

A REVIEW, OUTLOOK, AND INSIGHT, OF THE PROPERTIES AND CHARACTERISTICS OF *CALLISTEMON CITRINUS*

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Abstract

Callistemon citrinus, an invasive plant species that's native to Australia, is known for its uses by traditional healers in herbal medicinal formulations. Although the use of this plant species in alternative medicine strategies is varied, and well reported in the literature, there isn't enough information in a succinct format that can be accessed by the public, in general. Furthermore, very little information is accessible on the epidermal appendages found in this species of *Callistemon*. Therefore, in this paper, what's known and isn't about this plant species is being provided. Also, few observations on the epidermal appendages of *C. citrinus* is given as a means to compare it's morphology with its function.

Keywords: *C. citrinus*, constituents, epidermal appendages, eglandular, secretory tissues, leaf expansion theory, salt glands.

I. CLASSIFICATION OF CALLISTEMON

The family Myrtaceae is represented by over 35 species of *Callistemon* (commonly known as the bottle brush) plants that are endemic to Australia, of which each species differ in floral arrangement and organ morphology and anatomy (Spencer & Lumley, 1991; Cock, 2012; Shinde *et al.*, 2012). These once wide-spread, wet tropical, Australian species, have become an established invasive species in many countries like South America, Iran, Africa and tropical Asia (Shinde *et al.*, 2012). *C. comboynensis*, *C. linearis*, *C. pendulous*, *C. polandii*, *C. rigidus*, *C. speciosus*, *C. viminalis*, *C. viridiflorus* and *C. citrinus*, the species of interest in the present paper, are a few species of the *Callistemon* conglomerate that have successfully been assayed for many medicinal properties (Oyediji *et al.*, 2009; Ali *et al.*, 2011). Species of the genus *Callistemon*, in general, are traditionally used for treating coughs, bronchitis and haemorrhoids (the leaves) (Spencer & Lumley, 1991; Srivastava *et al.*, 2001), while many

species are also used in weed control and environmental management (such as crop protection), because they possess insecticidal (including larvicidal and pupicidal) and nematicidal properties, that are least toxic to human beings (Ali *et al.*, 2011). Antimicrobial, antifungal (the volatile oils), antistaphylococcal and antithrombin activities, in addition to the inhibitory activity of the volatile oils of *C. rigidus* (scirpusin B and piceatannol) on α -amylase in mice (Shinde *et al.*, 2012), have also been well discussed in the literature. In south Africa and other mild-temperate countries, *C. citrinus* is commonly used as an ornamental plant because it has attractive cylindrical brush-like flowers (Cock, 2012; de Andrade Wagner *et al.*, 2014), and leaves that are aromatic and lanceolate in shape; like most other *Callistemon* species (Spencer & Lumley, 1991). *C. citrinus* is usually found either as a shrub or tree that ranges between 0.5 to 7 m in height (Gilman & Watson, November 1993; Spencer & Lumley, 1991; Tantawy, 2004; Shinde *et al.*, 2012). The leaves range from 40 to 70 mm long, with a width of 3-6 mm (Spencer & Lumley, 1991; Srivastava *et al.*, 2001; Tantawy, 2004; Oyediji *et al.*, 2009; Shinde *et al.*, 2012; de Andrade Wagner *et al.*, 2014) and its leaf texture differs between young and mature types. Young leaves are soft, while mature ones are woody or hard in texture. *Callistemon* plant species have flowers with prominent stamens, and are borne in spikes that range from 40 to 150 mm in length (Spencer & Lumley, 1991; Gilman & Watson, November 1993; Shinde *et al.*, 2012). The flowers are rich in nectar and are fed on by birds. Petals are tiny, pale and greenish in colour (Spencer & Lumley, 1991; Cock, 2012). Though the only economic value of *Callistemon* plants is through their use as street trees or garden plants, nonetheless, these plants form an important part of South Africa's landscape, particularly because of their wide inter-continental and worldly distribution.

II. STUDIES ON *C. CITRINUS*– PRESENT AND PAST FINDINGS

Earlier studies on *Callistemon* species were based on their medicinal properties, use in crop protection, and on their nutritional value in Aborigines in Australia (Singh, personal statement). Later work dealt with their effects in mammalian models like mice and on the phytochemical composition of prepared *Callistemon* species extracts, including that of *C. citrinus*. Now, although new discoveries have been unleashed for *C. citrinus* hybrids, such as *C. citrinus viminalis*, many of these are repetitive in the methodologies used earlier, and the findings are conclusively inconsistent with published data; as they aren't collated in a completely coherent manner. Also, *C. viminalis* has been found to be the most frequently studied species, because of its availability in many countries more than in which *C. citrinus* co-exists. Comparing the anatomical structure of *C. citrinus* and *C. viminalis*, it seems that they are usually perceived as being identical; with leaf and stem surfaces being densely covered by trichomes. Thus far, there has been a limited amount of information on the exact role of trichomes in *C. citrinus* (Singh, personal statement), and there has only been one report on its type in the leaves of *C. citrinus viminalis* (Shinde *et al.*, 2012), which still remains inconclusive. These authors (Shinde *et al.*, 2012), while attempting to identify the phytoconstituents in the leaves of *C. citrinus viminalis*, had observed the presence of non-glandular trichomes. Since the early 1970's, it has been assumed that a high density of trichomes on plant leaves and stems (particularly young ones), like those found in *C. citrinus*, for example, are probably an adaptation to the temperate and persistently warm climates that plants are exposed to, so that they would be able to keep cool and prevent excessive transpiration from occurring (as a survival mechanism) (Uphof, 1962; Inamdar *et al.*, 1983; Werker, 2000). However, trichomes (to be defined later in this review), glandular and non-glandular (or eglandular), are well-known appendages that have many other functions in plant defence; and those roles have been published extensively for decades (Uphof, 1962; Spencer & Lumley, 1991; Antunes *et al.*, 2004; Agrawal & Fishbein, 2006). Microscopical structures such as stomata, glandular and eglandular trichomes (Panahi *et al.*, 2012), and the ultrastructure of leaves, have been noted for numerous plant species, other than *C. citrinus viminalis*. These species include *Tetradenia riparia*, *Salvia smyrnea* and *Plectranthus laxiflorus*, including other plants. These studies can be used as a base for microscopically evaluating the ultrastructure of trichomes found in *C. citrinus*.

III. TRICHOME CHARACTERISTICS, FUNCTION, AND COMPOSITION IN *C. CITRINUS*

Callistemon citrinus and *C. viminalis*, as mentioned, have been perceived to have identical appendages (non-glandular trichomes), though, it's uncertain whether their distribution, morphology and abundance at their prevailing locations are found at the same densities, since it has been suggested that such characteristics may vary for different plant species belonging to the same genus (Wagner, 1991; Werker, 2000). Apart from the non-glandular trichomes of these species being reported through microscopical observation, the ultrastructure and cytochemistry of them still remain quite a mystery. Reports have indicated that the type and density of trichomes differ between species, and that difference of trichome density on the organs of the same type plant species may exist. Scientists have, furthermore, reported the role of trichomes in the covering of reproductive and vegetative structures (Uphof, 1962; Ascensão *et al.*, 1995; Kaya *et al.*, 2007; Panahi *et al.*, 2012). A trichome, by definition, is a hair-like appendage or structure that extends from the epidermis of aerial tissues (Uphof, 1962). They have walls that are often similar or thicker than those of the surrounding epidermis (Uphof, 1962). Depending on the level of maturity of the plant species been studied, trichomes differ in the amount of cytoplasm they retain (Uphof, 1962; Werker, 2000). Reports have indicated that mature trichomes have little or no cytoplasm, while young or emerging trichomes have a lot of cytoplasm (Uphof, 1962). Apart from being glandular or eglandular, trichomes can also be straight, spiral, hooked or tortuous, simple, peltate, or stellate (Uphof, 1962; Pagne, 1978; de Andrade Wagner *et al.*, 2014). These trichome types are distinguished by stalk length and head size (Uphof, 1962; de Andrade Wagner *et al.*, 2014). It has also been suggested that trichomes play an important role in plant taxonomy; and in discriminating amongst different plant taxa (Uphof, 1962; Inamdar *et al.*, 1983; Agrawal & Fishbein, 2006). Glandular and non-glandular trichomes have widely different functions. Non-glandular trichomes, like the ones found in *C. citrinus viminalis*, reduce heat load of plants, assist in seed dispersal, increase tolerance against freezing, maintain leaf water balance, while glandular trichomes are secretory in nature; and they offer chemical protection to plants by preventing them from being invaded by insects, bacterial, and fungal pathogens (Uphof, 1962; Cornes, 2006; Cock, 2012; Zandi-Sohani *et al.*, 2013). The secretory products from glandular trichomes, are also utilised in pharmaceuticals, nutraceuticals, natural pesticides, flavourings, fragrances, and for non-food and fiber purposes (Uphof, 1962; Cock, 2012). Glandular trichomes also attract animals and salt accumulation (this with particular reference to salt dunes) (Uphof, 1962; Wagner, 1991).

IV. TRICHOME DISTRIBUTION, AND COMPOSITION AND ACTIVITIES OF *C. CITRINUS* OILS (OR EXTRACTS)

Pharmacognostic and phytochemical parameters, like macroscopy, microscopy, physicochemical constants, thin layer chromatography, and antimicrobial activity have been reported to be a few of the important factors that are required to identify and authenticate plant species. It has been reported that the leaves of *C. citrinus* contain more hydrodistillate-volatile oils compared to *C. viminalis* (Oyedemi *et al.*, 2009; Shinde *et al.*, 2012). The major components that were found in the leaves of *C. citrinus* were 1,8-cineole, α -pinene and β -pinene, with trace elements of α -terpinene, linalool, trans-pinocarveol, terpine-4-ol, and geraniol being present in the oils of both plant species (Antunes *et al.*, 2004; Oyedemi *et al.*, 2009). Furthermore, it has been reported that the oils of *C. citrinus* (and *C. viminalis*), exhibit antibacterial activity against a panel of bacterium species like *P. aeruginosa*, *S. aureus*, *B. cereus*, *E. cloacae*, *E. coli*, *S. faecalis*, *K. pneumonia*, *B. pumilus* and *S. marcescens*, and this has been attributed to the presence of 1,8-cineole, the important component used in medicinal essential oil classification (Oyedemi *et al.*, 2009; Cock, 2012). Although, the extracted oil of *C. citrinus* inhibited the growth of mostly gram-positive bacteria, this find (of inhibition occurring in a few gram-negative bacterium species) has been ascribed mainly to the presence of 1,8-cineole,

α -pinene, and linalool (Cock, 2012). The latter components have been known to exhibit antimicrobial and bacteriostatic activities, and thus play an important role in the overall medicinal, traditional and herbal applications, from a therapeutic and pharmaceutic perspective (Singh, personal statement), particularly because the essential oil of *C. citrinus* and *C. viminalis* exhibit a broad spectrum antimicrobial activity against gram-positive and gram-negative bacterium species.

Like in many plant species, such as *Xerophyta viscosa* and many plant species of the Lamiaceae family (Campbell *et al.*, 1997), the chemical constituents of trichomes confer additional therapeutic values to prepared and tested extracts on animal models, microorganisms (Shinde *et al.*, 2012) and fungi (Uphof, 1962; Levin, March 1973). These trichomes, as many reports have suggested, can cover either the entire surface of a plant or appear on the leaf surfaces in equal or unequal proportions (Basabalidis & Skoula, 1998). It has also been found in some species that trichomes appear only on one leaf surface i.e. either the abaxial or adaxial side (Basabalidis & Skoula, 1998). From personal observation of *C. citrinus*, the abaxial leaf surface of young leaves are more densely covered by long-pointed trichomes, compared to the abaxial surface of the same leaves, while this isn't the case in mature *C. citrinus* leaves. In the mature leaves, it has been observed that the abaxial surface has sparser trichome abundance, compared to adaxial leaf surfaces. The exact functional role for this difference has yet to be reported. It's uncertain whether this trichome density difference is due to the leaf expansion theory, or the maturity of leaves overtime. Reports on *Tetradenia riparia*, and others from this family, suggest that a very high density of trichomes are expected in young leaves; and that a rapid decline in trichome density is expected to occur, due to the leaf expansion theory (Maffei *et al.*, 1989; Ascensão *et al.*, 1995). It has been factually known that during the late stages of leaf development, and at maturity, plants shed their trichomes on senescence, since the functional role of trichomes become less important with aging (Werker, 2000). However, though there still are some plant species in which trichomes remain viable and functional in matured leaves, and during further leaf maturation (Werker, 2000). However, trichomes may also occur at specific locations on leaves, for example in *Plectranthus* species, non-glandular trichomes have also been found along the mid-vein in young leaves (Karabourniotis *et al.*, 1993), with a gradual decrease in their density during maturation on both surfaces (Karabourniotis *et al.*, 1993; Tattini *et al.*, 2000; Valkama *et al.*, 2003). This reinforces the protective role of non-glandular trichomes during the early stages of leaf development, i.e. protection against heat overloading, and against predation, by herbivorous insects during maturation (Levin, March 1973; Werker, 1991; Mauricio & Rauscher, 1997). This has also been reported for many other angiosperms, for example, in *C. citrinus*, the non-glandular trichome abundances could serve this protective role given that this angiosperm species is feasted in Australian aborigines (Cock, 2012).

V. CONTROVERSIES, AND NEW INFORMATION ON THE TRICHOMES IN *C. CITRINUS*

As it has already been established, *Callistemon citrinus* has non-glandular and elongated trichomes that cover the stem and abaxial and adaxial leaf surfaces. Many reports have identified eglandular trichomes with simplicity, suggesting that they possess a basal cell that's surrounded by a group of epidermal cells (Ascensão *et al.*, 1999). Other authors have reported that a presence of cuticular warts is an indication of maturity in that specifically examined plant region (Werker, 2000). In a study on *Plectranthus laxiflorus*, it was assumed that cuticular warts on the lowest regions of non-glandular trichomes (the septate region) began to shed off due to maturity (Werker, 2000; Cornes, 2006). Apart from the abundance of trichomes on plant surfaces, and secretory tissues, it has been reported that mixtures of glandular and eglandular trichomes may be present on the same plant organs, but in different proportions along the different regions of the root-stem-leaf continuum; in a single species (Marin *et al.*, 2006). In *C. citrinus*, a mixture of elongated (long and short), eglandular, trichomes were present in networks. This observation to date has not been reported in

literature. Glandular and non-glandular trichomes can be smooth or exhibit micro-ornamentation that either arises from the cell wall or the cuticle (Shinde *et al.*, 2012). The specific function of the non-glandular trichomes in *C. citrinus* is still unknown, except for its postulated roles from theory.

VI. PERSPECTIVE AND CONCLUSION

Although the leaves, stems, and roots, of *C. citrinus* have been largely investigated for their medicinal values, the contribution of any medicinally- or biochemically-important compounds (or components) from these abundant eglandular trichomes, or the tissues they emerge from (secretory or non-secretory), have not been studied to understand their functionality definitively. Still there is no information on the role, structure, and morphology of *C. citrinus* trichomes. It is hypothesised that the eglandular trichomes, and/or the tissues they emerge from in *C. citrinus*, prevent insect predation, contributes to medicinal efficacy (of the studied medicinal properties), and possess chemicals that are of biochemical importance.

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