

## Review

# Biological activities of species in the genus *Tulbaghia*: A review

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Since time immemorial, plants have been used by several communities to treat a large number of diseases. Numerous studies on the pharmacology of medicinal plants have been done. Medicinal plants constitute a potential source for the production of new medicines and may complement conventional antimicrobials and probably decrease health costs. Phytochemical compounds in plants are known to be biologically active aiding, for example, as antioxidants and antimicrobials. The overwhelming challenge of drug resistance has resulted in an increasing trend towards using medicinal plants to treat various diseases, especially in developing countries. Species of the genus *Tulbaghia* has been widely used in traditional medicine to treat various ailments such as rheumatism, fits, fever, earache, tuberculosis etc. It is believed that the species possess several therapeutic properties. This paper evaluates some of the biological activities of the genus *Tulbaghia*. It is evident from current literature that *Tulbaghia violacea* is the most promising species. The other species of *Tulbaghia* still require further studies to ascertain their medicinal potential.

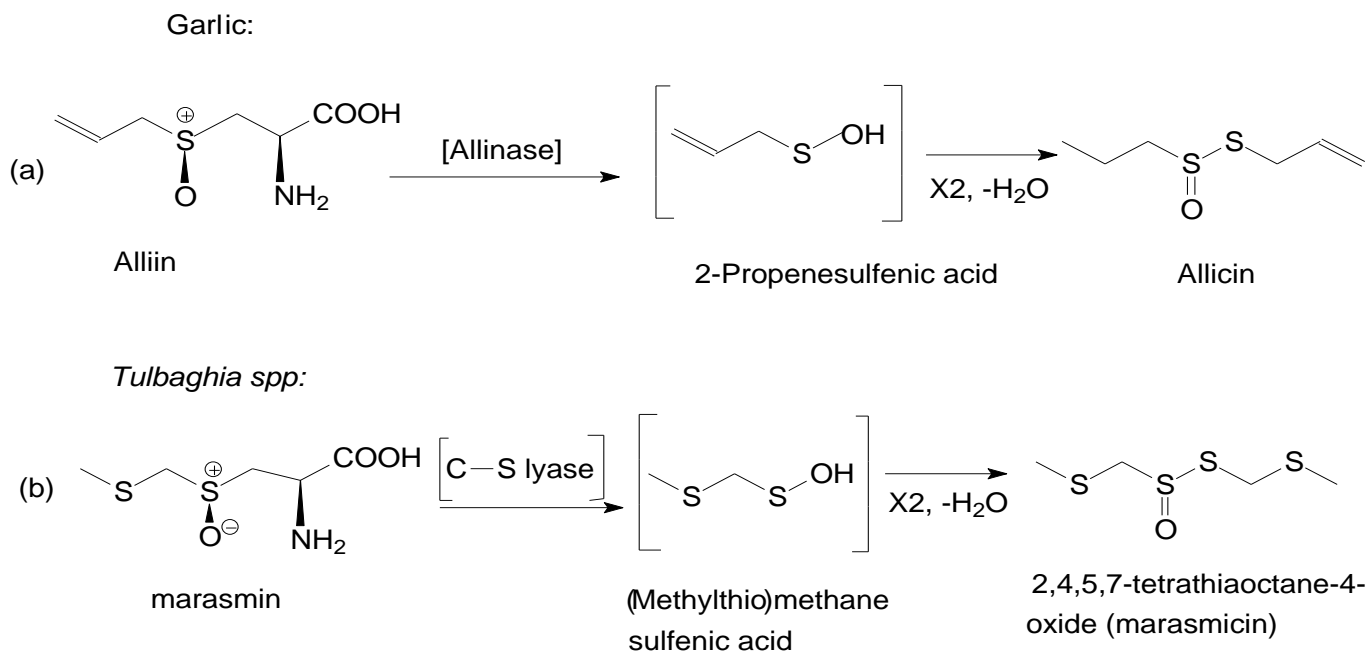
**Key words:** *Tulbaghia*, antioxidants, antimicrobial, anticancer, biological activities.

## INTRODUCTION

*Tulbaghia L.* is one of the 30 genera in the family *Alliaceae*. This genus comprises of approximately 63 species which are mostly rhizomatous plants with about 30 indigenous to the Southern Africa region (Lyantagaye, 2011). *Tulbaghia* is commonly known as “wild garlic”, “sweet garlic” or “pink agapanthus” due to its resemblance to the genus *Agapanthus* (Kubec et al., 2013). The majority of the species are found in South Africa, especially in the Eastern Cape. A distinctive feature of the genus is a corona, a ‘crown-shaped’ outgrowth or appendage of the perianth. Like other

members of the *Allioideae*, a distinctive ‘garlic-like odour’ is produced when the leaves or rhizomes are damaged, resulting in the release of cysteine-derived sulphur compounds (Jäger and Stafford, 2012). Researchers have highlighted the great potential of the genus *Tulbaghia* due to their nutritive, ornamental and medicinal value (Van Wyk, 2011). Medicinal plants are known to contain many chemical compounds possessing multiple biological activities (Aremu et al., 2012). It is believed that *Tulbaghia* may possess biological activities similar to garlic (*Allium sativum*) since both belong to the family

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**Figure 1.** Enzyme-mediated formation of allicin from alliin (a) and marasmicin from marasmin (b). Adapted from Van Wyk et al. (2013).

*Alliaceae* (Van Wyk and Gericke, 2000; Raji et al., 2012). The bioactive components of garlic are mainly responsible for the healing properties (Kallel et al., 2014). The major physiological roles of garlic are its antimicrobial, anticancer, antioxidant, immune boosting, antidiabetic, hepatoprotective, antifibrinolytic and anti-platelet aggregatory activity and its potential role in preventing cardiovascular diseases (Santhosha et al., 2013). However, most of the biological activities demonstrated by garlic remain to be investigated in *Tulbaghia* (Olorunnisola et al., 2012a).

## BIOACTIVE COMPONENTS OF WILD GARLIC

There are many chemical constituents that have been identified in the family *Alliaceae*. *Tulbaghia violacea* has been found to be rich in sulphur-containing compounds. These compounds, in most cases, account for the characteristic odours and the medicinal properties of both the *Tulbaghia* and *Allium* species (Lyantagaye, 2011). The characteristic flavour of garlic (*A. sativum*) and related alliums occurs when enzyme Allinase (EC.4.4.1.4) hydrolyses the S-alka(en)yl-L-Cys Sulfoxides (ACSOs) to form pyruvate, ammonia and sulphur containing volatiles (Lancaster et al., 2000; Fritsch and Keusgen, 2006).

Alliin is the precursor of allicin, formed by the action of alliinase enzyme (Figure 1a). Allicin has antibacterial, antiviral, antitumor, anticoagulation, antihypertensive, antiparasitic, hepatoprotective, etc., activities (Ankri

and Mirelman, 1999). It is also efficient against many fungal species, like *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans* and *Fusarium laceratum*. In *Tulbaghia* the precursor of the typical odour is a cysteine-derived amino acid, S-(methylthiomethyl) cysteine-4-oxide (marasmin) that is localized in the cytoplasm (Lyantagaye, 2011). When *Tulbaghia* tissue is disrupted, a C-S lyase present in cell vacuoles comes into contact with marasmin and catalyzes its breakdown into the thiosulfinate marasmicin (2,4,5,7-tetrathiaoctan-4-oxide, 2), Figure 1b, analogously to the formation of allicin from alliin in garlic (Kubec et al., 2002). According to (Ranglová et al., 2015) and (Kubec et al., 2002) marasmicin undergoes chemical decomposition into different sulphur-containing degradation products. Some of these compounds have been shown to possess antimicrobial, antifungal and anticancer properties (Jäger and Stafford, 2012).

## ANTIMICROBIAL ACTIVITY

Medicinal plants are a reservoir of a variety of bioactive molecules with diverse molecular structures and functions (Muleya et al., 2014). These molecules are primarily derived from the secondary metabolism of plants and protect them against predation by microorganisms, insects and herbivores. Since prehistoric times, people have used various plants to cure and prevent a range of ailments, and they are still found to be an effective source of remedy in traditional medical

practices. Therefore, evaluation of the activities of medicinal plants claimed for possessing antimicrobial activity has gained attention recently (Jeyaseelan et al., 2012). The antimicrobial properties of *Tulbaghia* species have been studied by numerous research groups. Various solvent extracts of *Tulbaghia* species have been tested against a range of microorganisms affecting both humans and plants as reported by Aremu and Van Staden (2013). One of the important findings of these studies is that there is no consensus on the antimicrobial activity of *Tulbaghia* among researchers. Whereas, some authors have found minimal antimicrobial activity (Bamuamba et al., 2008; Motsei et al., 2003; McGaw et al., 2008), others have observed significant antimicrobial properties in various *Tulbaghia* extracts (Lindsey et al., 2004; Buwa and Van Staden, 2006; Thamburan et al., 2006; Ncube et al., 2011a; Jäger and Stafford, 2012; Soyngbe et al., 2013; Netshiluvhi and Eloff, 2015; Ranglova et al., 2015).

The discrepancies in these results may be due to factors such as the age of plants and geographical location (van den Heever et al., 2008; Ncube et al., 2011b). Ranglova et al. (2015) suggested that the discrepancies could be mainly due to differences in sample preparation that ultimately determines the amount of the bioactive sulphur compounds present. For example, dichloromethane and water extracts of freshly homogenised *T. violacea* rhizomes exhibited relatively high activity against *C. albicans*, with minimum inhibitory concentration (MIC) values of 1.0 and 2.0 µl/ml, respectively, compared to dry extracts (Motsei et al., 2003; Buwa and Afoloyan, 2009). The reaction that results in the formation of marasmicin is mediated by the C-lyase, hence, extracts prepared from fresh plants are more likely to contain significantly higher levels of marasmicin compared to samples obtained from dried plant material. This is due to the fact that the enzyme involved in the formation of marasmicin may be inactivated through sample drying and application of heat or organic solvents thus effectively curtailing the formation of the bioactive thiosulfate (Ranglova et al., 2015).

Nonetheless, it has been established that *T. violacea* crude extract exhibit broad spectrum antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* (Gaidamashvili and Van Staden, 2002; Ncube et al., 2011a). The potential of the species as a remedy against the deadly tuberculosis causing *mycobacterium* has also been demonstrated (Buwa and Van Staden, 2006; Bamuamba et al., 2008; McGaw et al., 2008). *Tulbaghia alliacea* has also been shown to be fungicidal (Thamburan et al., 2006). Clearly, members of the *Tulbaghia* possess significant pharmacological potential. They have been used in traditional medicine, probably due to their content of sulphur compounds. The maximum activity of the extracts can possibly be obtained from fresh plant material by avoiding decomposition of

the sulphur compounds during extraction (Bungu et al., 2006; Jäger and Stafford, 2012).

## ANTIOXIDANT ACTIVITY

Numerous physiological and biochemical processes in the human body may produce oxygen-centred free radicals and other reactive oxygen species as by-products. Although the human body has inherent antioxidative mechanism, overproduction of such free radicals can cause oxidative damage to biomolecules eventually leading to many chronic diseases (Choi et al., 2002; Cai et al., 2004; Saeed et al., 2012). The antioxidant deficiency in the human body can be compensated by making use of natural antioxidants such as vitamin C, vitamin E, flavones, carotenes and natural products in plants (Cai et al., 2003). Antioxidants stabilize or deactivate free radicals, often before they attack targets in biological cells (Saeed et al., 2012). Clearly, the fact that the presence of reactive oxygen species is associated with many acute and chronic diseases in humans (Adewusi and Steenkamp, 2011) possibly explains usefulness of antioxidants as contributing remedies for such ailments. Furthermore, the antioxidant potential of the phenolic compounds especially those derived from medicinal plants are becoming the preferred choice for use when compared to the synthetic antioxidants (Aremu et al., 2013).

*T. violacea* is one of the commonly used plants in management of free radical related diseases. Several antioxidant screenings on *T. violacea* have been conducted. Most of the antioxidant activity has been done using various assays, taking into consideration the different mechanisms of antioxidant action (Zheng and Wang, 2001; Naidoo et al., 2008; Soyngbe et al., 2013). A study by Olorunnisola et al. (2012b) indicated that oral intake of *T. violacea* extract by normal rats may enhance the status of antioxidant defence enzymes, HDL (high density lipoprotein) - cholesterol and decrease serum concentration of malondialdehyde, thus suggesting that the extract may reduce the risk of oxidative induced diseases. It was also reported in the same study that 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities (% inhibition) of the *T. violacea* oil extract showed a concentration dependent activity pattern (Olorunnisola et al., 2012b). As the concentration increased, the scavenging effect also increased, reaching as high as  $89.2 \pm 1.5\%$  at 0.5 mg/mL. This value was very close to the activity of synthetic antioxidants BHT ( $92.4 \pm 3.20\%$ ) and vitamin C ( $98.1 \pm 1.30\%$ ) at the same concentration.

Moodley et al. (2014) demonstrated the antihypertensive as well as reno-protective effects of *T. violacea* administered in dahl salt sensitive (DSS) rats. The decreased blood pressure and reno-protective effects of *T. violacea* are most likely related to reduced

oxidative stress, increased nitric oxide (NO) production and decreased expressions of transforming growth factor beta (TGF- $\beta$ ) and nuclear factor kappa beta (NF- $\kappa$ B). Another study using a methanolic extract of *T. violacea* produced a protection against atherogenic diet induced aortic pathology, enzyme depletion glomerulosclerosis and hepatic damage by preventing hyperlipidemia and oxidative stress in rats (Olorunnisola et al., 2012c). *T. violacea* contains several ingredients such as steroidal saponins, quercetin, kaempferol, sugars, and / or sulfur-containing compounds (Duncan et al., 1999) that might be considered glucose lowering. Similar sulfur containing compounds in garlic have been shown to be hypoglycaemic in diabetic animals. This has been ascribed to their anti-oxidant activity and the interaction of these compounds with thiol-containing proteins. Accordingly, the aqueous extract of *T. violacea* (50  $\mu$ g/mL) showed significant increased glucose uptake activity into Chang liver cells (124.5%) and the ethanol extract (0.5 and 50  $\mu$ g/mL) into C2C12 muscle cells (140.5 and 117.7%, respectively) (van Huyssteen et al., 2011). In conclusion, antioxidants reduce the oxidative stress in cells and are therefore useful in the treatment of many human diseases, including cancer, cardiovascular diseases and inflammatory diseases. Natural antioxidants therefore represent a potentially side-effect free alternative to synthetic antioxidants for use in preventive medicine (Krishnaiah et al., 2011).

### ANTI-INFLAMMATORY ACTIVITY

Many inflammatory diseases are associated with the synthesis of prostaglandins, which are responsible for a sensation of pain (Fennell et al., 2004). The primary enzyme responsible for prostaglandins synthesis is the membrane – associated cyclooxygenase (COX), which occurs in two isoforms, COX-1 and COX-2. COX -1 is constitutively expressed while COX-2 is induced in the inflamed tissue. Modulation of the enzyme implies that the inflammation can be modified (Matu and van Staden, 2003). The commonly used anti-inflammatory drugs are becoming less acceptable due to serious adverse reactions such as gastric intolerance, bone marrow depression and water and salt retention resulting from prolonged use. This necessitates the continued search for potent anti-inflammatory agents with reduced or no side effects. Despite the extensive use of medicinal plants in traditional medicine, their anti-inflammatory activities have not been widely reported or investigated (Fawole et al., 2009).

Sulfur compounds isolated from garlic have been shown to exhibit anti-inflammatory properties (Ban et al., 2009; Lee et al., 2012; Bose et al., 2013). Currently, there is little information on the anti-inflammatory activity of *Tulbaghia* species. In one of the few studies, lectin-like proteins from *T. violacea* were examined for

prostaglandin synthesis inhibitory activity and they showed 11% inhibition (Gaidamashvili and Van Staden, 2006). High inhibitory levels have been observed against COX-2 by leaf and bulb extracts of *T. violacea* (Ncube et al., 2012). The results from this study demonstrated the potential of *T. violacea* as an anti-inflammatory agent as well as support for its traditional use in treatment of pain related ailments such as fever, stomach ache and cancer.

As a result of ulcerogenic and renal side effects of non-steroidal anti-inflammatory agents, search for new natural non-toxic substances with anti-inflammatory properties remains critical. A variety of plant extracts used as traditional medicines to treat inflammatory disorders strongly suggest that medicinal plants are the major source of important phytochemicals.

### ANTI-CANCER ACTIVITY

Many natural products isolated from medicinal plants, or secondary metabolites such as terpenoids, phenolic acids, lignans, tannins, flavonoids, quinones, coumarins, alkaloids which exhibit significant antioxidant and other activities, have played an important role in treatment of cancer. Studies have shown that many of antioxidant compounds possess antitumor, anti-mutagenic and anti-carcinogenic activities (Unnati et al., 2013; Senthilkumar et al., 2014; Tagne et al., 2014). The major challenges associated with currently available anticancer agents include selectivity, toxicity, resistance, and development of a secondary malignancy. These drawbacks have motivated the search for newer, more efficacious, and better tolerated antitumor drugs, especially natural products from plants which offer an inexhaustible reservoir for new drug discovery and development (Akindele et al., 2015). A study by Bungu et al. (2006) showed that *T. violacea* bulb and leaf extracts inhibited growth of MCF-7, WHCO3, HT29 and HeLa cancer cell lines. This is an indication that the inhibitory compounds are likely to modify common metabolic events that are not tissue specific.

Lyantagaye (2013) investigated the pro-apoptotic activity of *T. violacea* extracts in order to understand the mechanisms of action that might be related to its traditional use as anticancer medicine. An apoptosis-guided purification was used to isolate the active compound. The compound identified as Methyl- $\alpha$ -D-glucopyranoside was shown to kill Chinese hamster ovary cells, MCF7, and HeLa cells through the induction of apoptosis (Mthembu and Motadi, 2014) support that *T. violacea* does induce cell death in a p53 independent manner.

Saibu et al. (2015) also found that extracts of the leaves of *T. violacea* have cell growth inhibitory effects which are due to the induction of apoptosis. This is associated with production of reactive oxygen species

(ROS) and activation of caspase-3. The study also confirmed that the infusions of *T. violacea* have potential anticancer activity and that the bioactivity is contained in the leaf extract. The mode of action of many anticancer drugs is based on their ability to induce apoptosis (Alshatwi, 2010). From the studies carried out to date, it appears that *T. violacea* has compounds with potential anticancer properties as indicated by its cell growth inhibition which is due to induction of apoptosis. Further studies on non-cancerous cell lines still need to be done to determine the selectivity of *Tulbaghia* extracts. Furthermore, assessment of the exact molecular targets of the extracts needs to be conducted to understand the mechanism underlying tumor cytotoxicity.

## TOXICITY

The widely held accepted belief and assumption is that natural products are safe, based on their long-term usage by humans and livestock. However, recent investigations have revealed that some plants have mutagenic and perhaps toxic effects (Elgorashi et al., 2003). This raises concern about the potential mutagenic health hazards resulting from the long-term use of such medicinal plants. Even though medicinal plants have benefits such as low cost, availability and acceptability, their safety and toxicity need to be evaluated and documented (Aremu et al., 2012). Toxic substances from plants can affect the entire spectrum of vital human organs while some may affect key functional body systems like the central nervous system (CNS) thereby interfering with the coordination of nerve functions of the body. The most dominant toxins are neurotoxins that affect the brain and CNS, followed by cytotoxins and metabolic toxins that affect organs such as kidneys, the liver, heart and lungs. The severity of a toxic effect may depend on the route of administration, growth stage or part of the plant, the amount consumed, the species and susceptibility of the victim. Other factors that may influence the severity of toxins include the solubility of the toxin in body fluids, frequency of intoxication as well as the age of the patient (Ndhlala et al., 2013). Medication prepared from *T. violacea* is said to cause abdominal pain, gastro-enteritis, (Lewis, 1995) and cessation of gastro-intestinal peristalsis, contraction of the pupils and sloughing of the intestinal mucosa. The potential toxic effect of *T. violacea* was demonstrated in a study by (Olorunnisola et al., 2012b) using brine shrimp lethality test. It was shown that the oil extract of rhizomes of *T. violacea* is cytotoxic and that the toxicity is concentration dependent. The significant lethality of the oil extracts (LC50 value less than 100 µg/ml) against brine shrimp nauplii was assumed to be due to the presence of sulphur compounds and steroidal saponins which have been implicated as cytotoxic agents with potential anticancer, antimicrobial and antifungal activities (Olorunnisola et al.,

2012b). Cytotoxicity has also been observed at 62.5 and 125 µg/ml for the ethanol extract of *T. violacea* on Chang liver cells (van Huyssteen et al., 2011).

Kaempferol, one of the compounds that has been isolated from *Tulbaghia*, showed mutagenicity towards test strains in the bacterial reverse mutation (Ames) assay. It was also noted that it is necessary to clarify the conditions and the mechanisms that mediate the biological effects of flavonoids before treating them as therapeutical agents, since some compounds can be biotransformed into more genotoxic products; as is the case for kaempferol (Resende et al., 2012). On the other hand, some of the safety assessments of *Tulbaghia* have been found to be negative. A study on acute and sub-chronic toxicity of methanolic extract of *T. violacea* rhizomes in vista rats indicated that a single oral dose of 5 g/kg had no significant effect on behaviour and did not cause mortality within 14 days of observation (Soyingbe et al., 2013) found that the essential oil of *T. violacea* has low (1218 and 1641 µg/ml) cytotoxicity levels against HEK293 and HepG2 cell lines. The green parts and flowers of *T. violacea* are also known to be consumed as vegetables (Ncube et al., 2011b) and more recently, have shown an absence of genotoxicity in the Ames and VITOTOX® tests (Elgorashi et al., 2003). It is therefore possible that the reported adverse symptoms are due to extensive use and /or high dosage of the species. In view of the current evidence the safety of the genus *Tulbaghia* remains conflicting and inconclusive. Clearly, more studies are required to provide a better insight to the safety and toxicity (Jager and Stafford, 2012).

## CONCLUSION

*T. violacea* is so far the most promising species as it is the highly investigated species. From the current literature search there is an indication that *T. violacea* has potential against infectious diseases and cancerous conditions. The other *Tulbaghia* species require further studies to ascertain their medicinal potential. Therefore, more studies need to be conducted on the biological activities of *T. violacea* and the other less investigated species. Research aimed at identifying specific compounds responsible for the bioactivities of these medicinal plants could contribute positively to the discovery of new drugs.

## Conflict of interests

The authors have not declared any conflict of interest.

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