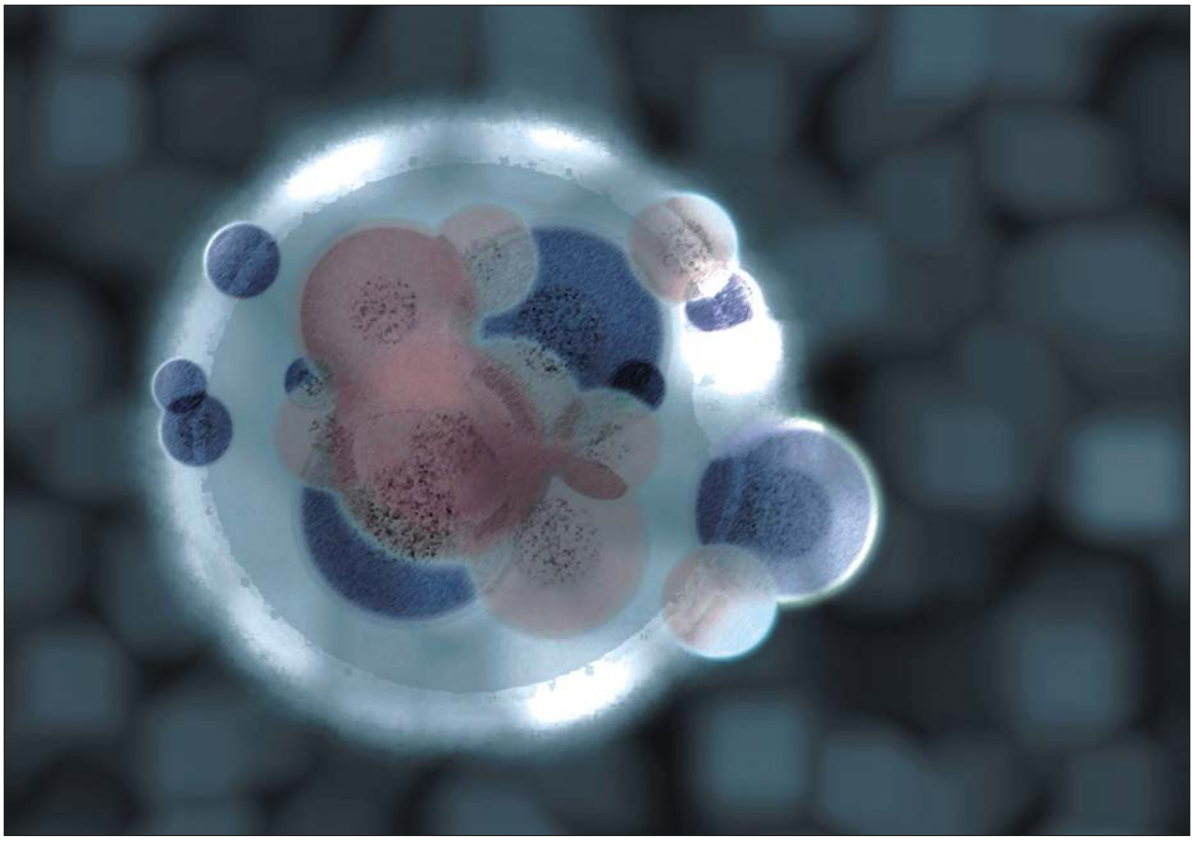

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Journal of Biological Sciences

ISSN 1727-3048
DOI: 10.3923/jbs.2019.280.289

Research Article
Influence of Plant Maturity on Antimicrobial Properties and Toxicity of *Celosia argentea*

Oluwafunmilayo Dorcas Adegbaju, Gloria Aderonke Otunola and Anthony Jide Afolayan

Medicinal Plants and Economic Development (MPED) Research Centre, Department of Botany, University of Fort Hare, Alice 5700, South Africa

Abstract

Background and Objectives: Maturity of a plant is preceded by complex reactions, which leads to the production of a wide range of bioactive compounds with different therapeutic properties and possible toxicity. This study evaluated the antibacterial activities and toxicity of *C. argentea* at three growth stages; pre-flowering, flowering and post-flowering of two trials. **Materials and Methods:** Agar dilution technique was used for evaluation of antimicrobial action of *C. argentea* against three Gram-negative, three Gram-positive pathogenic bacteria and four fungi; while ciprofloxacin and nystatin were used as standards, respectively. The preliminary toxicity assessment was evaluated using Brine Shrimp Lethality Assay (BSLA), with amoxicillin and sea water as the negative and positive controls, respectively. **Results:** Acetone extracts were unable to inhibit the growth of all the organisms tested in all growth stages and at both trials. *Streptococcus pyrogenes* and *P. aeruginosa* were susceptible to the methanol extracts of all the growing phases of both trials at 10 mg mL^{-1}, while *Klebsiella pneumoniae* was susceptible at 10 mg mL^{-1} to only the methanol post-flowering extracts of both trials. Moderate to high antifungal activity of the crude extracts of *C. argentea* were recorded on all the tested fungal strains. *Candida albicans* and *P. aurantiogriseum* were highly susceptible to the extracts with MIC value ranging from $\leq$0.625-5 mg mL^{-1} in both trials. The LC_{50} (>1 mg mL^{-1}) values for *C. argentea* extracts recorded for all the growth stages of *Artemia salina* indicated that the plant extracts were non-toxic. **Conclusion:** These findings indicated that maturity stage influence antimicrobial activity of *C. argentea*. The best time to harvest for antibacterial properties would be the post-flowering stage and the pre-flowering stage for antifungal. Toxicity evaluation showed that *C. argentea* is not toxic at any stage of maturity.

Key words: Microbes, growth stages, diseases, toxicity, *Celosia argentea*

Citation: Oluwafunmilayo Dorcas Adegbaju, Gloria Aderonke Otunola and Anthony Jide Afolayan, 2019. Influence of plant maturity on antimicrobial properties and toxicity of *Celosia argentea*. J. Biol. Sci., 19: 280-289.

Corresponding Author: Gloria Aderonke Otunola, Medicinal Plants and Economic Development (MPED) Research Centre, Department of Botany, University of Fort Hare, Alice 5700, South Africa Tel: +27745276130

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Developmental stages of plants are accompanied by series of secondary metabolism and the accumulation of secondary metabolites which demarcates the onset of one growth stage from another[1]. Plants synthesize different groups of biologically active compounds such as; phenols, flavonoids, tannins, resins and many other metabolites which mediate interactions with other organisms and their environment as they attain maturity. Furthermore, the composition of these compounds varies widely depending on the plant species, topography, seasonal changes, farming practices, age at harvest and their association with their micro-biome; hence, variation in chemical composition modifies their pharmacological activities[2].

The persistence of microbes in the environment and their interaction with humans is central to most infections and illnesses[3]. Clinical discoveries have provided an increased understanding of the complexity and emergence of infectious diseases caused by microbes; as a result, different groups of broad-spectrum antibiotics have been produced to inhibit or kill the growth of micro-organisms. The continuous and inappropriate use of antibiotics has led to microbial resistance, with increasing frequency of side effects. Thus, there is a renewed interest in exploring alternative antimicrobial models with different mechanisms of action and with little or no adverse effects[4].

More recently, plants extracts have been reported to possess excellent antimicrobial properties[5]. Antimicrobial metabolites produced by plants can be as a result of normal plant growth and development (anticipins) or response to microbial attack by transcriptional activation of genes for biosynthetic pathways (Phytoanticipins). These constitutive or triggered antimicrobial chemicals are usually stored in specialized organs or tissues such as; leaves, stems, roots and flowers of plants[6].

Interpreting the level of accumulation of antimicrobial metabolites across developmental stages in order to ascertain when the plant is most efficient against microbes and the level at which it becomes toxic to humans represents a substantial challenge for plant scientists.

In spite of various claims on the efficacy of plant medicines, preference of consumers for natural therapies and the belief that herbal products are superior to synthetic drugs; the problem of their safety, purity, efficacy and quantification continues to be a major concern[7]. Very little information is available regarding the toxicity of most phytomedicines; only a small fraction of the diversity of traditional plant-based medicines is published in pharmacopoeias[8]. Some medicinal plants have been reported to cause adverse reactions and contraindications which could lead to life-threatening injuries [9]. In order to reduce adverse effects caused by the use of herbal medicines, there is need for through scientific validation on the toxicity of these plants[10].

Several assays are used for the research of potential toxicity of plant extracts based on different biological models; of all the toxicological testing techniques on *in vivo* studies, one of the economical, easy to experiment and reproducible toxicity test is the brine shrimp lethality test. Several toxicological studies have reported the use of Brine Shrimp lethality assay for the evaluation of toxicity of heavy metals, medicines, plant extracts, dental materials, cyanobacteria toxins, pesticidal and antiviral compounds[11,12].

Celosia argentea belonging to the family Amaranthaceae, is one of the major green leafy vegetables widely known for its nutritional and medicinal values in the tropical, subtropical and temperate zones of Africa, south America and South East Asia[13]. The species are used in traditional remedies for various diseases including, haemorrhoids, gonorrhea, eczema, gynaecological disorder, piles, mouth sores etc[14]. It has also been found to be effective in the treatment of inflammations, antidote in snake poison, abscesses and skin eruptions[15]. *Celosia argentea* is also known pharmacologically for its antispasmodic, antifungal, antibacterial, nutritive, bile juice increase, antiurolithiatic and anti-inflammatory properties[16]. While, there are various reports on the nutritional folkloric and pharmacological uses of this plant, there is a dearth of information on the accumulation of anti-microbial metabolites and toxic chemical compounds across developmental stages of *C. argentea* and the correlation between the compounds responsible for antimicrobial action and the possible toxicity at different growth phases.

Therefore, this study was conducted to investigate the growth stage at which *Celosia argentea* is most potent for antimicrobial activity and to evaluate its possible potential toxicity using the brine shrimp lethality assay (BSLA).

MATERIALS AND METHODS

Plant source and study site: Mature seeds of *Celosia argentea* obtained from an Agro shop in Nigeria were planted in plastic pots filled with compost soil (Khanya Nursery, Alice Eastern Cape, South Africa) in the glass house of the University of Fort Hare, Alice 5700 Eastern Cape, South Africa. Study site lies at latitude 32°47'-19°26' S; longitude 26°50'-42°306'E and altitude of 514.70 m above sea level. Three harvest periods of two trials: pre-flowering (PRE, leaves and stem were harvested when the first flower was sighted), flowering (FLW, harvest

was done when 50% of all the plants had flowered) and post-flowering (PST, harvest was done when the flowers were few and dropping) stages were used for the investigation.

The first trial was conducted from October, 2017-January, 2018 and the second trial was from March-May, 2018. The plant was authenticated by a taxonomist while a voucher specimen (Ade/med/2017/01) was deposited at the Giffen Herbarium, University of Fort Hare, South Africa.

Preparation of crude extracts: After each harvest, the aerial part of the plants (stem and leaves for pre-flowering; stem, leaves and flowers for flowering and post-flowering) were rinsed with deionized water to remove dirt, then oven-dried at 40°C for 72 h. The dried leaves were pulverized with an industrial electric blender (Hamilton Beach, HBF 500s series, Canada). About 200 g of the ground sample was agitated constantly for 72 h using a shaker (Gallenkamp incubator orbital shaker) individually in 1.5 L of distilled water, acetone or methanol. The solution was thereafter filtered using Whatman No. 1 filter paper, a Buchner funnel and vacuum pump. The aqueous extract was concentrated by using a freeze dryer (Vir Tis Co, Vir Tis benchtop K, Gardiner, NY), while a rotary evaporator (Strike-202 Steroglass, Italy) was used to vaporize the acetone and methanol extracts at their respective boiling points. The dried extracts were stored at 4°C in a refrigerator (Polyscience AD15R-40-A12E, USA) until needed.

Antimicrobial assay: Antimicrobial activity of extracts from *C. argentea* was assessed against 3 Gram-positive (*Staphylococcus aureus* (OK), *Bacillus subtilis* KZN and *Bacillus cereus*) and 3 Gram-negative (*Klebsiella pneumonia* (ATCC 4352), *Pseudomonas aeruginosa* (ATCC 19582) and *Escherichia coli* (ATCC 8739) bacterial strains. The fungal strains used were *Penicillium chrysogenum* (ATCC 10106), *Candida glabarata* (ATCC 66032), *Candida albicans* (ATCC 10231) and *Penicillium aurantiogriseum* (ATCC 16025). The organisms were chosen primarily on the basis of their importance as opportunistic pathogens of humans and food deterioration and were obtained from the Microbiology Unit of the Medicinal Plants and Economic Development (MPED) Research Centre, Botany Department, University of Fort Hare.

The modified Clinical and Laboratory Standard Institute (CLSI) agar dilution assay as described by Wiegand *et al.*[17] and the European Committee for Antimicrobial Susceptibility Testing[18] were employed for the assays. Briefly, Mueller-Hinton and Sabouraud Dextrose agar were prepared by following the manufacturer's description, autoclaved and allowed to cool at 55°C. Stock solutions of the extracts were prepared in dimethyl sulfoxide (DMSO). Two-fold serial dilutions of *C. argentea* extracts and the standard drugs [(ciprofloxacin (antibacterial) and nystatin (antifungal)] were prepared to give concentration range of 12.5, 25, 50 and 100 mg mL^{-1} in the respective broth.

Direct colony suspension method was used to prepare the inocula to give colony suspensions of 1×10^6 and 5×10^5 CFU mL^{-1} for bacteria and fungi, respectively. The agar media containing the extracts and standards was poured into Petri dishes, swirled gently until the agar began to set and left overnight for solvent evaporation. Thereafter, 10 µL each from the prepared inocula was spotted on the surface of the solidified agar to give the desired final inoculum of 1×10^4 CFU/spot and 1×10^3 CFU mL^{-1} for the antibacterial and antifungal assays, respectively. The plates were incubated under aerobic conditions at 37°C and antibacterial readings were taken at 24 h, while the readings for the antifungal effect (incubated at 30°C) were taken at 72 h.

The Minimum Inhibitory Concentrations (MIC) were determined as the lowest concentrations of the extracts/standards at which no bacterial or fungal growth was visible after 24 h for bacterial and 36 h for fungal strains.

Toxicity assay using artemia salina: Toxicity was evaluated as described by Unuofin *et al.*[19] with slight modifications. Briefly, seawater (blank control) was filtered by using a vacuum pump to remove silt. Thereafter, 90 mL of varying concentrations (0.0625-1 mg mL^{-1}) of the crude extracts (dissolved first in 1 mL DMSO) in seawater was prepared. Amoxicillin (positive control) was also prepared in like fashion, but was dissolved directly in seawater. Ten Brine Shrimp nauplii (larvae) was introduced into the prepared crude extracts and controls. Reading was taken every 12 h and the amount of living nauplii was recorded. The experiment stood for 72 h and was well illuminated during the whole period. The percentage Lethality was calculated as:

$$\text{Lethality}(\%) = \frac{\text{Total nauplii} - \text{Alive nauplii}}{\text{Total nauplii}} \times 100$$

Half-lethal concentration (LC_{50}): Dose-response curves and the LC_{50} values were determined by using the data obtained from the percentage lethality of the extracts at different concentrations, growth phases and control experiments. The concentration required for generating 50% lethality of the nauplii (LC_{50}) were determined from the best-fit line obtained by regression analysis of the percentage lethality against concentration.

Data analysis : All data in the lethality assay were expressed as mean±standard deviation (SD) of three replicates. Statistical analysis was performed by using one-way analysis of variance (ANOVA), where data showed significance at $p<0.05$, means were separated by Fischer's LSD with the aid of MINITAB 17 statistical package.

RESULTS

Antibacterial activity: The minimum inhibitory concentration of the antibacterial activity of the different crude extracts of *C. argentea* are as shown in Table 1. Very low to no inhibitory activities were observed for all the organisms at all the concentrations tested. All the organisms were resistant to the acetone extracts at all stages of growth and trials, but susceptible to the aqueous extracts of the post-flowering stage in both trials and the flowering stage of the second trial. *Streptococcus pyogenes* and *P. aeruginosa* were susceptible to the methanol extracts of all the growing phases of both trials at 10 mg mL^{-1} except the post-flowering stage of the 2nd trial where the latter was inhibited at 5 mg mL^{-1}. *Klebsiella pneumoniae* was only susceptible at 10 mg mL^{-1} to the methanol post-flowering extracts in both trials. In contrast, all the organisms were highly susceptible to ciprofloxacin (the positive control) with MIC that ranged from ≤0.125–0.25 µg mL^{-1}.

Antifungal activity: The MIC of the antifungal investigation (Table 2) revealed moderate to high antifungal activity of the crude extracts of *C. argentea. Candida albicans* and *P. aurantiogriseum* were highly susceptible to all *C. argentea* extracts with MIC ranging from ≤0.625–5 mg mL^{-1} in both trials. However, the aqueous and methanol extracts of the pre-flowering stage gave the best inhibitory activity on these organisms for both trials.

Toxicity evaluation: Response of the Brine shrimp nauplii to extracts of *C. argentea,* amoxicillin and seawater for both trials is shown in Fig. 1: The percentage lethality of the extracts ranged from 0.83-5.5% in the first trail and from 0.83-5.17% in the second trial. The acetone extracts exhibited the highest lethality against brine shrimp at all stages of growth, while the aqueous extract was the least lethal for both trials. All the crude plant extracts of both trials and seawater did not show any statistical significance at $p<0.05$, but were, however, statistically different from the control ($p>0.05$) which had a lethality of 22.33±3.95%.

Table 1: Minimum Inhibitory Concentrations (MICs) of *C. argentea* extracts from different growth stages on selected Gram-negative and Gram-positive bacteria

	First trial									Second trial									
	Pre-flowering (mg mL^{-1})			Flowering (mg mL^{-1})			Post-flowering (mg mL^{-1})			Pre-flowering (mg mL^{-1})			Flowering (mg mL^{-1})			Post-flowering (mg mL^{-1})			Standard (µg mL^{-1})
	Aqu	Ace	Meth	Aqu	Ace	Meth	Aqu	Ace	Meth	Aqu	Ace	Meth	Aqu	Ace	Meth	Aqu	Ace	Meth	Cip
S. pyrogenes (+ve)	>10	>10	10	>10	>10	10	10	>10	10	>10	>10	10	10	>10	10	10	>10	10	≤ 0.125
B. subtilis (+ve)	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	≤ 0.125
S. aureus (+ve)	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	≤ 0.125
K. pneumoniae (-ve)	>10	>10	>10	>10	>10	>10	>10	>10	10	>10	>10	>10	>10	>10	>10	>10	>10	10	0.25
E. coli (-ve)	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	≤ 0.125
P. aeruginosa (-ve)	>10	>10	10	>10	>10	10	>10	>10	10	>10	>10	10	>10	>10	10	>10	>10	5	0.25

Aqu: Aqueous extract, Ace: Acetone extract, Met: Methanol extract, Cip: Ciprofloxacin, >: Value greater than the highest concentration tested and ≤: Value lesser than or equal to the lowest concentration tested

Table 2: Minimum Inhibitory Concentrations (MICs) of *C. argentea* extracts from different growth stages on selected fungi at 36 h

	First trial									Second trial									
	Pre-flowering (mg mL^{-1})			Flowering (mg mL^{-1})			Post-flowering (mg mL^{-1})			Pre-flowering (mg mL^{-1})			Flowering (mg mL^{-1})			Post-flowering (mg mL^{-1})			
	Aqu	Ace	Meth	Aqu	Ace	Meth	Aqu	Ace	Meth	Aqu	Ace	Meth	Aqu	Ace	Meth	Aqu	Ace	Meth	Nys
P. chrysogenum	>10	>10	>10	>10	5	>10	>10	>10	>10	>10	>10	>10	>10	5	>10	>10	>10	>10	8
C. albicans	1.25	5	≤0.625	2.5	2.5	≤0.625	2.5	2.5	1.25	≤0.625	5	≤0.625	2.5	2.5	1.25	5	5	1.25	8
C. glabrata	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	4
P. aurantiogriseum	≤0.625	5	≤0.625	2.5	1.25	≤0.625	2.5	2.5	≤0.625	≤0.625	5	≤0.625	≤0.625	≤0.625	1.25	5	2.5	≤0.625	8

Aqu: Aqueous extract, Act: Acetone extract, Met: Methanol extract, Nys: Nystatin, >: Value greater than the highest concentration tested) and ≤: Value lesser than or equal to the lowest concentration tested

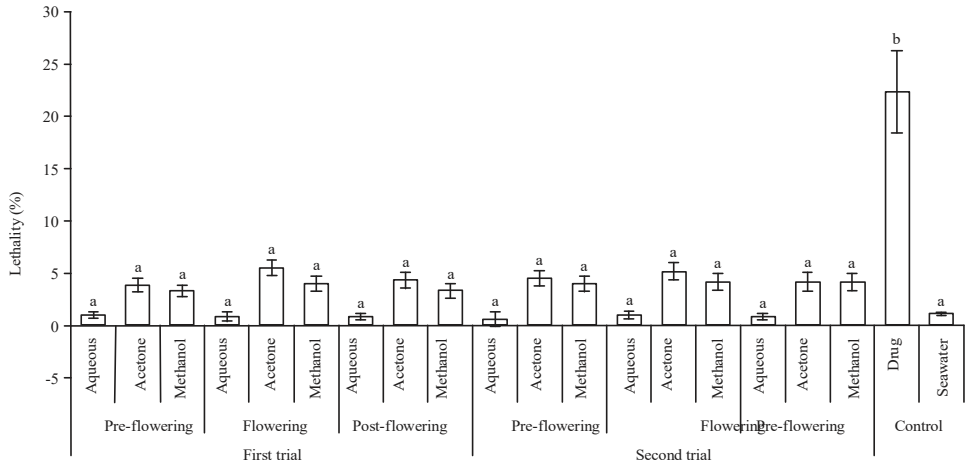

Fig. 1: Percentage lethality of crude extracts of *C. argentea* harvested at different growth stages and controls against *Artemia salina* nauplii

Means are values of five concentrations of the crude extracts/control±SD of three replications. Bars with different letters are significantly different from each other ($p<0.05$)

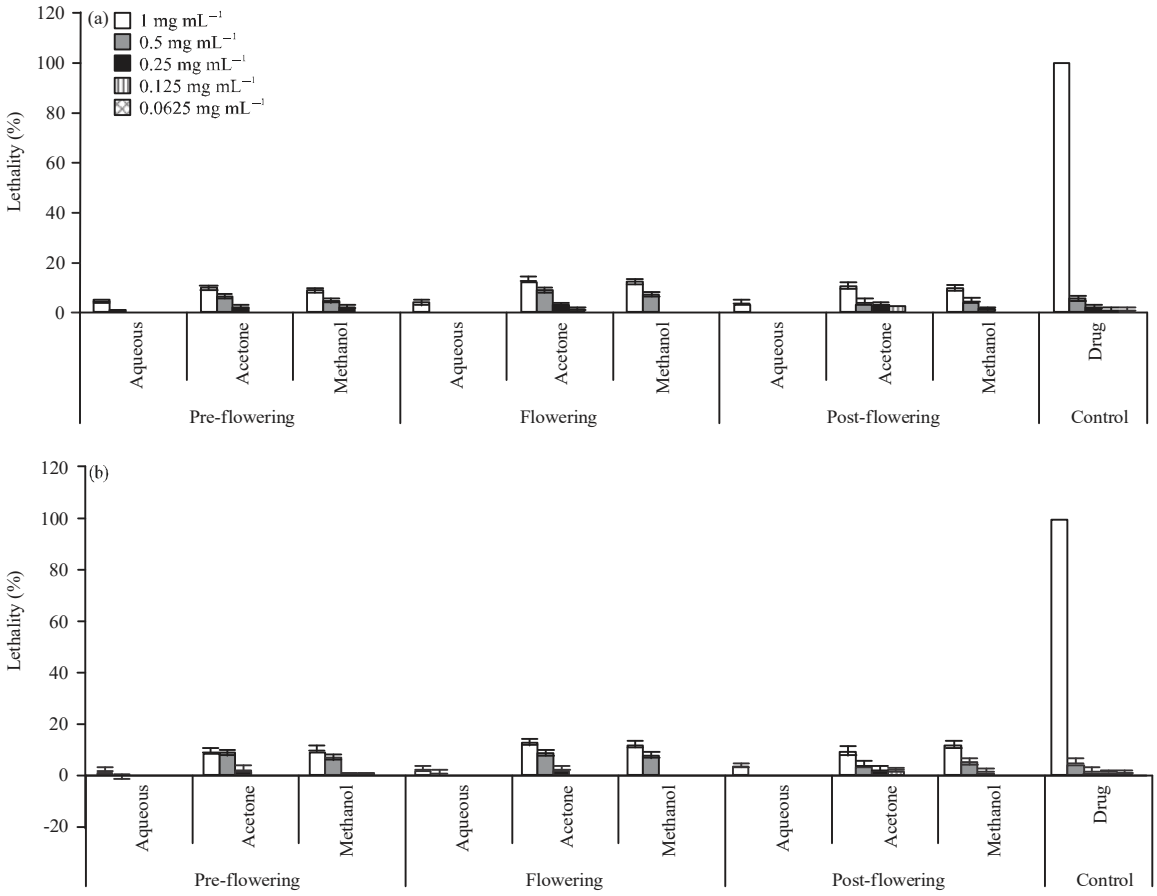

Fig. 2(a-b): Percentage lethality of varying concentrations of *C. argentea* extracts from different growing stages and control against *A. salina*, (a) First trial and (b) Second trial

Values are means of the replications (at different hours) for the concentrations±SD

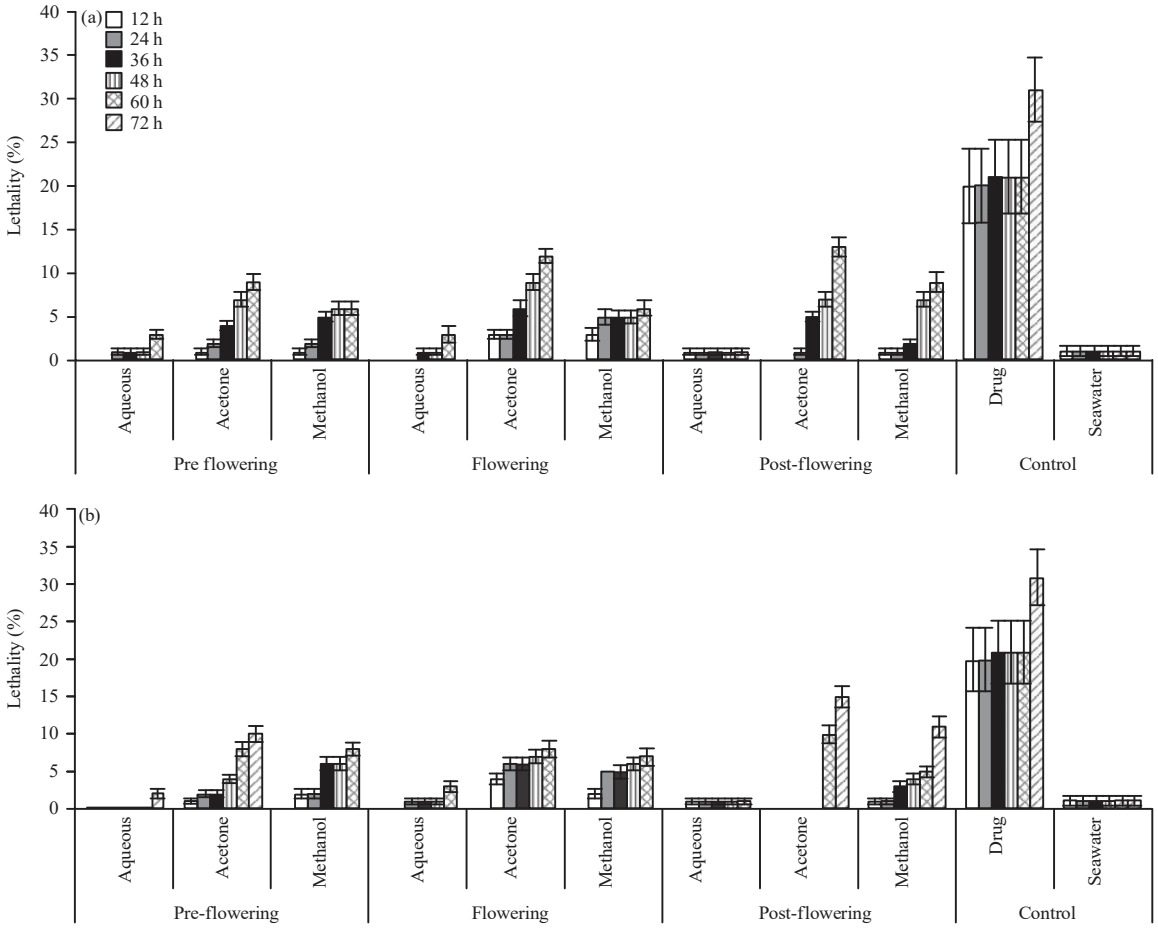

Fig. 3(a-b): Effect of exposure time to the crude extracts of *C. argentea* on the mortality of *A. salina*, (a) First trial and (b) Second trial

Values are means of the replications (of all the concentrations) for each extract/controls±SD

Table 3: LC_{50} of *C. argentea* on *Artemia salina*'s nauplii

Extracts		LC_{50} (mg mL^{-1})
First trial		
Pre-flowering	Aqueous	>1
	Acetone	>1
	methanol	>1
Flowering	Aqueous	>1
	Acetone	>1
	methanol	>1
Post-flowering	Aqueous	>1
	Acetone	>1
	methanol	>1
Second trial		
Pre-flowering	Aqueous	>1
	Acetone	>1
	methanol	>1
Flowering	Aqueous	>1
	Acetone	>1
	methanol	>1
Post-flowering	Aqueous	>1
	Acetone	>1
	methanol	>1
Standard drug	Amoxicillin	0.75±0.10

Figure 2 shows that lethality for all the extracts was concentration dependent and increased with concentration. At the highest concentrations (1 mg mL^{-1}), the aqueous extracts showed lethality of 4.17±0.51% at all stages of growth in the first trial and 2.92±0.52% (pre-flowering), 3.33±0.49% (flowering) and 4.17±0.51% (post-flowering) in the second trial. The highest lethality was recorded at 1 mg mL^{-1} of the acetone extracts for both trials.

Assessment of the extracts against nauplli incubated at different exposure times (Fig. 3) showed that lethality of extracts against nauplii was time dependent. The first trial showed 0% in all the crude extracts at 12 h, but in the second trial, there was 0.3% and 1±0.32% mortality in aqueous flowering and acetone pre-flowering/methanol post-flowering, respectively. The highest lethality of the extracts against the brine shrimp was recorded after 72 h of incubation with all the acetone extracts. Seawater maintained an average lethality of 1.11±0.57% all through the time of exposure.

The half minimal concentrations (LC_{50}) required to kill 50% of the larvae (nauplii) is shown in Table 3. All the extracts and seawater exhibited LC_{50} >1 mg mL^{-1}, while amoxicillin showed LC_{50} of 0.75±0.10 mg mL^{-1}.

DISCUSSION

Over the years, there has been a decrease in microbial susceptibility to existing antimicrobial agents responsible for critical point drug resistance in the healthcare sector[20]. As a result, secondary metabolites from natural sources such as plants have formed the basis for drug development, because they are being produced within the living systems, they are perceived to exhibit more similarities to drugs and show more biological friendliness than synthetic drugs[21]. However, various studies of plants in natural communities have found inconsistent patterns of allocation to secondary metabolites across the developmental stages of plant life[22,23]. Also, the diversity of secondary metabolites in plants, which may either be distributed throughout the plant or occur only in specific structures or locations are independent of each other; hence, quantifying the distribution of biologically active compounds at different stage of plant maturity could help identify when a plant is most potent for a particular medicinal property.

The low inhibitory activity recorded at the pre-flowering and flowering stage of the plant, is a pointer that harvest time of *C. argentea* has significant effect on the antibacterial potential of the plant. This knowledge is very imperative in decision making as it gives firsthand information on when in harvest this species for antibacterial purposes. The methanol extract showed better inhibitory activity than the other solvents used in this study.

This is an indication that different solvents have different ability to extract diverse bioactive compounds from plants. This is also important as it could serve as a guide to the right choice of solvent in bioactivity screening. The methanol extract at all growth stages in both trials inhibited *S. pyrogenes;* which is a group A *Streptococcus* that has developed complex virulence mechanism of maneuvering host defenses and is responsible for causing acute life threatening infections like pharyngitis, impetigo, rheumatic fever and cellulitis to mention a few[24-26]. This suggested that *C. argentea* could be an important therapeutic agent in the treatment and management of the above-mentioned infections. Also, inhibited was *P. aeruginosa* which is known to cause urinary tract infections (UTIs), respiratory, dermatitis, gastrointestinal infections and a variety of systemic infections. It is also responsible for nosocomial and ventilator-associated pneumonia[27]. Development of resistance to multiple antibiotics is also a known trend associated with *P. aeruginosa*; as it has the ability to form biofilms[28]. The ability of *C. argentea* to inhibit the growth of this organism could account for its ethnopharmacological report as an antispasmodic, anti-inflammatory and antiurolithiatic agent[29]. *Klebsiella pneumoniae* was susceptible to the methanol post-flowering extracts of both trials at 10 mg mL^{-1}. This also corroborated the results of Thakur[30], who reported the inhibition of *K. pneumoniae* by *C. argentea* plant extracts. This suggests that *C. argentea* methanol extracts could have the potential of managing infections caused by *K. pneumonia*. However, Okpako and Ajibesin[31] reported very good antimicrobial activity of *C. argentea* leaf, stem and root on selected bacteria and fungi with MIC that ranged from 12.5-50 µg mL^{-1} and MBC range of 25-50 µg mL^{-1}. This high level of activity is in contrast to the low antibacterial activity observed in this study and could be due in part to the plant material and location; while Okpako and Ajibesin[31] got their plant in the wild and different geographical region of Nigeria, the *C. argentea* used for this study was raised in a regulated nursery with no stress condition whatsoever in South Africa.

The antifungal evaluation of *C. argentea* showed very good inhibitory activity against selected fungi. Similar to the antibacterial activity, the methanol extracts of the first and second trial showed the best antifungal activities with MIC of ≤0.625 for *C. albicans* and *P. aurantiogriseum.*

Candida albicans is responsible for several infectious diseases which are not limited to vaginal yeast infections, thrush, diaper rash and candidemia; it causes the invasion of the organs throughout body as a result of the contamination of the bloodstream[32]. The susceptibility of *C. albicans* to *C. argentea* extracts justified the antifungal properties of the plant and could serve as the basis for producing a functional drug for the management of candidiasis.

Overall, minimal antibacterial activities were observed when compared to the antifungal activities of the plant extracts. Similar to our results, Kasim *et al.*[33] reported a low antibacterial activity of *C. argentea* methanol extract on 4 bacterial species with a MIC which ranged from 50-100 mg mL^{-1}. There was no positive correlation in the antibacterial and anti-fungal properties of *C. argentea*. These results indicates that *C. argentea* is likely to possess more than one active compound responsible for the inhibition of microbes.

Ethnopharmacologically, plant derived antimicrobial agents have been used in traditional medicine for the treatment of different kinds of infections. However, owing to the chemical diversity of plants secondary metabolites, plant extracts with efflux inhibitory activity against microbes could have other target sites than antimicrobial inhibition and

subsequently become toxic at a certain level[34]. This is because a desired activity of a crude plant extract is brought about by synergistive interactions rather than a single component[35]. Ginovyan *et al.*[36] reported that plant-derived antimicrobial chemotypes are far from ideal due to undesirable side effects or toxicity, hence, there is the urgent need to investigate potential toxicity from crude plant extracts.

Meyer *et al.*[37] and Bastos *et al.*[38] indices of toxicity states that LC_{50} greater than 1 mg mL^{-1} is not toxic; LC_{50} greater or equal to 0.5 mg mL^{-1}, but not greater than 1 mg mL^{-1} are considered to have weak toxicity, while LC_{50} values less than 0.5 mg mL^{-1} are toxic. The outcome of this study revealed that all the extracts from the different growth stages are not toxic as they all have LC_{50} values >1 mg mL^{-1}. Stemming from this premise, all the crude extracts of *C. argentea* in this study could be considered safe for consumption and can further be explored as a source of plant-based pharmaceutical. The effect of time of exposure to ascertain if prolonged exposure to the crude extracts would have adverse effect on the nauplii also showed that exposure in the extract up to 72 h did not result to significant lethality.

CONCLUSION

To the best of our knowledge, this is the first report of the effect of growth stages on the antimicrobial and toxicity of *C. argentea*. The study revealed that maturity could influence the bioactivity of *C. argentea* especially with respect to antimicrobial activity. The post-flowering stage of *C. argentea* exhibited the best anti-bacterial activity, while the pre-flowering stage exhibited the best anti-fungal activity. Also, the plant did not show a toxic effect on Brine shrimp even at the highest concentration used in this study. Therefore, *C. argentea* is considered safe for consumption and the development of natural therapeutic agents against opportunistic diseases of humans and animals.

SIGNIFICANCE STATEMENT

This study gives insights on the antimicrobial activities and possible toxicity of *Celosia argentea* across developmental stages. The study revealed that there is a significant variation in the antimicrobial activities of this plant at different growth stages. Furthermore, the stage of growth at which the plant is more potent against bacterial strains (post-flowering stage) differs from the stage of growth at which the plant showed best inhibitory activity against fungal strains (Pre-flowering stage). Extracts of *Celosia argentea* were found to be non-toxic at all stages of growth, even at the highest concentration tested. These findings can be beneficial for the development of natural therapeutic agents from *Celosia argentea* extracts against opportunistic diseases of humans and animals. Finally, this study will help plant scientist to identify and interpret the best stage of growth at which this plant accumulate phytocompounds responsible for certain biological activities and the level at which it becomes toxic to humans.

ACKNOWLEDGMENT

The authors wish to appreciate the financial support of Govan Mbeki Research Development Centre (GMRDC), University of Fort Hare, South Africa. Grant number C127.

REFERENCES

1. Lattanzio, V., P. Kroon, S. Quideau and D. Treutter, 2008. Plant Phenolics-Secondary Metabolites with Diverse Functions. In: Recent Advances in Polyphenol Research, Daayf, F. and V. Lattanzio (Eds.). Vol. 1, Blackwell Publishing Ltd., New York, USA., pp: 1-24.
2. Tsao, R., S. Khanizadeh and A. Dale, 2006. Designer fruits and vegetables with enriched phytochemicals for human health. Can. J. Plant Sci., 86: 773-786.
3. Upadhyay, A., I. Upadhyaya, A. Kollanoor-Johny and K. Venkitanarayanan, 2014. Combating pathogenic microorganisms using plant-derived antimicrobials: A minireview of the mechanistic basis. BioMed Res. Int., Vol. 2014. 10.1155/2014/761741.
4. Lederberg, J., M.A. Hamburg and M.S. Smolinski, 2003. Microbial Threats to Health: Emergence, Detection and Response. National Academies Press, USA., ISBN: 9780309278751, Pages: 397.
5. Chandra, H., P. Bishnoi, A. Yadav, B. Patni, A.P. Mishra and A.R. Nautiyal, 2017. Antimicrobial resistance and the alternative resources with special emphasis on plant-based antimicrobials-A review. Plants, Vol. 6. 10.3390/plants6020016.
6. Bednarek, P. and A. Osbourn, 2009. Plant-microbe interactions: Chemical diversity in plant defense. Science, 324: 746-748.
7. Kasilo, O.M.J. and J.M. Trapsida, 2011. Regulation of traditional medicine in the WHO African region. Afr. Health Monitor (Special Issue), 14: 25-31.

8. Upton, R., A. Graff, G. Jolliffe, R. Langer and E. Williamson, 2016. American Herbal Pharmacopoeia: Botanical Pharmacognosy-Microscopic Characterization of Botanical Medicines. CRC Press, USA., ISBN: 9781420073287, Pages: 800.
9. Ekor, M., 2014. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol., Vol. 4. 10.3389/fphar.2013.00177.
10. Ohikhena, F.U., O.A. Wintola and A.J. Afolayan, 2016. Toxicity assessment of different solvent extracts of the medicinal plant, *Phragmanthera capitata* (Sprengel) balle on brine shrimp (*Artemia salina*). Int. J. Pharmacol., 12: 701-710.
11. Harwig, J. and P.M. Scott, 1971. Brine shrimp (*Artemia salina* L.) larvae as a screening system for fungal toxins. Applied Microbiol., 21: 1011-1016.
12. Hamidi, M., B. Jovanova and T. Panovska, 2014. Toxic o logical evaluation of the plant products using brine shrimp (*Artemia salina* L.) model. Macedonian Pharm. Bull., 60: 9-18.
13. Adegbaju, O.D., G.A. Otunola and A.J. Afolayan, 2018. Effect of temperature, light and sowing depth on seed germination of *Celosia argentea* L. Asian J. Plant Sci., 17: 47-55.
14. Nidavani, R.B., A.M. Mahalakshmi, M. Seema and K.L. Krishna, 2014. Pharmacology of *Celosia argentea* L. J. Atoms Mol.: Int. Online J., 4: 635-644.
15. Kanu, C.L., O. Owoeye, I.O. Imosemi and A.O. Malomo, 2017. A review of the multifaceted usefulness of *Celosia argentea* Linn. Eur. J. Pharm. Med. Res., 4: 72-79.
16. Ghule, S., T. Prakash, D. Kotresha, R. Karki, V. Surendra and D. Goli, 2010. Anti-diabetic activity of *Celosia argentea* root in streptozotocin-induced diabetic rats. Int. J. Green Pharm., 4: 206-211.
17. Wiegand, I., K. Hilpert and R.E.W. Hancock, 2008. Agar and broth dilution methods to determine the Minimal Inhibitory Concentration (MIC) of antimicrobial substances. Nat. Protocol., 3: 163-175.
18. EUCAST., 2003. Determination of Minimum Inhibitory Concentrations (MICs) of antibacterial agents by broth dilution. Clin. Microb. Infect., 9: 1-7.
19. Unuofin, J.O., G.A. Otunola and A.J. Afolayan, 2018. Evaluation of acute and subacute toxicity of whole-plant aqueous extract of *Vernonia mespilifolia* Less. in wistar rats. J. Integr. Med., 16: 335-341.
20. Savoia, D., 2012. Plant-derived antimicrobial compounds: Alternatives to antibiotics. Future Microbiol., 7: 979-990.
21. Shoeb, M., 2006. Anticancer agents from medicinal plants. Bang. J. Pharmacol., 1: 35-41.
22. Schaefer, H.M., V. Schmidt and H. Winkler, 2003. Testing the defence trade off hypothesis: how contents of nutrients and secondary compounds affect fruit removal. Oikos, 102: 318-328.
23. Beckman, N.G., 2013. The distribution of fruit and seed toxicity during development for eleven Neotropical trees and vines in Central Panama. PLoS One, Vol. 8. 10.1371/journal.pone.0066764.
24. Lamagni, T. L., S. Neal, C. Keshishian, D.N. Powell and R. Potz *et al*., 2009. Predictors of death after severe *Streptococcus pyogenes* infection. Dispatches: Emerg. Infect. Dis., 15: 1304-1307.
25. Westbroek, M.L., C.L. Davis, L.S. Fawson and T.M. Price, 2010. Interactions of *Lactobacilli* with pathogenic *Streptococcus pyogenes*. Infect. Dis. Obstet. Gynecol., Vol. 2010. 10.1155/2010/289743.
26. McCann, M.T., B.F. Gilmore and S.P. Gorman, 2008. *Staphylococcus epidermidis* device related infections: Pathogenesis and clinical management. J. Pharm. Pharmacol., 60: 1551-1571.
27. Dantas, R.C., M.L. Ferreira, P.P. Gontijo-Filho and R.M. Ribas, 2014. *Pseudomonas aeruginosa* bacteraemia: Independent risk factors for mortality and impact of resistance on outcome. J. Med. Microbiol., 63: 1679-1687.
28. Gierhart, S. and U. Chukwuma, 2017. Annual surveillance summary: *Pseudomonas aeruginosa* infections in the Military Health System (MHS), 2015. Prepared March 2017, EpiData Center Department, NMCPHC-EDC-TR-195-2017er. https: //apps.dtic.mil/dtic/tr/fulltext/u2/1040552.pdf
29. Shelke, T., S. Wayal, S. Gunjegaokar, S. Gaikwad, A. Shirsath and S. Hadke, 2014. An overview on Indian medicinal plants with antiurolithiatic activity. J. Pharm. Res. Clin. Pract., 4: 33-40.
30. Thakur, H.A., 2017. Phytochemical screening, antibacterial and antifungal activity of Pinda concanensis (Dalzell) P.K. Mukh. and Constance (Apiaceae) an endemic plant from Nasik district (M.S.) India. Ann. Plant Sci., 6: 1886-1892.
31. Okpako, E. and K.K. Ajibesin, 2015. Antimicrobial activity of *Celosia argentea* L. amaranthaceae. Am. J. Res. Commun., 3: 123-133.
32. Nobile, C.J. and A.D. Johnson, 2015. *Candida albicans* biofilms and human disease. Annu. Rev. Microbiol., 69: 71-92.
33. Kasim, L.S., O. Ayodele, H.I. Effedua, O.E. Adejumo, M. Ekor and T.O. Fajemirokun, 2012. Antimicrobial activity of six selected plants against some strains of pathogenic organisms. J. Microbiol. Antimicrob., 4: 54-59.
34. Fiamegos, Y.C., P.L. Kastritis, V. Exarchou, H. Han and A.M.J.J. Bonvin *et al*., 2011. Antimicrobial and efflux pump inhibitory activity of caffeoylquinic acids from *Artemisia absinthium* against gram positive pathogenic bacteria. PLoS One, Vol. 6. 10.1371/journal.pone.0018127.
35. Baravalia, Y., K. Nagani and S. Chanda, 2011. Evaluation of pharmacognostic and physicochemical parameters of *Woodfordia fruticosa* Kurz. Flowers Pharm. J., 2: 13-18.

36. Ginovyan, M., M. Petrosyan and A. Trchounian, 2017. Antimicrobial activity of some plant materials used in Armenian traditional medicine. BMC Complement. Alternat. Med., Vol. 17. 10.1186/s12906-017-1573-y.
37. Meyer, B.N., N.R. Ferrigni, J.E. P utnam, L.B. Jacobson, D.E. Nichols and J.L. McLaughlin, 1982. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Med., 45: 31-34.
38. Bastos, M.L.A., M.R.F. Lima, L.M. Conserva, V.S. Andrade, E.M.M. Rocha and R.P.L. Lemos, 2009. Studies on the antimicrobial activity and brine shrimp toxicity of *Zeyheria tuberculosa* (Vell.) Bur. (Bignoniaceae) extracts and their main constituents. Ann. Clin. Microbiol. Antimicrob., Vol. 8. 10.1186/1476-0711-8-16.