of natural antioxidants which can be used as nutraceuticals.

*M. fragrans* seeds and *C. terminalis* leaves were collected from Tamil Nadu (Southern India). The extracts were prepared in 70% methanol and the extract was standardized by physicochemical and biochemical methods. A detailed phytochemical screening of *M. fragrans* and *C. terminalis* extracts were carried out. In addition to this, it was found that the both the extracts possess alpha amylase inhibitory effect and antibacterial activity.

However in order to know the extent of contribution of polyphenolic compounds towards antioxidative capacity, herein methanolic (methanol-water) and water extracts prepared from seeds of *M. fragrans* and leaves of *C. terminalis* were investigated by liquid chromatography coupled to online ultra violet spectroscopic detection, combined with mass spectrometry (LC-UV-MS). High performance LC was carried out in reverse phase mode and mass spectrometric data acquisition was by electrospray ionization (ESI). The resulting mass spectral data were interpreted utilizing database of LIPID MAPS to elucidate the flavonoid subclasses in these extracts.

The methanolic (methanol-water) and water extracts from seeds of *Myristica fragrans* and leaves of *Cordyline terminalis* were investigated by liquid chromatography (reverse phase) coupled to electrospray ionization mass spectrometry (LC-ESI MS) to evaluate polyphenolic composition in these extracts. It was striking to find out that different types of polyphenolic compounds were present in the form of different
subclasses of main class- ‘flavonoids’, within the category of ‘polyketides’, as per Lipid Metabolites and Pathways Strategy consortium (LIPID MAPS). Hence, the database of LIPID MAPS was utilized to interpret the mass spectrometric data. *M. fragrans* seem to possess relatively a higher content of ‘isoflavonoids’, ‘chalcones and dihydrochalcones’ and ‘other polyketides’ subclasses, whereas the subclass ‘flavones and flavonols’ seem to be more abundant in *C. terminalis*. Analyses of HPLC-ESI-MS data indicated ~232 flavones and Flavonols, 32 flavanones, 33 isoflavonoids, 12 chalcones & dihydrochalcones and 20 anthocyanidins in *C. terminalis* extracts. In the crude extracts of *M. fragrans*, 162 flavones and flavonols 29 flavanones, 45 isoflavonoids, 23 chalcones & dihydrochalcones and 3 anthocyanidins as revealed by HPLC-ESI-MS. This may be attributed to family, age and geographical regions. Further, the mass spectrometric data were indicative of some isoprenoids too in *M. fragrans*. This is the first report on the elucidation of flavonoid subclasses from these plants. Also, this is perhaps the first attempt on the utilization of LIPID MAPS database for identifying flavonoid (polyphenol) subclasses.

Polyphenols are generally prone to degradation on storage. Hence encapsulation of the polyphenols in *C. terminalis* and *M. fragrance* extracts using Casein beads were carried out. In this study, we have developed an encapsulation procedure which retains the antioxidant properties and polyphenol content from two novel plants and is stable over a period of one year at room temperature at non-toxic level. The stability of the encapsulated extracts was studied over a period of 12 months. We observed that the polyphenols content and antioxidant activity remained stable in the encapsulated beads compared to the unencapsulated extracts. The DPPH radical-scavenging activity was stable for a period of twelve months after encapsulation compared to unencapsulated extracts of both the plants. The release of polyphenols from encapsulated casein beads was maximum at 60° C for 7 hours. In addition, there was no significant colour change of encapsulated casein beads over a period of twelve months. Sodium-caseinate beads prove to be a promising technique for food supplementation with natural antioxidants.
In conclusion, our present study elucidates that *C. terminalis* and *M. fragrans* possesses following activities: antioxidant, α- amylase inhibition and antibacterial. Thus, *C. terminalis* and *M. fragrans* has a potential to be used as a nutraceutical. The LC-ESI-MS data were useful to identify the potential polyphenols compounds and their subclasses. It will be interesting to determine the antioxidant activity of each of the subclass alone and compare each of their contribution to the total antioxidant activity. By comparing the tandem mass spectrometric data (MS/MS) and/or nuclear magnetic resonance (NMR) spectroscopic data of standards with that of the compounds isolated from natural extracts, the prospective identifications can be ascertained. Gas-chromatography (GC)-MS can also aid in confirming the compounds identified herein. Such investigations might be pursued in future.