

# Evaluation of antimicrobial potential and phytochemicals in *Acmella* (*A. oleracea*) flower pod extracts subjected to different drying techniques

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## Abstract

The study was aimed to evaluate how drying methods and extracting solvents can preserve antimicrobial properties of *Acmella* flower pods. Four drying techniques (sun drying [SD], air drying [AD], oven drying [OD], and cooling with dehumidifying [CWD]) and three different solvent extractions (ethanol extracts [EE], water extracts [WE], and pet ether extracts [PEE]) were employed to evaluate extraction yield (EY), phytochemical analysis, and in vitro antibacterial activity. The highest EY was observed in CWD dried WE. Alkaloids, tannin, and quinone were detected in all extracts while flavonoid only in SD and CWD dried EE. CWD dried WE comprised all tested phytochemicals, except flavonoids. CWD dried WE showed higher zones of inhibitions (ZOI) 18.8, 14.0, 12.0, 20.2, and 17.3 mm for *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*, respectively. CWD dried pod extracts showed higher effectivity against bacteria and fungus while lowest given by SD extracts. CWD dried WE gave 20 volatile compounds in GC-MS, including dilauryl thiodipropionate, clionasterol, and spilanthol.

## Practical applications

This study provided a comprehensive evaluation of how the drying method and solvent used for extraction of *Acmella oleracea* flower pods can effect on the extraction yield, available phytoconstituents, and antimicrobial activity. Through this study, it was recognized that CWD drying followed by water extraction is the best method to preserve antimicrobial potential and bioactive constituents in preserving raw *Acmella oleracea* flower pods. Phytochemical availability and ability to inhibit Gram-negative, positive bacteria, and fungus is an indication of *Acmella* flower pods' antimicrobial potential which can be employed to control food pathogenic microorganisms in food industry.

## KEYWORDS

*Acmella oleracea*, antimicrobial activity, cooling with dehumidification, drying methods, phytochemicals, spilanthol

## 1 | INTRODUCTION

The genus *Acmella* (family Asteraceae) comprises 30 species (Maimulyanti & Prihadi, 2016; Nascimento et al., 2013; Uthpala & Navaratne, 2020). One of the most distinguished species of the genus is *Acmella oleracea* (L.) R.K. Jansen, which consisted of flowers with large cylindrical discoid capitula in a unique golden yellow color with a red tip (Figure 1). It is a flowering herb with rich source of antimicrobial and antifungal constituents and commonly known as toothache plant which is found in various parts of the world including tropical and subtropical regions around the world (Bedi et al., 2017; Dubey et al., 2013; Jansen, 1985; Molina-Torres et al., 2004; Uthpala, Navaratne, et al., 2020). Paracress, akarkara, akmella, sichuan buttons, buzz buttons, ting flowers, eye ball plant, and electric daisy are some of the other common names used for this plant (Matyushin & Evdokimova, 2017; Nascimento et al., 2013; Uthpala, Navaratne, et al., 2020). This plant is growing abundantly in the wild and still unexploited. Thus, it is only restricted to ayurvedic medicine in most of Asian countries. The decoctions obtained from the leaves and flowers of this plant are used in the treatment of rheumatism, as a sialagogue for stammering, tongue paralysis, antipyretic, sore throat, and gum infections (Uthpala, Navaratne, et al., 2020). Moreover, leaves and flowers of these plants have sensorial properties (pungency, tingling, numbing, mouth-watering) that make it a popular spice and ingredient in several Brazilian dishes (Barbosa, 2016; Uthpala, Navaratne, et al., 2020).

The effects of the plant extract may differ depending on the type of extraction method, temperature applied in extraction, and solvents used in the extraction process (Dhanani et al., 2017; Rani & Murty, 2005; Uthpala, Navaratne, et al., 2020). Therefore, more in-depth studies of the biological activities of this plant are needed since this is an edible plant which shows beneficial activities in multidisciplinary applications. Notwithstanding, the major cause of foodborne illnesses is caused by pathogenic bacteria and people living in the southeast Asian region fall ill and die from this every year than in any other region (World Health Organization, 2015; Uthpala & Navaratne, 2019). Foodborne illnesses are very common in Sri Lanka, and major causes for this national issue are pathogens multiplication in food products. Currently, synthetic chemicals are applied to control foodborne pathogens in the food handling business. Therefore, it is essential to investigate the potential of natural herbal extracts from edible plants like acmella which is restricted only for indigenous

medical uses in our country, even though it is easily grown and wide prospectus in the arena of the food field is available.

Thus, the aim of this study is to investigate the potential use of *Acmella oleracea* flower bud extract in controlling of microbial growth related to food borne pathogens. Flower inflorescences of *Acmella oleracea* (L.) R. K. Jansen were harvested and subjected to dehydration using four types of drying techniques and subjected to extraction by use of three different solvents separately. Then, the extraction yield was measured, and extracts were subjected to phytochemical screening. Simultaneously, antimicrobial potential was evaluated for aforementioned 12 types of extracts using selected food pathogenic Gram-positive, Gram-negative strains, and a yeast using disc diffusion method. Finally, chemical composition was obtained through gas chromatography-mass spectrometry analysis for crude extracts obtained from the best drying method. According to authors' knowledge, this is the first attempt, a research study of this manner has been conducted for the flower pods of this plant. In addition, these findings will be beneficial for both commercial scale *Acmella* producers and persons who will carry out future studies related to the field of food safety and especially, controlling food pathogenic microbes.

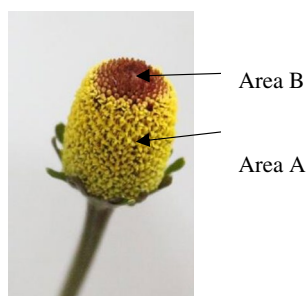
## 2 | MATERIALS AND METHODS

### 2.1 | Plant material

The flowers of *Acmella oleracea* were collected in May of 2019 from a home garden, Colombo, western province, Sri Lanka (6.843° N, 79.955° E). The voucher specimen (TG-Acmella-01) was deposited in the Department of National Botanic gardens, National Herbarium, Peradeniya, Sri Lanka. Disease-free mature *Acmella oleracea* flowers were collected. Color of the collected mature pods (Figure 1) was measured using Chromameter (Lovibond LC 100, China). The color of the selected flowers at A and B regions (Figure 1) was within the chromameter values (lightness ( $L^*$ ), redness ( $a^*$ ), yellowness ( $b^*$ ), chroma ( $C^*$ ), and hue angle (H)) as shown in Table 1.

### 2.2 | Drying methods

Collected *Acmella* flower pods were washed with distilled water and dried using four different drying methods (sun drying



**FIGURE 1** Image of a flower pod of *Acmella oleracea* plant

**TABLE 1** Chromameter value of collected *Acmella* flower pods

| Value | Area A       | Area B       |
|-------|--------------|--------------|
| $L^*$ | 50.32 ± 2.93 | 33.03 ± 1.93 |
| $a^*$ | 7.10 ± 1.58  | 15.15 ± 2.75 |
| $b^*$ | 45.23 ± 5.71 | 30.62 ± 2.05 |
| $C^*$ | 44.82 ± 5.63 | 33.60 ± 0.97 |
| H     | 81.04 ± 1.90 | 68.71 ± 3.46 |

Note: Values are mean ± SD,  $n = 20$ .

[40°C–44°C], dehydration by oven drying [40°C], air drying [30°C–34°C], and cooling with dehumidifying [4°C–7°C]) for a period of 2 days. Then, the dried pods were pulverized until a fine powder was obtained.

### 2.3 | Preparation of the extract and measuring extraction yield

The dried flower powder was used to obtain the extracts by maceration at room temperature, separately with assays of ethanol, petroleum ether, and water. Extraction was done by slight modifications in the maceration process of Holetz et al., (2002; Maimulyanti & Prihadi, 2016). A sample of 10 g of dried powder was macerated in 200 ml of respected solvent (ethanol, water, and pet ether) separately for 48 hr, with six replicates at a time. The extract obtained was filtered using Whatman grade 1 filter papers and was concentrated in a rotary evaporator. This procedure was followed to obtain 12 different extractions. The extraction yields were calculated according to Equation (1), and yields were recorded. Those crude extracts which were collected and stored at 4°C for the subsequent analysis, particularly for water extracts were frozen at –80°C and freeze-dried to obtain extraction yield.

$$\text{Extraction yield} = \frac{\text{Weight of the extract after evaporating the solvent}}{\text{Weight of the initial dried sample}} \times 100\% \quad (1)$$

### 2.4 | Antimicrobial assay

The disk diffusion method was employed for the determination of antimicrobial activity of 12 extracts of *Acmella oleracea* flower pod extracts. The minimum inhibitory concentrations (MICs) of the samples against the test microorganisms were determined by the broth micro-dilution method.

### 2.5 | Microbial strains and maintenance

Five microbial strains (Gram-positive (2), Gram-negative (2), and a yeast) were employed in the study. Gram-positive strains were *Bacillus subtilis* (ATCC 6623) and *Staphylococcus aureus* (ATCC 29213). The Gram-negative strains included *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) along with *Candida albicans* (ATCC 10231) was used as the fungus. The microbial stock cultures were obtained from Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka.

### 2.6 | Preparation of inocula

Inocula were prepared as per the method described by Chandrasekaran and Venkatesalu (2004) with some modification.

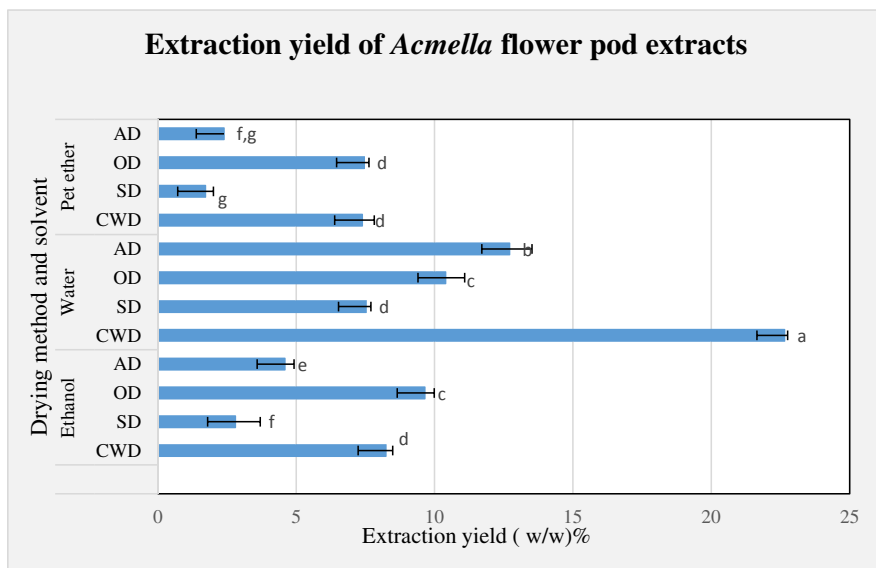
Briefly, selected bacteria and yeast were grown for 24 hr in nutrient broth (NB) at 37°C and Sabouraud dextrose broth (SDB) at 28°C, respectively, and then mixed with sterile saline and the turbidity was adjusted at a McFarland turbidity standard of 0.5 (10<sup>6</sup> CFU/ml). The fungal isolates were subcultured on SDB and incubated at 28°C for 7–14 days. The growth was aseptically macerated thoroughly in sterile distilled water and the absorbance of the fungal suspension was adjusted to absorbance of 0.60 at 450 nm.

### 2.7 | Disk diffusion assay

The disc diffusion method (DDM) was employed to determine the zones of inhibition exhibited by different extracts against the bacterial and fungal pathogens as per the method described by Chandrasekaran and Venkatesalu (2004) with some modifications. Sterile paper disks (6 mm) were impregnated with 600 µg of each extract for bacteria and 1,200 µg for *Candida* species and allowed to dry at room temperature (27°C). A blank disc with respective solvent was set as negative control and commercial discs (MASTDISCS™) with gentamycin (10 µg) and fluconazole (25 µg) were set as positive controls for the antibacterial and antifungal assays, respectively. Bacterial and fungal inocula (0.1 ml) were swabbed on NA and Sabouraud dextrose agar (SDA), respectively. After the discs impregnated with extracts, positive and negative controls were placed onto the microbial lawns. The bacterial plates were incubated at 37°C while the fungal plates were incubated at 28°C. The zone of inhibition (ZOI) were measured after 24 hr in millimetres.

### 2.8 | Determination of minimum inhibitory concentration

Extraction yield values and ZOI values were subjected to analysis of variance using Minitab 17 software at 0.05 significance level and the best drying method was selected. The extracts obtained from the best drying method using different solvents were taken for MIC determination by microdilution method (Clinical & Laboratory Standards Institute, 2018). In vitro antibacterial and antifungal activity were determined using NB and SDB medium, respectively. Bacterial and fungal cells were treated with different concentrations of the extract and MIC values were determined using the 96 well microtiter plate-based method with 2,3,5, triphenyl tetrazolium chloride (TTC) dye as described by Mahmoud et al., (2019) with some modifications. By using sterile distilled water 0.2 mg/ml TTC dye was prepared. Gentamycin and fluconazole were used as positive controls for antibacterial and antifungal assays, respectively, while the relevant solvent was set as the negative control for both the assays. This test was only done for the dried *Acmella* flower pod extracts which were obtained from the CWD drying method as it was the best drying method.



**FIGURE 2** Extraction yield of *Acmeella* flower pod extracts obtained by four different drying techniques and three solvents. Values are mean  $\pm$  standard deviation,  $n = 6$ , means that do not share a letter are significantly different

Briefly, 100  $\mu$ l of extract (5,000  $\mu$ g/ml in relevant solvent) was pipetted into the first row of the plate. Then with NB or SDL half dilutions were prepared in serially descending concentrations. Then 10  $\mu$ l of bacterial suspensions were added to each well. A final volume of 100  $\mu$ l was achieved in each well using NB or SDL. Each plate had a set of controls: a column with a positive control (gentamycin for bacterial strains or fluconazole for yeast). The plates were incubated at 37°C for 24 hr, 28°C for 48 hr for bacteria and yeast, respectively. Then, 50  $\mu$ l of TTC was added to each well and the plates were re-incubated for 1 hr. The red color indicated the presence of viable bacteria while wells with no color change indicated inhibition of microorganisms. The MIC value was determined as the lowest concentration at which no color change occurred.

## 2.9 | Phytochemical analysis

### 2.9.1 | Phytochemical screening

The extracts were subjected to phytochemical tests for plant secondary metabolites such as tannin, alkaloids, flavonoids, quinone, phlobatanin, saponin, total phenols, gum, and mucilage in accordance with methods of Harborne, Trease, and Evans with slight modifications (Harborne, 1980, 1998; Sadiq et al., 2015). The presence of saponins, tannins and phenols, alkaloids, flavonoids was done by frothing test, ferric chloride test, Hager's test and Shinoda test, respectively (Sadiq et al., 2015; Uthpala et al., 2020).

### 2.9.2 | Chemical composition of the CWD dried plant extracts—GCMS analysis

Chemical composition of the plant extract was analyzed by gas chromatography mass spectrometry (GC-MS) analysis method for the samples obtained by CWD drying method. The water extracts

which was lyophilized was dissolved in ethanol and filtered with 0.45- $\mu$ m filter before used in GC-MS procedure. Ethanol extract was dissolved in ethanol and filtered with 0.45- $\mu$ m filter and pet ether extract was dissolved in *n* hexane, filtered with 0.45- $\mu$ m filter before used in GC-MS procedure.

GC-MS program was carried out according with modified method of Payum (2017). GC-MS analysis was performed in Agilent Technologies 7890A GC system, MS 5975C (triple access detector) system. Volatile compounds were separated from HP5MS 5% phenyl methyl silox capillary column (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m). Helium was used as carrier gas at a constant flow rate 1 ml/min. The operating conditions of the column were as follows: oven temperature program from 80°C for 2 min, 210°C at 4°C/min withhold time of 5 min and from 300°C at 15°C/min for 8 min, and the final total time was 53.5 min. The injector temperature was maintained at 270°C, the volume of injected sample was 1  $\mu$ l; pressure 85.4 kPa, column flow 1.21 ml/min, linear velocity 40.5 cm/s, purge flow 3.0 ml/min, split ratio: 50:1; ion source temperature 230°C; scan mass range of  $m/z$  20–550 and interface line temperature 280°C.

The identification of compounds was performed by comparing their mass spectra with data from NIST 11 (National Institute of Standards and Technology, US) and WILEY 8 using the criterion of at 80% similarity for the mass spectra. Relative abundance (RA) of each compound was calculated using total sum normalization (Maduwanthi & Marapana, 2019) using the following formula;

$$\text{Relative abundance (\%)} = \frac{\text{Area of the peak}}{\text{Sum of areas of all peaks}} \times 100\% \quad (2)$$

### 2.9.3 | Statistical analysis

All the extraction and antimicrobial trials were done using 6 replicates. Data were expressed as mean  $\pm$  standard deviation. Significant differences ( $p < .05$ ) among the mean values of extraction results were determined by one-way analysis of variance

(ANOVA) followed by Tukey pairwise comparison test, using Minitab 17 version.

testing, 1,200 µg disc<sup>-1</sup> of extract was used with fluconazol (25 µg disc<sup>-1</sup>) as the positive control.

### 3 | RESULTS

#### 3.1 | Extraction yield

The extraction yields (EY) of *Acmella* flower pod extracts obtained by four different drying methods namely, sun drying (40°C–44°C), oven drying (40°C), air drying (30°C–34°C) and cooling with dehumidifying (4°C–7°C) as well as using three solvents (ethanol, water, and pet ether) are shown in Figure 2. The EY of CWD drying was higher than that of OD, AD, and SD irrespective to the solvent. The highest EY was observed from CWD dried water extracts.

#### 3.2 | Antimicrobial assays

##### 3.2.1 | Disk diffusion assay

Table 2 shows the results obtained from the DDM for the *Acmella* flower pod extracts which were subjected to different drying methods. Among them CWD method has shown higher ( $p < .05$ ) ZOI (mm) values. For assessing antimicrobial potential, 600 µg disc<sup>-1</sup> of *Acmella* flower pod extract was used against bacteria while gentamicin (10 µg disc<sup>-1</sup>) was taken as the positive control. For fungal

##### 3.2.2 | Determination of minimum inhibitory concentration

MIC values of pet ether, ethanol, and water extracts of CWD dried *Acmella* flower pod extracts are shown in Table 3 in micrograms per one milliliter. These MIC values were determined by micro dilution method using twofold dilution for the dilution series. The MIC of tested *Acmella* flower extracts were in the range of 312.5 to 2,500 µg/ml against all tested bacterial strains as in Table 3.

**TABLE 3** MIC of extracts obtained from *Acmella* flower pods, subjected to cooling with dehumidified drying against test microorganisms

| Microbial species             | MIC (µg/ml) |       |         |
|-------------------------------|-------------|-------|---------|
|                               | Pet ether   | Water | Ethanol |
| <i>Staphylococcus aureus</i>  | 1,250       | 625   | 1,250   |
| <i>Escherichia coli</i>       | 1,250       | 625   | 1,250   |
| <i>Bacillus subtilis</i>      | 1,250       | 625   | 1,250   |
| <i>Pseudomonas aeruginosa</i> | 1,250       | 312.5 | 625     |
| <i>Candida albicans</i>       | 2,500       | 625   | 1,250   |

**TABLE 2** Zone of inhibition (mm) of *Acmella* flower pod extracts subjected to different drying methods and extracted using different solvents

| Solvent   | Drying method | Zone of inhibition (mm)      |                            |                           |                               |                            |
|-----------|---------------|------------------------------|----------------------------|---------------------------|-------------------------------|----------------------------|
|           |               | <i>Staphylococcus aureus</i> | <i>Escherichia coli</i>    | <i>Bacillus subtilis</i>  | <i>Pseudomonas aeruginosa</i> | <i>Candida albicans</i>    |
| Pet ether | CWD           | 8.80 <sup>d</sup> ± 0.50     | 7.20 <sup>c</sup> ± 0.60   | 7.00 <sup>d</sup> ± 0.20  | 9.30 <sup>e</sup> ± 0.31      | 11.60 <sup>d</sup> ± 0.43  |
|           | SD            | ND <sup>g</sup>              | ND <sup>e</sup>            | ND <sup>f</sup>           | ND <sup>g</sup>               | ND <sup>h</sup>            |
|           | OD            | 6.50 <sup>f</sup> ± 0.50     | ND <sup>e</sup>            | ND <sup>f</sup>           | ND <sup>g</sup>               | 7.80 <sup>f</sup> ± 0.52   |
|           | AD            | 6.10 <sup>f</sup> ± 0.20     | ND <sup>e</sup>            | ND <sup>f</sup>           | ND <sup>g</sup>               | 7.20 <sup>f,g</sup> ± 0.30 |
| Water     | CWD           | 18.80 <sup>b</sup> ± 0.29    | 14.00 <sup>b</sup> ± 0.9   | 12.00 <sup>b</sup> ± 0.48 | 20.20 <sup>a</sup> ± 0.30     | 17.30 <sup>b</sup> ± 0.70  |
|           | SD            | 6.10 <sup>f</sup> ± 0.08     | ND <sup>e</sup>            | ND <sup>f</sup>           | ND <sup>g</sup>               | 7.70 <sup>f,g</sup> ± 0.40 |
|           | OD            | 7.50 <sup>e</sup> ± 0.40     | 6.30 <sup>d</sup> ± 0.50   | 6.20 <sup>e</sup> ± 0.18  | 7.80 <sup>f</sup> ± 0.45      | 8.00 <sup>e</sup> ± 0.34   |
|           | AD            | 10.30 <sup>c</sup> ± 0.31    | 6.50 <sup>c,d</sup> ± 0.44 | 6.30 <sup>e</sup> ± 0.20  | 11.50 <sup>d</sup> ± 0.12     | 7.50 <sup>f</sup> ± 0.30   |
| Ethanol   | CWD           | 10.20 <sup>c</sup> ± 0.21    | 8.00 <sup>c</sup> ± 0.80   | 7.70 <sup>c</sup> ± 0.50  | 12.50 <sup>c</sup> ± 0.45     | 14.60 <sup>c</sup> ± 0.22  |
|           | SD            | ND <sup>g</sup>              | ND <sup>e</sup>            | 6.30 <sup>e</sup> ± 0.20  | ND <sup>g</sup>               | 7.00 <sup>g</sup> ± 0.40   |
|           | OD            | 7.20 <sup>e</sup> ± 0.50     | 6.10 <sup>d</sup> ± 0.08   | 6.20 <sup>e</sup> ± 0.05  | ND <sup>g</sup>               | 7.30 <sup>f,g</sup> ± 0.10 |
|           | AD            | 7.00 <sup>e</sup> ± 0.45     | 6.20 <sup>d</sup> ± 0.20   | 6.10 <sup>e</sup> ± 0.40  | 7.50 <sup>f</sup> ± 0.30      | 7.70 <sup>f</sup> ± 0.10   |
| Positive  |               | 23.70 <sup>a</sup> ± 0.30    | 22.20 <sup>a</sup> ± 0.23  | 23.30 <sup>a</sup> ± 0.40 | 19.30 <sup>b</sup> ± 0.20     | 15.00 <sup>a</sup> ± 0.05  |
| Negative  |               | ND <sup>g</sup>              | ND <sup>e</sup>            | ND <sup>f</sup>           | ND <sup>g</sup>               | ND <sup>h</sup>            |

Note: Mean ± SD (n = 6), different superscript letters in the same column denote a significant difference under Tukey pairwise comparison test ( $p < .05$ ). For bacterial testings –600 µg disc<sup>-1</sup> *Acmella* extracts were used with gentamicin (10 µg disc<sup>-1</sup>) as positive control; fungal testing –1,200 µg disc<sup>-1</sup> of *Acmella* extracts were used with fluconazol (25 µg disc<sup>-1</sup>) as the positive control.

Abbreviation: ND, not detected.

### 3.3 | Phytochemical analysis

#### 3.3.1 | Phytochemical screening

Evaluation of the phytochemical profile of each *Acmella* extracts obtained by different drying techniques and different solvents showed marked differences in the phytochemical content (Table 4). Phytochemical screening gave positive results for alkaloids, tannin, and quinone in all extracts while flavonoid only in SD and CWD dried EE. Phlobatanin, saponins, and gum mucilage were absent in EE. The CWD dried pods subjected to WE comprised with all tested phytochemicals, except flavonoids.

#### 3.3.2 | Chemical composition of the CWD dried plant extracts

Table 5 shows phytoconstituents detected by GC-MS on HP5MS capillary column in CWD dried *Acmella oleracea* flower pod extracts obtained through water, pet ether and ethanol solvents. Total number of compounds detected in CWD dried *A. oleracea* flower pod extracts was 29. Any compounds found less than 0.05% or less than 80% of the similarity with the NIST library were not considered for the identification.

## 4 | DISCUSSION

The drying is a complex process that entails mass and heat transfer along with physical and structural variations (Alves-Filho, 2002; Uthpala et al., 2020). Although the foremost objective of food drying is preservation, it is affected by the drying method eventually ending up raw material into a completely different material with significant variation in product quality (Chua et al., 2001; Uthpala, Navaratne,

et al., 2020). The CWD dried aqueous extracts obtained from maceration showed the highest ( $p < .05$ ) extraction yield (22.65%) while SD pet ether extracts reported the lowest (1.72%) among the other extraction combinations. The extraction yield of aqueous extracts was comparably higher than that obtained from pet ether and ethanol extracts. Irrespective of the solvents, the lowest extraction yields were reported from the sun-dried samples. There was no significant difference ( $p > .05$ ) between extraction yields of AD and SD pet ether extracts with SD ethanol extract under Tukey pairwise comparison. Recent studies have found that particle size of the material, solid-to-solvent ratio, solvent type, and extraction procedure have a significant impact on the extractable phytoconstituents, while the extraction time is having a lower impact (Jovanović et al., 2017).

In the CWD process, air is cooled sensibly and cold dry air removes the moisture from food material (Harriman, 2002; Uthpala, Navaratne, et al., 2020). CWD dryers provide multiple advantages over typical conventional oven drying for the drying of food products, covering higher energy efficiency, better product quality, and the ability to work independently of outside ambient weather conditions (Uthpala, Navaratne, et al., 2020).

Table 2 shows the antibacterial potential of *Acmella* flower pod extracts obtained by PEE, WE, and EE subjected to different drying methods. Prepared extracts were tested against gram positive and negative bacteria and yeast. Sun-dried PEE did not display any antimicrobial effects against the tested bacteria while extracts obtained from other drying and extraction methods have shown antimicrobial effect against more than one tested pathogen. *Acmella* flower pods extracts subjected to four drying methods were active against *C. albicans* instead of SD pet ether extract. Highest ZOI against *C. albicans* was noted in CWD water extract ( $17.3 \pm 0.70$  mm) followed by CWD ethanol ( $14.6 \pm 0.22$  mm) and CWD pet ether ( $11.6 \pm 0.43$  mm) extraction. CWD aqueous extracts showed higher ZOI values against all the tested bacterial isolates leading *P. aeruginosa* ( $20.2 \pm 0.30$  mm) followed by

**TABLE 4** Phytoconstituents present in *Acmella* pod extracts obtained through different drying methods and solvent extractions

| Solvent   | Drying method | Alkaloids | Flavonoids | Tannin | Total phenol | Quinone | Phlobatanin | Saponin | Gum and mucilage |
|-----------|---------------|-----------|------------|--------|--------------|---------|-------------|---------|------------------|
| Pet ether | CWD           | +         | -          | +      | -            | +       | +           | -       | +                |
|           | SD            | +         | -          | +      | -            | +       | +           | -       | +                |
|           | OD            | +         | -          | +      | -            | +       | +           | -       | +                |
|           | AD            | +         | -          | +      | -            | +       | +           | -       | +                |
| Water     | CWD           | +         | -          | +      | +            | +       | +           | +       | +                |
|           | SD            | +         | -          | +      | +            | +       | -           | +       | +                |
|           | OD            | +         | -          | +      | +            | +       | -           | +       | +                |
|           | AD            | +         | -          | +      | +            | +       | -           | +       | +                |
| Ethanol   | CWD           | +         | +          | +      | +            | +       | -           | -       | -                |
|           | SD            | +         | +          | +      | +            | +       | -           | -       | -                |
|           | OD            | +         | -          | +      | +            | +       | -           | -       | -                |
|           | AD            | +         | -          | +      | +            | +       | -           | -       | -                |

Note: (+) Denotes available and (-) denotes not detected.

**TABLE 5** Chemical composition of CWD dried *Acemella oleracea* flower pod extracts obtained through water, pet ether, and ethanol solvents

| No. | Name of the compound  | Molecular formula  | CWD dried WE |        | CWD dried PEE |        | CWD dried EE |        |
|-----|---|--|--------------|--------|---------------|--------|--------------|--------|
|     |   |  | Rt (min)     | RA (%) | Rt (min)      | RA (%) | Rt (min)     | RA (%) |
| 1   | (S,1Z,6Z)-8-Isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene   | C <sub>15</sub> H <sub>24</sub>  | 30.290       | 2.440  | 28.501        | 3.210  | 28.279       | 39.437 |
| 2   | 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester:   | C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>                               | ND           | ND     | ND            | ND     | 40.555       | 0.222  |
| 3   | 1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester     | C <sub>21</sub> H <sub>25</sub> NO <sub>4</sub>                              | ND           | ND     | 43.080        | 0.941  | ND           | ND     |
| 4   | 2-(difluoromethyl)-1H-benzimidazole   | C <sub>8</sub> H <sub>6</sub> F <sub>2</sub> N <sub>2</sub>                  | 44.345       | 0.465  | ND            | ND     | ND           | ND     |
| 5   | 6,7-dihydropyrrolo[3,4-b]pyridin-5-one  | C <sub>13</sub> H <sub>16</sub> N <sub>2</sub> O <sub>2</sub>                | 48.709       | 1.330  | ND            | ND     | ND           | ND     |
| 6   | 2,4,7-Trimethylcarbazole  | C <sub>15</sub> H <sub>15</sub> N  | 44.130       | 0.478  | D             | D      | ND           | ND     |
| 7   | 2-Oxo-4-phenyl-6-(4-chlorophenyl)-1,2-dihydropyrimidine   | C <sub>16</sub> H <sub>11</sub> ClN <sub>2</sub> O                           | D            | D      | 46.416        | 11.965 | ND           | ND     |
| 8   | 5'-Methyl-[2,2']bithiophenyl-5-carboxylic acid (2-oxo-1,2-dihydroindol-3-ylidene)hydrazide              | C <sub>18</sub> H <sub>13</sub> N <sub>3</sub> O <sub>2</sub> S <sub>2</sub> | 44.071       | 0.586  | ND            | ND     | ND           | ND     |
| 9   | 6-Amino-5-cyano-4-(5-cyano-2,4-dimethyl-1H-pyrrol-3-yl)-2-methyl-4H-pyran-3-carboxylic acid ethyl ester | C <sub>17</sub> H <sub>18</sub> N <sub>4</sub> O <sub>3</sub>                | 48.764       | 0.464  | ND            | ND     | ND           | ND     |
| 10  | 9,10-Methanoanthracen-11-ol, 9,10-dihydro-9,10,11-trimethyl   | C <sub>18</sub> H <sub>18</sub> O  | ND           | ND     | 46.683        | 7.589  | ND           | ND     |
| 11  | 9H-Fluorene-4-carboxylic acid, 9-oxo-, (2,6-dimethylphenyl)amide  | C <sub>22</sub> H <sub>17</sub> NO <sub>2</sub>                              | 48.243       | 1.494  | ND            | ND     | ND           | ND     |
| 12  | Adamantane, 1-isothiocyanato-3-methyl   | C <sub>12</sub> H <sub>17</sub> NS   | D            | D      | 48.785        | 0.377  | 43.941       | 0.050  |
| 13  | A-Neoeleane-3(5),12-diene   | C <sub>30</sub> H <sub>48</sub>  | 45.324       | 2.905  | D             | D      | 45.889       | 0.316  |
| 14  | Benz[b]-1,4-oxazepine-4(5H)-thione, 2,3-dihydro-2,8-dimethyl  | C <sub>11</sub> H <sub>13</sub> NOS  | ND           | ND     | 47.120        | 1.805  | D            | D      |
| 15  | beta-Amyrin   | C <sub>30</sub> H <sub>50</sub> O  | D            | D      | 51.555        | 0.181  | 52.329       | 0.156  |
| 16  | Cannabinol, pentafluoropropionate   | C <sub>24</sub> H <sub>25</sub> F <sub>5</sub> O <sub>3</sub>                | 49.628       | 0.302  | 49.623        | 4.140  | ND           | ND     |
| 17  | Cholest-8-en-3.beta.-ol, acetate  | C <sub>29</sub> H <sub>48</sub> O <sub>2</sub>                               | 45.408       | 5.669  | ND            | ND     | ND           | ND     |
| 18  | dilauryl thiodipropionate   | C <sub>30</sub> H <sub>58</sub> O <sub>4</sub> S                             | 47.190       | 15.080 | ND            | ND     | ND           | ND     |
| 19  | gamma.-Sitosterol (Clionasterol)  | C <sub>29</sub> H <sub>50</sub> O  | 46.078       | 10.747 | ND            | ND     | ND           | ND     |
| 20  | methyl hexacosanoate  | C <sub>27</sub> H <sub>54</sub> O <sub>2</sub>                               | 46.601       | 6.605  | ND            | ND     | ND           | ND     |
| 21  | N-(9-Acridinyl)valine   | C <sub>18</sub> H <sub>18</sub> N <sub>2</sub> O <sub>2</sub>                | 45.931       | 5.676  | ND            | ND     | ND           | ND     |
| 22  | N-Isobutyl-2(E),6(Z),8(E)-decatrienamamide (spilanthol)   | C <sub>14</sub> H <sub>23</sub> NO   | 44.964       | 8.110  | D             | D      | 47.059       | 0.099  |
| 23  | Pentadecane   | C <sub>15</sub> H <sub>32</sub>  | 31.301       | 0.766  | 30.350        | 1.020  | 28.701       | 10.070 |
| 24  | Pyrrrol-2(5H)-one, 4-acetyl-5-(2-chlorophenyl)-1-(2-furfuryl)-3-hydroxy                                 | C <sub>17</sub> H <sub>14</sub> ClNO <sub>4</sub>                            | 48.043       | 1.200  | ND            | ND     | ND           | ND     |
| 25  | Quinoclamine  | C <sub>10</sub> H <sub>6</sub> ClNO <sub>2</sub>                             | 51.903       | 0.494  | ND            | ND     | 46.947       | 0.050  |
| 26  | Sitostenone   | C <sub>29</sub> H <sub>48</sub> O  | 47.760       | 7.991  | ND            | ND     | D            | D      |
| 27  | trans-Ferulic Acid  | C <sub>10</sub> H <sub>10</sub> O <sub>4</sub>                               | 41.741       | 0.827  | ND            | ND     | 41.556       | 0.950  |
| 28  | Urs-12-en-24-oic acid, 3-oxo-, methyl ester   | C <sub>31</sub> H <sub>48</sub> O <sub>3</sub>                               | ND           | ND     | ND            | ND     | 51.073       | 0.085  |
| 29  | β-Caryophyllene   | C <sub>15</sub> H <sub>24</sub>  | D            | D      | 15.480        | 1.300  | 15.285       | 0.059  |
| 30  | Total RA%   |  |              | 73.640 |               | 32.530 |              | 51.320 |

Abbreviations: D, detected but with less than 80% similarity or less than 0.05% RA; ND, not detected; RA, relative abundance (%); Rt, retention time.

*S. aureus* (18.8 ± 0.29 mm), *E. coli* (14.0 ± 0.9 mm) and *B. subtilis* (12.0 ± 0.48 mm) as shown in Table 2. These pathogens cause foodborne diseases can lead to severe health issues among

the residing community, particularly in developing countries. Moreover, frequent appearance of antibiotic-resistant microorganisms has inspired scientists to explore further antimicrobial

agents that are more effective against the microbial pathogens (Uthpala & Navaratne, 2019; Voon et al., 2012).

The MIC is the lowest concentration of extract that completely inhibits the growth of tested microorganisms. The WE showed the lowest MIC values when compared to PEE and EE. The MIC values of CWD dried *Acemella* pod extracts against *E. coli* and *B. subtilis* were lower in the current study than study reported by Lalthanpuui et al., (2017). *Pseudomonas aeruginosa* had shown the lowest MIC value (312.5 µg/ml) for WE while *Candida albicans* had given higher MIC (2,500 µg/ml) value for PEE of CWD dried *Acemella* flower pod. The WE showed lower MIC values against *S. aureus*, *P. aurogenosa*, and *C. albicans* than previously reported MIC values by Holetz et al., (2002). The reported difference might be due to the use of different water-ethanol combinations as well as the drying method applied for raw material preservation. Moreover, those values were in accordance with the antimicrobial properties of pet ether and ethanol extractions irrespective of the solvents used in previous studies (Bedi et al., 2017; Holetz et al., 2002; Uthpala, Navaratne, et al., 2020). Previous studies found that the organoleptic qualities of dried agricultural products using CWD drying were better than those products using conventional hot-air dryers (Prasertsan & Saen-saby, 1998; Uthpala, Navaratne, et al., 2020). Therefore, the CWD drying method leads to better preservation while water is an efficient solvent to obtain effective extracts. The most susceptible bacterium was *P. aurogenosa*. It was found that all extracts obtained from the CWD drying method showed better antibacterial activity against tested pathogens.

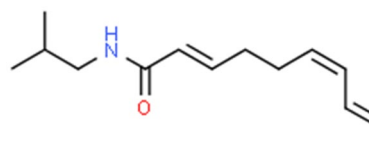
As described by Harborne (1999) phytochemicals can be basically categorized into four major classes as terpenoids, phenolic metabolites, alkaloids, and other nitrogen containing compounds (Dillard & German, 2000; Harborne, 1999). Recent researches in phytoconstituent extractions have revealed that water extractions are more economical and practical in tannin extractions than other solvents (Shakeri et al., 2020). Alkaloids, tannins, and quinones were detected in all of the pod extracts irrespective of the solvent or drying method applied. Flavonoids were only detected in ethanol extracts which had been subjected to the CWD drying and SD process. The total phenols were positive both in all the EE and WE, except PEE. Saponin was only available in WE, while gum and mucilage were only absent in EE. Phlobatanin showed inconsistent patterns across the various solvents and drying methods applied (Table 4). Among all the extracts, WE taken from CWD drying technique has exhibited the richer in all of the tested phytoconstituents excluding flavonoids. Sefidkon et al., (2006) have found that different drying methods created some variation of the relative proportions of the components available in the extractions obtained from aerial parts of the flowering plant of *Satureja hortensis* (Sefidkon et al., 2006). Costa et al., (2016) have studied that the drying operation of microalgae by CWD drying can retain bioactive compounds in the dried product because of the regulated temperature and humidity conditions. This rich profile of phytochemical available in CWD dried, WE of *Acemella* flower might be the reason for its higher antimicrobial potential. Conversely, *Acemella* pods subjected to OD and SD followed by the EE showed lower phytochemical profile compared to other drying and solvent extraction combinations.

GC-MS analysis for CWD dried *Acemella oleracea* flower pod extracts resulted in the identification of 29 compounds, as shown in Table 5. According to the results obtained, the total relative abundance percentages of compounds identified from WE, PEE, and EE were respectively 73.64%, 32.53%, and 51.32%. The CWD dried WE of *Acemella* pods had 20 volatile compounds while PEE and EE had 10 and 11 peaks, respectively.

(S,1Z,6Z)-8-Isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene (2.44%, 3.21%, 39.44%) and pentadecane (0.77%, 1.02%, 10.07%) were commonly identified in WE, PEE, and EE, respectively. (S,1Z,6Z)-8-Isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene is a sesquiterpene which is generally named as D germacrene (Cincotta et al., 2015). Jirovetz et al., (2006) have found that *Acemella radicans* (Jacq.) R.K. Jansen var. *radicans* are comprised with D germacrene which gives dry woody aromatic characteristics and pentadecane which gives weak fatty floral aroma.

Notable amount of dilauryl thiodipropionate (15.08%) followed by gamma-sitosterol (10.75%), spilanthal (8.11%), sitostenone (7.99%), methyl hexacosanoate (6.61%), N-(9-Acridinyl) valine (5.68%), and cholest-8-en-3.βeta.-ol, acetate (5.67%) were identified in WE obtained by CWD drying. Peaks with considerable relative abundancies of 2-oxo-4-phenyl-6-(4-chlorophenyl)-1,2-dihydropyrimidine (11.97%) and 9,10-methanoanthracen-11-ol, 9,10-dihydro-9,10,11-trimethyl (7.59%) were recorded with CWD dried PEE. High amount of D germacrene (39.44%) and pentadecane (10.07%) were noted in CWD dried *Acemella* EE. According to previous research studies, N-Isobutyl-2(E),6(Z),8(E)-decatrienamide which is commonly known as spilanthal (Figure 3) has been identified as the major active alkamide present in *Acemella* plants which are responsible for unique sensorial properties and functional properties including antioxidant, insecticidal, antimicrobial, and etc. (Greger, 2016; Ramsewak, 1999; Uthpala, Navaratne, et al., 2020). Spilanthal was notably identified both in aqueous and ethanol extracts rather than pet ether extract. This may be due to the amphiphilic nature of spilanthal. It is an unsaturated alkamide with a relative polar amide and a less polar fatty acyl which leads to its amphiphilic nature (Abeysinghe et al., 2014; Ramsewak, 1999; Uthpala, Navaratne, et al., 2020).

Twelve compounds out of 20 in WE including 2-(difluoromethyl)-1H-benzimidazole, 2,3-dimethyl-7,7-tetramethylene-6,7-dihydro-1H-pyrrolo[3,4-b]pyridin-4,5-dione, 2,4,7-trimethylcarbazole, 5'-Methyl-[2,2']bithiophenyl-5-carboxylic acid (2-oxo-1,2-dihydroindol-3-ylidene) hydrazide, 6-Amino-5-cyano-4-(5-cyano-2,4-dimethyl-1H-pyrrol-3-yl)-2-methyl-4H-pyran-3-carboxylic acid ethyl ester, 9H-fluorene-4-carboxylic acid, 9-oxo-, (2,6-dimethylphenyl)amide,



**FIGURE 3** 2D image of Spilanthal—(2E,6Z,8E)-N-Isobutyl-2,6,8-decatrienamide



cholest-8-en-3.beta.-ol, acetate, dilauryl thiodipropionate, clonasterol, methyl hexacosanoate, N-(9-acridinyl)valine and pyrrol-2(5H)-one, 4-acetyl-5-(2-chlorophenyl)-1-(2-furfuryl)-3-hydroxy were only detected in CWD dried WE. Among 10 peaks in the PEE four of them were identified namely 1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester and 9,10-Methanoanthracen-11-ol, 9,10-dihydro-9,10,11-trimethyl only in the PEE. Both 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester and urs-12-en-24-oic acid, 3-oxo methyl ester compounds were only detected in EE of CWD dried *Acmella* flower pods. Further, previous studies found that the drying methods had a significant effect on the proportion of the phytoconstituents available in a plant (Hassanpouraghdam et al., 2010; Mohamed Hanaa et al., 2012; Okoh et al., 2008). The GC-MS study revealed that the only WE and EE obtained from CWD dried samples have detectable spilanthol content while PEE have noted the minute amount of spilanthol. These GC-MS outputs further ensure the qualitative analysis of phytoconstituents available (phytochemical screening) in *Acmella* pods subjected to the CWD drying method.

## 5 | CONCLUSION

This study provided a comprehensive evaluation of how the drying method and solvent used for extraction of *Acmella oleracea* flower pods can affect the extraction yield, available phytoconstituents, and antimicrobial activity. Pertaining to both drying methods and solvents, WE obtained from CWD showed the better extraction yield, antimicrobial potential against tested Gram-positive, Gram-negative, and fungal strains. Further, CWD dried flower pods were rich in phytoconstituents including alkaloids, tannins, phenolics, quinones, and other tested compounds except flavonoids. Moreover, GC-MS analysis proved that CWD dried WE of *Acmella* pods have significantly high bioactive compounds including spilanthol, dilauryl thiodipropionate, gamma-sitosterol and, others than extracts obtained from ethanol or pet ether. Concerning the raw material drying method in preserving *Acmella oleracea* flower pod until extraction, CWD drying followed by WE is the best method in order to preserve antimicrobial potential and bioactive constituents. Moreover, pod extracts obtained by this method can be used to control food pathogenic microorganisms in the field of food quality and safety. Future studies should be performed to determine how antimicrobial properties are affected by the synergistic action of phytoconstituents of *Acmella* pod extract against isolated phytoconstituents.

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## CONFLICT OF INTEREST

The authors have declared no conflict of interest.

## AUTHOR CONTRIBUTION

**Uthpala T G G:** Data curation; Formal analysis; Investigation; Methodology; Software; Writing-original draft; Writing-review & editing. **Munasinghe H H:** Methodology; Resources; Supervision; Validation; Writing-review & editing. **Peiris L D C:** Investigation; Supervision; Validation; Writing-review & editing. **Navaratne S B:** Conceptualization; Data curation; Funding acquisition; Investigation; Project administration; Resources; Supervision; Visualization; Writing-review & editing.

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