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VARIATION IN ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES IN *LANTANA CAMARA* L. FLOWERS IN RELATION TO EXTRACTION METHODS

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ABSTRACT

Background. The present work was designed to appraise how different extraction solvents and techniques affect the extractability of antioxidant and antimicrobial components from *Lantana camara* (*L. camara*) flowers.
Material and methods. Four extraction solvents including 100% methanol, 80% methanol, 100% ethanol and 80% ethanol coupled with three extraction techniques namely stirring, microwave-assisted stirring and ultrasonic-assisted stirring employed to isolate extractable components from the flowers of *L. camara*. The extracts produced were evaluated for their antioxidant and antimicrobial attributes.
Results and discussion. The yield of extractable components varied over a wide range 4.87-30.00% in relation to extraction solvent and techniques. The extracts produced contained considerable amounts of total phenolics (8.28-52.34 mg GAE/100 g DW) and total flavonoids (1.24-7.88 mg CE/100 g DW). Furthermore, a promising antioxidant activity in terms of DPPH° scavenging, inhibition of linoleic acid peroxidation and reducing power, as well as antimicrobial potential of the extracts were recorded against the selected bacterial and fungal strains.
Conclusions. It was concluded that both extraction solvent and techniques employed affected the antioxidant and antimicrobial attributes of the extracts from *L. camara* flowers. With few exceptions, overall methanolic extracts produced by ultrasonic-assisted stirring offered superior activities followed by the microwave-assisted stirring and then stirring. The results advocate the use of appropriate extraction strategies to recover potent antioxidant and antimicrobial agents from the flowers of *L. camara* for nutraceutical and therapeutic uses.

Key words: *Lantana camara*, extractable components, total phenolics, total flavonoids, effective extraction, radical scavenging

INTRODUCTION

Lantana camara L., a member of family Verbenaceae, is an evergreen, aromatic weed, native to tropical America, but it is now cultivated in many other parts of the world [Raghu et al. 2004]. Almost all parts of this plant have been used traditionally for treatment of several ailments due to their multiple biological activities such as anthelmintic [Patel et al. 2011], bechic, anti-leukemia [Badakhshan et al. 2009], larvicidal

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[Kumar and Maneemegalai 2008], antioxidant [Bhakta and Ganjewala 2009], antibacterial [Ganjewala et al. 2009], antiproliferative [Gomes-de Melo et al. 2010], antiulcerogenic [Thamotharan et al. 2010], hemolytic [Kalita et al. 2011], antimutagenic activity, antihypertensive [Kaur et al. 2010] and hepatoprotective activities [Abou El-Kassem et al. 2012]. Most importantly, the flower extracts of *L. camara* are used in folk medicine for the management of several disorders including cancers, asthma, tumors, bilious fevers, chicken pox, eczema, measles, ulcers, swellings, high blood pressure, catarrhal infections, rheumatism, tetanus, malaria and abdominal viscera [Ghisalberti 2000, Day et al. 2003]. These medicinal properties and therapeutic uses of this herb are attributed to the presence of several triterpenoids, napthaquinones, flavonoids, alkaloids and glycosides with diverse biological activities [Raghu et al. 2004, Anwar et al. 2013].

Recently, there is a revival of interest in the use of plants as natural remedy for medication of several health disorders due to the reason that they possess multiple biological activities, compatibility with system biology, potential physiological functions and protective role against several degenerative diseases [Suhaj 2006, Iqbal and Bhanger 2007, Tadhani et al. 2007, Espin et al. 2007, Wolfe et al. 2009, Lifschitz 2012, Gaweł 2012].

The extraction of antioxidant components from a plant material is a crucial step so as to accomplish further fractionation, isolation, purification and characterisation of biologically active compounds. A variety of extraction techniques such as orbital shaker, stirring, accelerated solvent extraction, microwave assisted extraction and supercritical fluid extraction etc., are in use to recover antioxidant and nutraceutical components from plant matrices [Wang and Weller 2006, Shabbir et al. 2011, Sultana et al. 2009, Mariod et al. 2012, Anwar and Przybylski 2012]. All techniques have some advantages and disadvantages over others, but none of these is claimed to be perfect in all aspects.

In view of the above-mentioned reports, this study was planned to explore the availability of potent phenolic antioxidants as well as antimicrobial agents of *L. camara* flowers using different extraction techniques and solvents with the major aim to devise an appropriate extraction strategy for isolation of potent antioxidant and antimicrobial extracts.

MATERIAL AND METHODS

Collection of samples

Flowers of *L. camara* were collected from the vicinity of the University of Agriculture, Faisalabad and further identified and authenticated by Dr. Mansoor Hameed, Department of Botany, University of Agriculture, Faisalabad. The flowers were manually washed with distilled water and dried under ambient conditions.

Reagents and standards

All the standard antibiotic and culture media were purchased from Oxoid Ltd. (Hampshire, UK). 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT) (99.0% ascorbic acid, trichloro-acetic acid, catechin, gallic acid, Folin-Ciocalteu reagent, sodium nitrite, aluminium chloride, ferric chloride, potassium ferricyanate, linoleic acid and various reference chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals such as anhydrous sodium carbonate, ferrous chloride, ammonium thiocyanate, chloroform, ethanol and methanol of analytical grade were purchased from Merck (Darmstadt, Germany), unless stated otherwise.

Extracting solvents

The dried flower samples of *L. camara* were ground into a fine powder (80-mesh) using a blender (Anix, Germany). Four solvent systems, 100% methanol, 80% methanol (methanol:water, 80:20 v/v), 100% ethanol and 80% ethanol (ethanol:water, 80:20 v/v) were employed for the extraction of antioxidant components.

Extraction techniques

We used magnetic stirring, and ultrasonic and microwave assisted stirrings for the extraction of antioxidant/antimicrobial components. Briefly, 20 g of finely ground powder of *L. camara* flowers were mixed separately with 200 mL of different extraction solvents and subjected to ultrasonication (30 min) and microwave (5 min) treatment in independent experiments followed by magnetic stirrer (3 h) under ambient temperature (26°C ±1). The extracts were filtered through Whatman No. 1 filter paper and the residues re-extracted twice with fresh solvent following the

same practice. The pooled extracts were freed of solvent at 45°C under reduced pressure, using a rotary evaporator (EYELA, SB-651, Rikakikai Co. Ltd. Tokyo, Japan) and stored at –4°C until used for analyses. The percent yield of extracts was determined gravimetrically.

Total phenolic content (TPC)

The total phenolic contents (TPC) in the *L. camara* flower extracts were estimated following the slightly modified Folin-Ciocalteu reagent method [Chaovanalikit and Wrolstad 2004]. Briefly, crude extract (50 mg) of each extract was mixed with Folin-Ciocalteu reagent (0.5 mL), diluted with deionized water (7.5 mL), incubated at room temperature for 10 min and then mixed with 20% sodium carbonate (1.5 mL) solution. The mixture was heated at 40°C (water bath) for 20 min, cooled and absorbance measured at 755 nm (U-2001, Hitachi Instruments Inc., Tokyo, Japan) to calculate the amount of TP as gallic acid equivalents using a standard curve within range of 10-100 ppm ($R^2 = 0.9986$).

Total flavonoid contents (TFC)

Total flavonoid contents (TFC) in *L. camara* flower extracts were determined by the spectrophotometric method as previously described by Dewanto et al. [2002]. Briefly, 1 mL (0.1 mg/mL) of each extract was diluted with 4 mL of water. To this mixture 0.3 mL of 5% $NaNO_2$, 0.3 mL of 10% $AlCl_3$ and 2 mL of 1.0 M NaOH were added at 5, 6 and 10 min, respectively. The mixture was then diluted with water (2.4 mL) and absorbance read at 510 nm to calculate TFC (g/100 g of DW) as catechin equivalents (CE).

DPPH° Scavenging assay

The 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging potential of biological components extracted from *L. camara* flowers was evaluated spectrophotometrically as described by Tepe et al. [2006]. Aliquots (50 µl) of the extract samples at various concentrations (10-100 µg/ml) were mixed with 5 ml of 0.004% methanol solution of DPPH, incubated for 30 min at room temperature and then absorbance recorded at 517 nm against a blank. Scavenging (%) of DPPH° by different extracts was calculated by the following formula:

$$I\,(\%) = 100 \times (A_{blank} - A_{sample}/A_{blank})$$

Where A_{blank} and A_{sample} denote the absorbance of control and test compounds, respectively.

Antioxidant activity in linoleic acid system

The antioxidant activity of *L. camara* flower extracts was also assessed in terms of measurement of percentage inhibition of peroxidation using linoleic acid system as documented earlier by Iqbal et al. [2007]. Accurately weighed 5 mg extract was transferred to a mixture of 0.13 mL linoleic acid, 10 mL ethanol (99.8%) and 10 mL 0.2 M sodium phosphate buffer (pH 7). The resulting solution was diluted to 25 mL with deionised water. The mixture was incubated at 40°C for 175 h and the extent of oxidation was monitored by the following equation:

$$100 - [(\text{Abs. increase of sample at 175 h / Abs. increase of control at 175 h}) \times 100]$$

Determination of reducing power

The reducing power of different extracts of *L. camara* flowers was assessed using the procedure reported by Yen et al. [2007] with slight modifications. Briefly, 2.5-10.0 mg extract was mixed with 5.0 mL of 0.2 M sodium phosphate buffer (pH 6.6) and potassium ferricyanide (1.0%), and incubated the mixture at 50°C for 20 min. Then, 5 mL of trichloroacetic acid (10%) were added, centrifuged at 5°C in a refrigerated centrifuge machine at 980 X g for 10 min (CHM--17; Kokusan Denki, Tokyo, Japan). The supernatant (5.0 mL) was collected, decanted, diluted with 5.0 mL of distilled water, mixed with 1.0 mL ferric chloride (0.1%) and absorbance read at 700 nm using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan).

Antimicrobial activity

The antimicrobial activity of *L. camara* flower extracts against four bacterial strains: *Escherichia coli*, *Staphylococcus aureus*, *Pasturella multocida*, and *Bacillus subtilis*, and four pathogenic fungi: *Aspergillus flavous*, *Aspergillus niger*, *Alternaria alternata*, and *Rhizopus solani* was assessed by measuring minimum inhibitory concentration (MIC) using the disc diffusion method [National... 1997]. The discs (6 mm in diameter)

were infused with 30 mg/mL of different extracts placed on the inoculated agar. Antibiotics; Amoxycillin and Flumequine (30 μg/disc) were used as positive control for bacteria and fungi, respectively, whereas a disc without samples was used as a negative control.

All the extracts of *L. camara* flowers were tested in Nutrient broth (NB) and Sabouraud dextrose broth (SDB) supplemented with Tween-80 detergent to a final concentration of 0.5% (v/v) for bacteria and fungi, respectively. Growth control (NB/SDB + Tween 80), sterility control (NB/SDB + Tween-80 + test oil) and 20 μL of the test solution were added to 96 well microplates having 160 μL NB and SDB for bacteria and fungi, respectively. The microplates were inoculated with 20 μL (5×10^5 CFU/mL (colony forming units) of standard microorganism suspension and incubated for 24 h at 37°C (bacteria) and 30°C (fungi).

Statistical analysis

All the data acquisition tests were conducted in triplicate and statistical analyses including one-way ANOVA were performed using Microsoft Excell 2010 and Minitabl 13 portable (Minitab Inc., State College, PA) with probability value of $p \leq 0.05$ considered to be statistically significant.

RESULTS AND DISCUSSION

Extract yield

The quantity of available antioxidant components from a plant depends on the nature of solvent, components and target material, as well as on their assimilation during extraction procedure [Hsu et al. 2006]. In the present work, we used methanol and ethanol (100 and 80%, respectively) as extraction media and magnetic stirring (MS), ultrasonic assisted magnetic stirring (UMS) and microwave assisted magnetic stirring (MMS) as extraction techniques to extract biologically active components from *L. camara* flowers. The percentage yield (g/100 g of dry weight) of extractable components from *L. camara* flowers varied significantly in relation to extraction solvents and techniques employed. A maximum yield of extract (30.00%) was obtained with 80% aqueous methanol using UMS, while the minimum (4.87%) in the case of 100% ethanol with MS (Table 1). The variation in percentage extract yields might be attributed to the nature and polarity of extraction solvent, as well as the efficacy of extraction technique towards solubilization and recovery of extractable components [Sultana et al. 2009, Hsu et al. 2006]. Based on the extract yield data, the overall extraction potential of the tested solvents/techniques followed the order: 80% methanol UMS > 80% ethanol MMS > 100% methanol UMS > 80% ethanol MMS > 80% ethanol MS > 80% methanol UMS > 100% methanol MMS > 100% methanol MS > 100% ethanol UMS > 80% methanol MS > 100% ethanol MMS > 100% ethanol MS. The UMS technique with both solvents was found to be much efficient among others that might be linked to its better efficacy towards rupturing plant cell walls, facilitating solvent access to the cell contents,

Table 1. Percentage yield of *L. camara* flower extracts, g/100 g of dry weight

Solvent system	Extraction techniques		
	magnetic stirring (MS)	microwave assisted magnetic stirring (MMS)	ultrasonic assisted magnetic stirring (UMS)
100% methanol	$17.13 \pm 0.34^{A}_{a}$	$17.00 \pm 0.34^{C}_{a}$	$21.53 \pm 0.46^{B}_{b}$
100% ethanol	$4.87 \pm 0.14^{C}_{b}$	$9.87 \pm 0.39^{D}_{a}$	$15.00 \pm 0.20^{D}_{b}$
80% methanol	$14.60 \pm 0.59^{B}_{c}$	$19.53 \pm 0.56^{B}_{b}$	$30.00 \pm 0.60^{A}_{a}$
80% ethanol	$18.87 \pm 0.46^{A}_{b}$	$21.87 \pm 0.44^{A}_{a}$	$18.53 \pm 0.37^{C}_{b}$

Values (mean ±SD) are average of three samples analysed individually in triplicate. Small alphabets in subscript within the same row show significant difference at $P < 0.05$ among extraction techniques. Upper case alphabets in superscript within the same column show significant difference at $P < 0.05$ among extraction solvents.

enhanced mass transfer and provision of high contact surface area between the material and the liquid phase [Novak et al. 2008]. Our present trends revealing best efficacy of aqueous methanol (80% methanol) in conjunction with UMS offering highest extract yield can be supported by the results of Sun et al. [2011] who observed that ethanol coupled with ultrasonic is more efficient than the classical extraction system.

Total phenolic contents (TPC)

Total phenolic contents (TPC) of extracts from *L. camara* flowers produced by different extraction solvents and technique ranged from 8.28-52.34 mg GAE/100 g DW (Table 2). The 80% aqueous methanol when used with UMS (80% methanol-UMS) extracted the highest TP (52.34 mg/100 g DW), while the lowest (8.28 mg/100 g DW) was observed in the case when 100% ethanol was employed with magnetic stirring (100% – MS). This variability in TPC available from the flowers of *L. camara* for different solvents and extraction practices in the present study might be attributed to varying degree of effectiveness of the extraction technique, as well as the solvent employed. The 80% methanol-UMS might have recovered highest amount of total phenolics due to improved cell wall rupturing, better chemical solubilization, facilitated solvent access to the cell contents, enhanced mass transfer and higher contact surface area between both extraction phases during ultrasonication (REFS). Higher recovery of phenolics in the case of 80% methanol-UMS during the present experiments is in accordance with the earlier literature reports [Ghafoor et al. 2009, Khan et al. 2010].

Total flavonoids contents (TFC)

Total flavonoid contents (TFC) of different extracts from *L. camara* flowers ranged from 1.24-7.88 (mg CE/100 g DW). Aqueous methanol (80%) when used along with UMS gave maximum TFC (7.88 mg/100 g DW), while minimum (1.24 mg/100 g DW)

Table 2. Total phenolic content (TPC) of *L. camara* flowers, mg GAE/100 g DW

Solvent system	Extraction technique		
	MS	MMS	UMS
100% methanol	$33.57 \pm 0.67^{A}_{a}$	$22.10 \pm 0.66^{C}_{b}$	$24.90 \pm 0.48^{B}_{b}$
100% ethanol	$8.28 \pm 0.25^{D}_{b}$	$12.00 \pm 0.30^{D}_{a}$	$15.70 \pm 1.71^{D}_{b}$
80% methanol	$25.40 \pm 1.02^{B}_{b}$	$52.20 \pm 1.57^{A}_{a}$	$52.34 \pm 1.56^{A}_{a}$
80% ethanol	$11.70 \pm 0.23^{C}_{b}$	$37.61 \pm 1.50^{B}_{a}$	$13.34 \pm 0.53^{C}_{b}$

Explanations as in Table 1.

Table 3. Total flavonoid contents (TFC) of *L. camara* flowers, mg CE/100 g DW

Solvent system	Extraction technique		
	MS	MMS	UMS
100% methanol	$4.36 \pm 0.08^{A}_{b}$	$3.31 \pm 0.06^{C}_{a}$	$3.74 \pm 0.07^{B}_{a}$
100% ethanol	$1.24 \pm 0.04^{B}_{b}$	$2.25 \pm 0.04^{D}_{a}$	$2.85 \pm 0.03^{B}_{c}$
80% methanol	$3.81 \pm 0.15^{A}_{b}$	$7.85 \pm 0.24^{A}_{a}$	$7.88 \pm 0.16^{A}_{a}$
80% ethanol	$1.76 \pm 0.07^{B}_{b}$	$5.64 \pm 0.22^{B}_{a}$	$2.60 \pm 0.08^{B}_{b}$

Explanations as in Table 1.

with absolute ethanol during MS. Total flavonoid contents of *L. camara* flower extracts were lower than those present in *Boerhaavia diffusa* (9.20 mg/100 g) [Olaleye et al. 2010], however, these values are within the range of green tea (2.37 and 22.5 mg CE/100 g) [Tsai et al. 2008]. A higher level of TFC in the UMS produced extracts during the present analysis is in agreement with previous studies by Ghafoor et al. [2009] and Khan et al. [2010].

DPPH radical scavenging assay

Antiradical activity of the *L. camara* flower extracts was assessed by investigating their potential to scavenge DPPH° and found to be 31.32-60.24% (Table 4). Highest DPPH radical scavenging capacity (60.24%) was noted for the UMS produced aqueous methanol extract (60.24%), while the lowest by the MS absolute methanol extract (31.32%). The variation in DPPH° scavenging ability of *L. camara* flower extracts in relation to different extraction solvents and techniques might be related to the availability of biologically active components as function of extraction media.

When compared with sole related studies, the presently recorded radical scavenging activity of *L. camara* flower extracts was found to be lower than that of leaves of *Salvia miltiorrhiza* (70%) [Zhang et al. 2010], however, it was greater than that of peanut skins (31.5-32.59%) as reported by Nepote et al. [2002].

Antioxidant activity in linoleic acid system

The inhibition of linoleic acid peroxidation by the extracts varied over a wide range 40.85-72.00% versus butylated hydroxyl toluene (BHT) being used as a positive control (90.76%). The extract of *L. camara* flowers obtained using 80% aqueous methanol and UMS showed the highest inhibition (72.00%) of linoleic acid peroxidation, whereas the lowest for MS produced using 100% ethanol.

Overall, the results of the present study indicated that methanol solvent extraction accomplished with UMS offered highest inhibition of linoleic acid

Table 4. DPPH° radical scavenging activity of *L. camara* flower extracts

Solvent system	Extraction technique		
	MS	MMS	UMS
100% methanol	$31.32 \pm 0.60^{C}_{c}$	$52.77 \pm 1.60^{A}_{a}$	$43.61 \pm 1.74^{B}_{b}$
100% ethanol	$34.94 \pm 1.00^{B}_{b}$	$33.01 \pm 1.32^{C}_{b}$	$38.19 \pm 1.12^{C}_{a}$
80% methanol	$40.48 \pm 0.81^{A}_{c}$	$54.22 \pm 1.63^{A}_{b}$	$60.24 \pm 2.40^{A}_{a}$
80% ethanol	$35.16 \pm 0.58^{B}_{b}$	$39.75 \pm 1.19^{B}_{a}$	$39.52 \pm 0.79^{C}_{a}$

Explanations as in Table 1.

Table 5. Percentage inhibition of peroxidation activity of *L. camara* flower extracts

Solvent system	Extraction technique		
	MS	MMS	UMS
100% methanol	43.00 ± 0.90^{Cc}	47.14 ± 1.48^{Cb}	49.71 ± 1.22^{Ca}
100% ethanol	40.85 ± 1.43^{Cb}	42.86 ± 2.11^{Db}	48.57 ± 1.74^{Ca}
80% methanol	45.00 ± 1.80^{Bc}	56.43 ± 1.13^{Ab}	72.00 ± 1.50^{Aa}
80% ethanol	51.29 ± 2.07^{Aa}	52.86 ± 1.28^{Bb}	62.14 ± 1.04^{Ba}

Explanations as in Table 1.

peroxidation among others. The same behaviour of antioxidant activity in linoleic acid system was observed by Toma et al. [2001] who revealed that ultrasonic-assisted extraction considerably increased the recovery of potent antioxidant components.

Reducing power

The reducing potential of extracts (2.5-7.5 mg/mL) from the flowers of *L. camara* yielded by different extraction solvents and techniques (in terms of absorbance data) varied between 0.549 and 0.781 (Table 6) showing a concentration dependent trend. Aqueous ethanol (80% ethanol) extract yielded UMS exhibited the highest reducing power while the lowest for absolute ethanol extract with MS. Furthermore, the reducing potential shown by *L. camara* flower extracts (0.343 to 0.781) was found to be greater than anise (*Pimpinella anisum* L.) seed extracts (0.276) as explored by Gulcin et al. [2003], however, slightly lower than that investigated by Chen et al. [2007] for aqueous methanol extracts of *Psidium guajava* leaves (0.820).

Antimicrobial activity

The results for antimicrobial activity of different extracts from *L. camara* flowers against selected food-borne and pathogenic bacteria and fungi are presented in Tables 7-8. The results show that all the extracts of *L. camara* flowers possess notable antimicrobial activity against bacterial and fungal strains. The minimum inhibitory concentration (MIC) values observed for different extracts by the disc diffusion method, showed that aqueous methanol extract produced by UMS presents the best antimicrobial potential (MIC values 0.07-0.15 μg/ml), against *S. aureus* and *A. flavous* strains. The 80% ethanol in combination with magnetic stirring also extracted a significant antimicrobial activity against *P. multocida* and *A. alternata*

Table 6. Reducing power of *L. camara* flower extracts

Solvent	Concentration mg/mL	Extraction technique		
		MS	MMS	UMS
100% methanol	2.5	0.419 ±0.008^{Aa}	0.496 ±0.014^{Ba}	0.523 ±0.010^{Ca}
	5.0	0.525 ±0.016^{Ab}	0.534 ±0.016^{Bb}	0.568 ±0.014^{Cb}
	7.5	0.678 ±0.027^{Ac}	0.607 ±0.012^{Bc}	0.740 ±0.020^{Cc}
100% ethanol	2.5	0.343 ±0.010^{Aa}	0.421 ±0.017^{Ba}	0.410 ±0.013^{Ca}
	5.0	0.416 ±0.016^{Ab}	0.477 ±0.019^{Bb}	0.511 ±0.015^{Cb}
	7.5	0.549 ±0.011^{Ac}	0.665 ±0.020^{Bc}	0.628 ±0.025^{Bc}
80% methanol	2.5	0.409 ±0.016^{Aa}	0.525 ±0.010^{Ba}	0.421 ±0.008^{Ba}
	5.0	0.457 ±0.009^{Ab}	0.570 ±0.022^{Bb}	0.620 ±0.018^{Cb}
	7.5	0.606 ±0.018^{Ac}	0.706 ±0.028^{Bc}	0.701 ±0.018^{Bc}
80% ethanol	2.5	0.345 ±0.007^{Aa}	0.460 ±0.014^{Ba}	0.527 ±0.015^{Ca}
	5.0	0.404 ±0.01^{Ab}	0.527 ±0.021^{Bb}	0.554 ±0.022^{Cb}
	7.5	0.565 ±0.022^{Ac}	0.654 ±0.026^{Bc}	0.781 ±0.031^{Cc}

Values (mean ±SD) are average of triplicate samples analysed individually in triplicate, whereas 100% and 80% denote absolute and aqueous, respectively. Small alphabets in superscript within the same column show significant difference at $P < 0.05$ among extraction solvents. Upper case alphabets in superscript within the same row show significant difference at $P < 0.05$ among extraction technique used.

Table 7. Antibacterial activity of extracts from *L. camara* flowers

Samples extract	Bacterial strain							
	E. coli		*P. multocida*		*B. subtilis*		*S. ureus*	
	mm[L]	mg/mL[M]	mm	mg/mL	mm	mg/mL	mm	mg/mL
Flumequine	19.70 ±0.8[b]	0.5 ±0.0[d]	21.70 ±1.1[b]	0.4 ±0.0[c]	23.10 ±0.9[d]	0.2 ±0.0[b]	*S. ureus*	0.1 ±0.0[c]
100% M-MS	20.50 ±0.6[b]	0.20 ±0.0[a]	22.00 ±0.4[b]	0.34 ±0.0[b]	22.00 ±0.4[d]	0.15 ±0.0[a]	22.30 ±1.2[a]	0.11 ±0.0[c]
100% E-MS	–	0.23 ±0.0[a]	19.75 ±0.6[a]	0.45 ±0.0[c]	20.00 ±0.6[c]	0.22 ±0.0[b]	25.50 ±1.1[b]	0.09 ±0.0[a]
80% M-MS	20.75 ±0.4[b]	0.34 ±0.0[b]	18.00 ±0.7[a]	0.23 ±0.0[a]	20.50 ±0.8[c]	0.30 ±0.0[c]	28.00 ±0.8[c]	0.12 ±0.0[c]
80% E-MS	18.50 ±0.7[a]	0.52 ±0.0[d]	25.00 ±0.5[c]	0.40 ±0.0[c]	23.50 ±0.7[d]	0.18 ±0.0[ab]	25.00 ±0.5[b]	0.14 ±0.0[c]
100% M-MMS	19.00 ±0.4[a]	0.40 ±0.0[c]	21.00 ±0.6[b]	0.35 ±0.0[b]	17.00 ±0.6[a]	0.25 ±0.0[bc]	22.50 ±0.9[a]	0.13 ±0.0[c]
100% E-MMS	20.75 ±0.6[b]	0.35 ±0.0[b]	30.00 ±1.2[d]	0.40 ±0.0[c]	19.75 ±0.4[b]	0.33 ±0.0[d]	20.00 ±0.6[a]	0.08 ±0.0[a]
80% M-MMS	22.00 ±0.6[c]	0.45 ±0.0[cd]	24.50 ±0.5[c]	0.45 ±0.0[c]	22.00 ±0.8[d]	0.15 ±0.0[a]	24.50 ±0.5[b]	0.19 ±0.0[d]
80% E-MMS	–	0.52 ±0.0[d]	16.50 ±0.3[a]	0.62 ±0.0[d]	19.75 ±0.3[c]	0.21 ±0.0[b]	30.00 ±1.2[d]	0.08 ±0.0[a]
100% M-UMS	19.25 ±0.4[b]	0.60 ±0.0[d]	22.50 ±0.9[b]	0.52 ±0.0[cd]	21.00 ±0.4[bc]	0.24 ±0.0[bc]	30.00 ±1.2[d]	0.13 ±0.0[c]
100% E-UMS	–	0.43 ±0.0[c]	25.00 ±1.0[c]	0.43 ±0.0[c]	18.00 ±0.5[a]	0.23 ±0.0[bc]	25.00 ±0.7[c]	0.15 ±0.0[d]
80% M-UMS	21.00 ±0.8[c]	0.53 ±0.0[d]	28.00 ±0.8[cd]	0.55 ±0.0[cd]	23.00 ±0.7[d]	0.25 ±0.0[bc]	20.00 ±0.6[a]	0.07 ±0.0[a]
80% E-UMS	18.75 ±0.3[a]	0.25 ±0.0[a]	25.50 ±1.1[c]	0.37 ±0.0[b]	22.00 ±0.6[d]	0.14 ±0.0[a]	30.00 ±1.2[d]	0.16 ±0.0[cd]

Values (mean ±SD) are: [L] average diameter of inhibition zone (mm), [M] minimum inhibitory concentration (mg/mL) of triplicate samples analysed. MS – magnetic stirring, MMS – microwave assisted magnetic stirring, UMS – ultrasonic assisted magnetic stirring, M – methanol, E – ethanol. Small alphabets in subscript within the same column show significant difference at $P < 0.05$ among extraction techniques and solvent combinations practiced.

with t inhibition zones (16.50 and 16.48 mm) and MIC value (0.62 and 0.65 µg/ml), respectively.

In general, the antimicrobial activity of the tested *L. camara* flower extracts was found to be comparable with the standard drugs, amoxicillin and flumequine. In comparison with some other related studies, the antimicrobial activity of *L. camara* flower extracts was slightly smaller than that of basil (*Ocimum basilicum* L.) as reported by Hussain et al. [2008]. The methanolic and ethanolic extracts of *Punica granatum* were equally effective against *Bacillus cereus, Escherichia coli*, and *Salmonella aureus* as shown by *L. camara* flower extracts in the present work [Voravuthikunchai and Kitpipit 2005]. Kim et al. [2008] found that the extracts of *Polygonum cuspidatum* strongly inhibited the growth of *B. cereus*, *S. aureus*, and *E. coli*, but lesser than *L. camara* flower extract. It was also found that *L. camara* flower extract exhibited significantly higher activity against *E. coli* and *S. aureus* than that of *Cassia auriculata* reported by Samy and Ignacimuthu [2000].

CONCLUSION

Overall, *L. camara* flowers contained considerable amount of total phenolics and flavonoids, which rendered *L. camara* extracts as potential antioxidant and antimicrobial agents. Of the extraction solvents and extraction systems used, ultrasonic assisted magnetic stirring in combination with aqueous methanol was found to be the most efficient for the maximum recovery of antioxidant and antimicrobial components. Overall, this reveals that *L. camara* flowers contain valuable antioxidant and antimicrobial components that can be isolated for further uses as nutraceuticals and functional food ingredients.

Table 8. Antifungal activity of extracts from *L. camara* flowers

Sample extract	Fungal strain							
	A. niger		*A. flavous*		*R. solani*		*A. alternata*	
	mm	mg/mL	mm	mg/mL	mm	mg/mL	mm	mg/mL
Flumequine	17.80 ±0.7^{a}	0.40 ±0.0^{c}	16.30 ±1.1^{a}	0.60 ±0.0^{d}	19.60 ±1.0^{a}	0.30 ±0.0	15.90 ±0.8^{a}	0.70 ±0.0
100% MS	16.50 ±0.3^{a}	0.35 ±0.0^{b}	18.50 ±0.7^{b}	0.25 ±0.0^{b}	21.00 ±0.6^{b}	0.32 ±0.0	16.75 ±0.7^{a}	0.62 ±0.0
100% ES	18.00 ±0.5^{b}	0.56 ±0.0^{d}	19.00 ±0.5^{b}	0.38 ±0.0^{c}	20.75 ±0.8^{a}	0.28 ±0.0	17.00 ±0.3^{a}	0.52 ±0.0
80% MS	18.50 ±0.7^{b}	0.23 ±0.0^{a}	21.00 ±0.8^{bc}	0.34 ±0.0^{b}	19.00 ±0.4^{a}	0.19 ±0.0	19.50 ±0.6^{b}	0.45 ±0.0
80% ES	21.50 ±0.4^{bc}	0.22 ±0.0^{a}	22.50 ±0.9^{c}	0.28 ±0.0^{b}	21.00 ±0.6^{b}	0.15 ±0.0	21.00 ±0.8^{b}	0.32 ±0.0
100% MMS	18.00 ±0.5^{b}	0.180 ±0.0^{a}	19.00 ±0.6^{b}	0.36 ±0.0	20.75 ±0.8^{b}	0.29 ±0.0	22.75 ±0.4^{c}	0.41 ±0.0
100% EMS	16.50 ±0.6^{a}	0.27 ±0.0^{b}	17.50 ±0.3^{a}	0.59 ±0.0^{d}	19.50 ±0.4^{a}	0.21 ±0.0	18.00 ±0.5^{a}	0.29 ±0.0
80% MMS	24.50 ±0.5^{d}	0.32 ±0.0^{b}	25.00 ±1.0^{d}	0.17 ±0.0^{a}	21.50 ±0.3^{b}	0.15 ±0.0	19.50 ±0.8^{b}	0.57 ±0.0
80% EMS	18.50 ±1.0^{b}	0.21 ±0.0^{a}	18.75 ±0.4^{b}	0.47 ±0.0^{c}	19.00 ±0.6^{a}	0.25 ±0.0	16.50 ±0.3^{a}	0.65 ±0.0
100% MUS	20.00 ±0.4^{b}	0.38 ±0.0^{c}	24.00 ±0.5^{d}	0.23 ±0.0^{a}	22.50 ±0.9^{c}	0.21 ±0.0	23.00 ±0.7^{c}	0.52 ±0.0
100% EUS	20.00 ±0.6^{b}	0.17 ±0.0^{a}	19.00 ±0.6^{a}	0.43 ±0.0^{c}	21.00 ±0.4^{b}	0.34 ±0.0	21.50 ±0.8^{b}	0.27 ±0.0
80% MUS	25.00 ±1.0^{d}	0.15 ±0.0^{a}	26.00 ±0.8^{d}	0.15 ±0.0^{a}	22.75 ±0.7^{bc}	0.21 ±0.0	22.00 ±0.4^{b}	0.65 ±0.0
80% EUS	20.00 ±0.9^{b}	0.32 ±0.0^{b}	21.00 ±0.7^{bc}	0.29 ±0.0^{b}	19.50 ±0.6^{a}	0.25 ±0.0	24.75 ±1.2^{d}	0.55 ±0.0

Explanations as in Table 7.

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