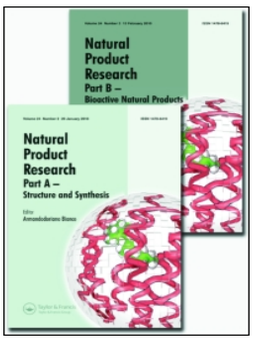


Natural Product Research

Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: http://www.tandfonline.com/loi/gnpl20

Phytochemical and biological study of callus cultures of Tulbaghia violacea Harv. Cultivated in Egypt

Hanaa H. Eid & Ghada F. Metwally

To cite this article: Hanaa H. Eid & Ghada F. Metwally (2017) Phytochemical and biological study of callus cultures of Tulbaghia violacea Harv. Cultivated in Egypt, Natural Product Research, 31:15, 1717-1724, DOI: 10.1080/14786419.2017.1289206

To link to this article: https://doi.org/10.1080/14786419.2017.1289206

NATURAL PRODUCT RESEARCH, 2017
VOL. 31, NO. 15, 1717–1724
https://doi.org/10.1080/14786419.2017.1289206

Taylor & Francis
Taylor & Francis Group

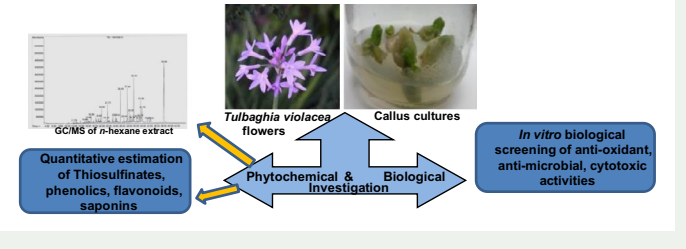
Phytochemical and biological study of callus cultures of *Tulbaghia violacea* Harv. Cultivated in Egypt

Hanaa H. Eid[a] and Ghada F. Metwally[b]

[a]Faculty of Pharmacy, Department of Pharmacognosy, Cairo University, Cairo, Egypt; [b]Department of Plant Tissue Culture, National Organization for Drug Control & Research (NODCAR), Giza, Egypt

ABSTRACT

As *in vitro* plant cultures are used extensively to produce bioactive metabolites, our goal was to establish calli from *Tulbaghia violacea* Harv. flowers and assess the tissue phytochemically and biologically. Murashige & Skoog medium(MS) + 22.6 µM 2,4-dichlorophenoxyacetic acid +2.2 µM benzylaminopurine induced callus from flowers. Gas chromatography/mass spectrometry(GC/MS) analyses of *n*-hexane extracts of calli(HC) and flowers(HF) revealed 33 and 32 components(92.6 and 98.5%, respectively). Hydrocarbons were predominant in HC (55.0%), whereas a higher percentage of oxygenated compounds was found in HF(74.6%). *Trans*(E)-anethole(39.1%) and 16-hentriacontanone (30.3%) dominated in HF and HC, respectively. However, sulphur compounds were only detected in HF. Quantitative estimation of thiosulphinates, phenolics, flavonoids and saponins in ethanolic extracts of calli(EC) and flowers(EF) showed much higher contents in EF. Antioxidant, antimicrobial and cytotoxic screening of extracts demonstrated that EF was the most potent, followed by HF and EC; conversely, HC was inactive. Although HC and EC were less biologically active, these calli could be an alternative source of bioactive metabolites.



ARTICLE HISTORY
Received 3 September 2016
Accepted 12 January 2017

KEYWORDS
Tulbaghia violacea Harv.; callus cultures; GC/MS; secondary metabolites; biological activities

1. Introduction

Tulbaghia violacea Harv. is a small bulbous herb belonging to Amaryllidaceae family (formerly: Alliaceae), indigenous to KwaZulu–Natal, Gauteng and the Eastern Cape region in South Africa, and are cultivated for medicinal, ornamental and culinary purposes (Chase

CONTACT Ghada F. Metwally ghadafarouk70@hotmail.com
Supplemental data for this article can be accessed http://doi.dx.org/10.1080/14786419.2017.1289206.

et al. 2009). It has tubular mauve or pale purple flowers occurring in umbels up to 20 at the tip of the slender stalk. The plant is closely related to garlic (*Allium sativum*) and is commonly known as wild garlic, society garlic or sweet garlic. It also has a garlic-like odour when the leaves are bruised (Van Wyk & Gericke 2000; Lyantagaye 2011). It has traditionally been used for fever, colds, asthma, tuberculosis (Van Wyk 2008), and had also been reported to possess anticancer (Bungu et al. 2006), antithrombotic (Bungu et al. 2008), antimicrobial (Ncube et al. 2011), antidiabetic and anticoagulant properties (Davison 2012).

As with the genus *Allium,* volatile sulphur-containing compounds are responsible for the characteristic smell and taste of *Tulbaghia* species (Kubec et al. 2013). However, Ranglová et al. (2015) confirmed that such compounds were different from those found in *Allium* species, indicating their uniqueness to *Tulbaghia violacea* Harv. Marasmicin, an unstable thiosulphinate present in the plant under study, decomposes to generate various degradation products, such as 2,4,5,7-tetrathiaoctane-2,2-dioxide, 2,4,5,7-tetrathiaoctane-4,4-dioxide and 2,4,5,7-tetrathiaoctane-2,2,7,7-tetraoxide (Kubec et al. 2002). In contrast to *Allium* species, there are few studies to date regarding the chemical constituents of the studied plant. Available researches report the presence of saponins (Watson & Dallwitz 1992), flavonoids and tannins (Ncube et al. 2011). In a previous publication, the influence of extraction methods on the composition and antimicrobial activity of volatile constituents of leaves and flowers of *Tulbaghia violacea* Harv. cultivated in Egypt (Eid 2015), was studied.

Currently, *in vitro* cultures (callus and cell suspension) are frequently used as alternative sources for producing secondary metabolites in high yields and a reasonable timeframe. Except for few studies focusing on the micropropagation of the investigated plant (Phelan et al. 2007; Ncube et al. 2011), to our knowledge, there is no research thus far on the establishment of callus cultures or the investigation of their secondary metabolites. Therefore, as a continuation of our previous work on locally cultivated plant (Eid 2015), the aim of the present study was to induce callus from *Tulbaghia violacea* Harv. and to assay extracts compared to field-grown plants; the *n*-hexane extracts were examined for non-polar compounds and ethanolic extracts were assessed quantitatively for polar secondary metabolites (thiosulphinates, phenolics, flavonoids and saponins). Moreover, *n*-hexane and ethanolic (70%) extracts of the calli and field-grown plants were investigated for antioxidant, antimicrobial and cytotoxic activities.

2. Results and discussion

2.1. Callus induction and biomass yield

Toaima et al. (2003) and Sundarasekar et al. (2012) recommend a combination of the plant growth regulators; 2,4-dichlorophenoxyacetic acid (2,4-D) and benzylaminopurine (BAP) in callus initiation for some members of Amaryllidaceae. In this study, a whitish-green compact undifferentiated callus was successfully initiated from flowers of field-grown plants on Murashige & Skoog (MS) medium supplemented with 22.6 µM 2,4-D and 2.2 µM BAP; other explants (leaves and stalks of the inflorescence) showed no callus induction. Figure S1 (a–d) shows the stages of callus development from *Tulbaghia violacea* Harv. flowers during eight-week incubation period. Growth commenced at the basal part of the flower within the first two weeks and reached its maximum after eight weeks. The survival percentage on the medium used ranged from 40 to 80%. The growth curve of the calli was investigated to

determine its capacity to increase biomass and continue to multiply (Robledo-Paz et al. 2006). The fresh and dry weights followed a sigmoidal pattern with lag, exponential and stationary phases, during the eight weeks (Figure S2). The lag phase occurred from the beginning of the culture until the end of the third week, with an increase of 6.3 mg (calculated on a dry weight basis). The exponential phase (the period of maximum cell division) occurred after the 3rd–6th week, with an increase in weight up to 99 mg (calculated on a dry weight basis). The stationary phase started after the sixth week and continued until the eighth week, at which no cell division or increase in weight occurred. The produced sigmoid growth curve is in accordance with previous studies (Ling et al. 2009), which also reported the three phases. The curve indicated that the highest growth rate, cell division and biomass yield were attained between the third and the sixth weeks, after which an increase in secondary metabolites rather than cell division could be afforded (Tan et al. 2010). Thus, the callus cultures were harvested for further phytochemical and biological studies after ensuring that the stationary phase has begun (at the end of the eighth week).

2.2. Determination of moisture content

The moisture content (Figure S3) in the period from the first week until the beginning of the third week (88.1–91.8%) was higher than that in the period after the third week to the end of the incubation period (85.7–88.8%).

2.3. Preliminary phytochemical screening of callus cultures and flowers

The data presented in Table S1 reveal the presence of volatile constituents, carbohydrates and/or glycosides, coumarins, flavonoids, and saponins and the absence of alkaloids, anthraquinones, cardiac glycosides and tannins. Sterols were present in trace amounts. These results revealed some similarity between the flowers of the field-grown plants and its callus cultures with respect to secondary metabolites; an exception was volatile constituents, flavonoids and saponins, which were present in higher amounts in the flowers of the field-grown plants than in the calli. These differences in secondary metabolite concentrations are in agreement with previous studies (Koua et al. 2011; Bagratishvili & Jikia 2014). Furthermore, the similarity in secondary metabolites in the calli and flowers of the same plant suggested that callus cultures may be an alternative source for these phytochemicals.

2.4. GC/MS analysis of n-hexane extracts of calli and flowers

A yellowish white semi-solid residue with faint garlic-like odour (0.1% w/w) was produced from *n*-hexane extracts of calli (HC), whereas *n*-hexane extracts of flowers (HF) afforded a yellowish green semi-solid residue with a strong garlic-like odour (4% w/w). Results of gas chromatography/mass spectrometry (GC/MS) analyses revealed qualitative and quantitative variability for the chemical profiles of the investigated samples (HC and HF). A total of 54 compounds (33 in HC and 32 in HF) were identified in both extracts, with only 11 being common; the identified components represented 92.6 and 98.5% of HC and HF, respectively. These compounds included variable classes *viz.* non-oxygenated compounds such as hydrocarbons (aliphatic, monoterpene and sesquiterpene), oxygenated compounds (alcohols, aldehydes, ketones, phenolics, fatty acid esters and aromatics) and sulphur-containing

compounds. Hydrocarbons were the major components in HC (55.0%), but oxygenated compounds were predominant in HF (74.6%). All classes were abundant in HF, except for aldehydes. On contrast, HC was devoid of monoterpene hydrocarbons, sesquiterpene hydrocarbons and sulphur-containing compounds. 16-Hentriacontanone (30.3%) and nonacosane (12.6%) were major compounds in HC, whereas *trans*(E)–anethole (39.1%) and 16-hentriacontanone (28.2%) were found at high levels in HF. This variation could be attributed to the fact that differentiation in callus cultures is essential for the production of secondary metabolites (Hagimori et al. 1982; Kutchan et al. 1983) and could partly explain the absence of sulphur-containing compounds, such as those responsible for the garlic-like odour; 2,3,5-trithiahexane, 2,4,5,7-tetrathiaoctane and dimethyl trisulfide (Eid 2015), in addition to monoterpene and sesquiterpene hydrocarbons from HC. Aliphatic hydrocarbons were predominant in HC, among which nonacosane was a main component; this indicates that calli may be an accessible source for this biologically active component that acts as a vasodilator and saluretic metabolite (Mihailović et al. 2011). Additionally, the bioactive compound 16-hentriacontanone (palmitone), which is reported to have anticonvulsant (Gonzalez-Trujano et al. 2001) and anxiolytic (Gonzalez-Trujano et al. 2006) activities, could be easily supplied by either HC or HF.

The results of GC/MS analysis of HF in this study are in agreement with Eid (2015) regarding the content of sulphur-containing compounds (8.8 and 8.6%) and aliphatic hydrocarbons (14.2 and 14.9%), additionally, 16-hentriacontanone (28.2 and 13.6%) and *trans*(E)-anethole were major components (39.1 and 6.4), respectively. Conversely, Eid (2015) described oxygenated non-aromatic compounds as major components, at 37.6%, whereas in our study, HF was found to be largely composed of oxygenated aromatic compounds (44.7%). Finally, the chemical profile of HF in this study quantitatively differed to a great extent from that reported by Eid (2015) and this variation could be directly related to the extraction method, as the previous report used only cold percolation, but in this study sonication was used. These results emphasise that ultrasound-assisted extraction, which is proposed as an alternative method to conventional extraction, provides higher recovery of volatile constituents, lower solvent consumption and a high content of bioactive compounds (Santos et al. 2009).

2.5. Evaluation of secondary metabolites in ethanolic extracts of callus and flowers

Ethanolic extracts of callus (EC) and flowers (EF) were brownish in colour, with yields of 1.5 and 10.8% (calculated on a fresh weight basis), respectively. According to the data shown in Table S3, among all of the investigated metabolites, EC exhibited lower contents of thiosulphinates, phenols, flavonoids and saponins than EF.

Due to their medicinal importance, we sought to assess the amount of thiosulphinates in EC compared to EF. The thiosulphinate marasmicin is an enzymatic product of marasmin, which is present in *Tulbaghia violacea* Harv. (Kubec et al. 2002), exhibiting antimicrobial activity against various micro-organisms, including *Escherichia coli, Micrococcus luteus, Bacillus cereus, Candida albicans, Saccharomyces cerevisiae, Fusarium solani* and *Aspergillus niger* (Ranglová et al. 2015). This activity is comparable to that of allicin. The thiosulphinate content in EF (0.5 ± 0.02 µg mg^{-1}, F. wt**)** was fivefold higher than that in EC (0.1 ± 0.02 µg mg^{-1}, F. wt), which is in accordance with Nasim et al. (2009), who noted that the allicin content of non-differentiated callus of *Allium sativum* L. was lower than that of differentiated callus.

The phenolic and saponin contents were approximately 10-fold higher in EF (14.8 ± 3 and 53.8 ± 1.8 µg mg^{-1}, F. wt, respectively) than in EC (1.7 ± 0.7 and 5.3 ± 0.2 µg mg^{-1}, F. wt, respectively). Compared to EF (132.6 ± 1.5 µg mg^{-1}, F. wt), flavonoids were only found in trace amounts in EC (2.3 ± 1.7 µg mg^{-1}, F. wt). These results are in agreement with Mustapha and Harun (2015), who reported lower amounts of phenolic compounds and flavonoids in leaf-derived calli of *Ficus deltoidea* than in the leaves of the same plant. Additionally, our results agreed with those of Collin (2001), who stated that differentiation of any tissue is associated with increased synthesis of secondary metabolites under *in vitro* conditions, which could be due to the appearance of complex cells and tissues that are more metabolically competent.

2.6. Evaluation of the biological activities of n-hexane and ethanolic extracts of callus and flowers

The antioxidant activities of HC, HF, EC and EF were assessed (Table S4) based on the free radical-scavenging activity (the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) method). According to IC_{50} results, HC exerted no activity compared to HF (2502.8 µg ml^{-1}). In addition, EC showed less (7349.5 µg ml^{-1}) *in vitro* antioxidant activity than EF (1932.5 µg ml^{-1}). The activity of EF could be attributed to the presence of *trans*(E)-anethole (Wong et al. 2014) as well as higher amounts of antioxidant metabolites, such as phenolics and flavonoids (Tsao 2010).

The antimicrobial activities of the *n*-hexane and ethanolic extracts of the callus cultures and flowers of field-grown plants were investigated. As shown in Table S5, it could be concluded that HC was inactive against all of the tested organisms. Conversely, HF exhibited moderate activity against *Escherichia coli* (14.2 ± 0.8 mm) and *Bacillus subtilis* (19.7 ± 0.6 mm) compared to ciprofloxacin (32.0 ± 0.5 and 35.0 ± 1.5 mm, respectively) but nearly similar activity against *Candida albicans* (31.0 ± 1.0 mm) as nystatin (30.0 ± 1.0 mm) and slightly higher activity against *Staphylococcus aureus* (30.3 ± 1.5 mm) than ciprofloxacin (27.0 ± 1.0 mm). The strong activity of HF could be explained by the presence of high levels of *trans*(E)-anethole, in agreement with Mohammed (2009), who reported activity of the compound against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* but no activity against *Pseudomonas aeruginosa*. However, EF showed higher activity against the tested microorganisms than EC, which exhibited moderate activity (19.0 ± 0.6 mm) that was slightly higher than that of EF (16.8 ± 0.3 mm) against *Escherichia coli*. Weak activity was exerted by EC against *Bacillus subtilis*, *Staphylococcus aureus* and *Candida albicans* (13.0 ± 1.0, 11.5 ± 0.5 and 10.8 ± 0.3 mm, respectively), whereas very high activity was observed for EF (36.3 ± 1.5, 33.2 ± 0.8 and 30.3 ± 0.6 mm, respectively), approaching that of the standard antimicrobial compounds (the antibacterial: ciprofloxacin and antifungal: nystatin), which was even higher in the case of *Staphylococcus aureus* (35.0 ± 1.5, 27.0 ± 1.0 and 30.0 ± 1.0 mm, respectively). The high antimicrobial activity of EF compared to EC is due to the presence of higher amounts of thiosulphinates (Ranglová et al. 2015) and saponins (Sparg et al. 2004), which are known for their antimicrobial activity, in the former. Nonetheless, the results of HF antimicrobial activity disagreed with Eid (2015), who reported that the extract obtained via cold percolation was inactive against *Escherichia coli* and *Candida albicans*; in the present study, both micro-organisms were sensitive to the extract, and also both extracts were active against *Staphylococcus aureus* and *Bacillus subtilis*, though with variable responses. These variations in activity could

be attributed to the presence of antimicrobially active compounds such as eugenol (Burt 2004), *trans*(E)-anethole (Mohammed 2009), 2,4,5,7-tetrathiaoctane (Kubota et al. 1994) and 16-hentriacontanone (Shanker et al. 2007) in different concentrations. Additionally, the antimicrobial activity of HF against *Escherichia coli* and *Candida albicans* is consistent with the results of Ncube et al. (2011), who investigated the activity of non-polar extracts (prepared similarly using sonication) against these micro-organisms. All of the tested extracts proved to be inactive against *Pseudomonus aeruginosa* and *Aspergillus niger*, partially agreeing with Eid (2015) with regard to the former micro-organism not being sensitive to HF.

Screening of the cytotoxic activity of *n*-hexane and ethanolic extracts for each of the callus cultures and the flowers of field-grown plants (HC, HF, EC, EF, respectively) was performed at a dose of 250 µg ml^{-1}, according to Bungu et al. (2006). Activity was evaluated using three different types of human carcinoma cell lines: hepatocellular (HepG2), prostate (PC-3) and breast (MCF-7). The results presented in Table S6 show that HF, EC and EF at a concentration of 250 µg ml^{-1} exhibited variable cytotoxic activities against the tested cancer cell lines; in contrast, HC displayed almost no activity under the tested conditions. The highest growth inhibition by the investigated extracts was achieved by EF against HepG2 (74.3 ± 0.9) and MCF-7 (73.3 ± 0.9) cells, whereas HF exerted the strongest inhibition of PC-3 cells (63.3 ± 2.0); the least activity was observed for EC (52.4 ± 1.0, 21.7 ± 3.1 and 19.6 ± 0.6, respectively). Furthermore, the inhibition results were almost as great as that of the anticancer drug doxorubicin**.** The different responses achieved by the extracts in the cancer cell lines could be due to variation in bioactive metabolites in each extract. Our results are in agreement with Bungu et al. (2006), who reported that methanolic extracts of *Tulbaghia violacea* Harv. leaves and bulbs inhibited the growth of MCF-7 cells at a concentration of 250 µg ml^{-1} (43.9 ± 4.7 and 49.6 ± 2.6%, respectively), and Lyantagaye (2013a, 2013b), who stated that 0.5 mg ml^{-1} of a crude aqueous extract of *Tulbaghia violacea* Harv. is able to induce apoptosis in MCF-7 cells (59%). This work provides an important basis for further investigation of callus cultures of *Tulbaghia violacea* Harv. with regard to isolating and identifying metabolites that could be responsible for the observed biological activities and further screening of the *in vivo* pharmacological activity of the plant and its *in vitro* cultures. Moreover, from the tissue culture point of view, different growth factors and techniques can be assessed to increase the levels of bioactive metabolites. To the best of our knowledge, this is the first report on callus induction using flowers of *Tulbaghia violacea* Harv. and evaluation of their secondary metabolites.

3. Conclusion

Flowers of field-grown plants were suitable material for initiating *in vitro* callus cultures on MS medium supplemented with 22.6 µM 2,4-D and 2.2 µM BAP. Although these cultures were found to be less biologically active than the flowers of field-grown plants, this study could be considered a starting point for further investigation and for enhancing the production of secondary metabolites in *in vitro* callus cultures for future use in pharmaceutical applications as antioxidant, cytotoxic or antimicrobial preparations. Additionally, the establishment of differentiated callus cultures is recommended because they could be a potential source of higher amounts of bioactive metabolites.

ORCID

Ghada F. Metwally http://orcid.org/0000-0002-9507-7697

References

Bagratishvili D, Jikia R. 2014. Formation of the phenolic compounds in the tissue culture of *Rhododendron Caucasicum* pall. Bull Georg Natl Acad Sci. 8:85–88.

Bungu L, Frost CL, Brauns SC, Van de Venter M. 2006. *Tulbaghia violacea* inhibits growth and induces apoptosis in cancer cells in vitro. Afr J Biotechno. 5:1936–1943.

Bungu L, Frost CL, Van de Venter M. 2008. Evidence for an in vitro anticoagulant and antithrombotic activity in *Tulbaghia violacea*. Afr J Biotechno. 7:681–688.

Burt S. 2004. Essential oils: their antibacterial properties and potential applications in foods: a review. Int J Food Microbiol. 94:223–253.

Chase MW, Reveal JL, Fay MF. 2009. A subfamilial classification for the expanded asparagalean families Amaryllidaceae, Asparagaceae and Xanthorrhoeaceae. Bot J Linn Soc. 161:132–136.

Collin HA. 2001. Secondary product formation in plant tissue cultures. Plant Growth Regul. 34:119–134.

Davison C. 2012. A biochemical study of the antidiabetic and anticoagulant effects of *Tulbaghia violacea* [MSc thesis]. Port Elizabeth: Nelson Mandela Metropolitan University.

Eid HH. 2015. The Influence of extraction methods on the composition and antimicrobial activity of the volatile constituents of *Tulbaghia violacea* Harv. cultivated in Egypt. J Pharmacogn Phytochem. 4:118–125.

Gonzalez-Trujano ME, Navarrete A, Reyes B, Cedillo-Portugal E, Hong E. 2001. Anticonvulsant properties and bio-guided isolation of palmitone from leaves of *Annona diversifolia* Saff. Planta Med. 67:136–141.

Gonzalez-Trujano ME, Martinez AL, Reyes-Ramirez A, Reyes-Trejo B, Navarrete A. 2006. Palmitone isolated from annona diversifolia induces an anxiolytic-like effect in mice. Planta Med. 72:703–707.

Hagimori M, Matsumoto T, Obi Y. 1982. Effect of light and plant growth substances on digitoxin formation by undifferentiated cells and shoot-forming cultures of *Digitalis purpurea* L. grown in liquid media. Plant Physiol. 69:653–656.

Koua FHM, Babiker HAA, Halfawi A, Ibrahim RO, Abbas FM, Elgaali EI, Khalafallah MM. 2011. Phytochemical and biological study of *Striga hermonthica* (Del.) Benth Callus and Intact Plant. Res Pharma Biotechnol. 3:85–92.

Kubec R, Velíšek J, Musah RA. 2002. The amino acid precursors and odor formation in society garlic (*Tulbaghia violacea* Harv.). Phytochemistry. 60:21–25.

Kubec R, Krejčová P, Mansur L, García N. 2013. Flavor precursors and sensory-active sulfur compounds in alliaceae species native to South Africa and South America. J Agric Food Chem. 61:1335–1342.

Kubota K, Ohhira S, Kobayashi A. 1994. Identification and antimicrobial activity of the volatile flavor constituents from scorodocarpus borneensis Becc. Biosci Biotech Biochem. 58:644–646.

Kutchan TM, Ayabe S, Krueger RJ, Ciscia EM, Coscia CJ. 1983. Cytodifferentiation and alkaloid accumulation in cultured cells of *Papaver bracteatum*. Plant Cell Rep. 2:281–284.

Ling APK, Kuok YT, Jualang AG, Sobri H. 2009. Induction and maintenance of callus from leaf explants of *Mirabilis jalapa* L. Med Aromat Plant Sci Biotechnol. 3:42–47.

Lyantagaye SL. 2011. Ethnopharmacological and phytochemical review of *Allium* species (sweet garlic) and *Tulbaghia* species (wild Garlic) from Southern Africa. T J Science. 37:58–72.

Lyantagaye SL. 2013a. Methyl-α-D-glucopyranoside from *Tulbghia violacea* extract induces apoptosis in vitro in cancer cells. Bangladesh J Pharmacol. 8:93–101.

Lyantagaye SL. 2013b. Two new pro-apoptotic glucopyranosides from *Tulbaghia violacea*. J Med Plants Res. 7:2214–2220.

Mihailović V, Vuković N, Niforović N, Solujić S, Mladenović M, Mašković P, Stanković MS. 2011. Studies on the antimicrobial activity and chemical composition of the essential oils and alcoholic extracts of *Gentiana asclepiadea* L. J Med Plant Res. 5:1164–1174.

Mohammed MJ. 2009. Isolation and identification of anethole from *Pimpinella anisum* L. fruit oil. An antimicrobial study. J Pharm Res. 2:915–919.

Mustapha Z, Harun H. 2015. Phytochemical constituents in leaves and callus of *Ficus deltoidea* Jack var. Kunstleri (RIng) Corner. Walailak J Sci Tech. 12:431–439.
Nasim SA, Mujib A, Rashmi K, Samar F, Junaid A, Mahmooduzzafar M. 2009. Improved alliin yield in somatic embryos of *Allium sativum* L. (cv Yamuna safed) as analyzed by HPTLC. Acta Biol Hungarica. 60:441–454.
Ncube B, Ngunge VNP, Finnie JF, Van Staden J. 2011. A comparative study of the antimicrobial and phytochemical properties between outdoor grown and micropropagated *Tulbaghia violacea* Harv. plants. J Ethnopharmacol. 134:775–780.
Phelan S, Douglas G, Hunter A. 2007. Micropropagation and growth regulation of *Tulbaghia violacea* 'Silver lace'. Acta Hortic. 764:113–118.
Ranglová K, Krejčová P, Kubec R. 2015. The effect of storage and processing on antimicrobial activity of *Tulbaghia violacea*. S Afr J Bot. 97:159–164.
Robledo-Paz A, Vázquez-Sánchez MN, Adame-Alvarez RM, Jofre-Garfias AE. 2006. Callus and suspension culture induction, maintenance, and characterization. In: Loyola-Vargas VM, Vázquez-Flota F, editors. Methods in Molecular Biology, Plant Cell Culture Protocols. 2nd ed. Totowa, NJ: Humana Press Inc; p. 59–70.
Santos HM, Lodeiro C, Capelo-Martinez JL. 2009. The power of ultrasound. In: Capelo-Martinez JL, editor. Ultrasound in chemistry: analytical applications. Weinheim: WILEY-VCH Verlag GmbH & Co. KGaA; p. 1–16.
Shanker KS, Kanjilal S, Rao BV, Kishore KH, Misra S, Prasad RB. 2007. Isolation and antimicrobial evaluation of isomeric hydroxy ketones in leaf cuticular waxes of *Annona squamosa*. Phytochem Anal. 18:7–12.
Sparg SG, Light ME, van Staden J. 2004. Biological activities and distribution of plant saponins. J Ethnopharmacol. 94:219–243.
Sundarasekar J, Anthony JJJ, Murugaiyah V, Subramaniam S. 2012. Preliminary responses of 2, 4-D and BAP on callus initiation of an important medicinal-ornamental *Hymenocallis littoralis* plants. J Med Plants Res. 6:2088–2093.
Tan SH, Musa R, Ariff A, Maziah M. 2010. Effect of plant growth regulators on callus, cell suspension and cell line selection for flavonoid production from Pegaga (*Centella asiatica* L. urban). Am J Biochem Biotechnol. 6:284–299.
Toaima N, Novak E, Schumann G. 2003. Callus induction from different explants of commercial cultivars of leek, *Allium ampeloprasum* var. Porrum L. Acta Hortic. 597:303–309.
Tsao R. 2010. Chemistry and biochemistry of dietary polyphenols. Nutrients. 2:1231–1246.
Van Wyk EB. 2008. A broad review of commercially important southern African medicinal plants. J Ethnopharmacol. 119:342–355.
Van Wyk EB, Gericke N. 2000. People's plants: a guide to useful plants of southern Africa. 1st ed. Pretoria: Brizza Publications; p. 138.
Watson L, Dallwitz MJ. 1992. The families of flowering plants: descriptions, illustrations, identification, and information [cited 2000 Dec 14]. Available from: http://biodiversity.uno.edu/delta/
Wong YC, Lee PP, Wan Nurdiyana WA. 2014. Extraction and antioxidative activity of essential oil from star anise (*Illicium verum*). Orient J Chem. 30:1159–1171.