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Original Article

# Biological Activity of *Cymbopogon citratus* (DC) Stapf and Its Potential Cosmetic Activities

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**Abstract**

The present work makes a general bibliographical review around a variety of issues around *Cymbopogon citrates* and tries to summarize the most important aspect and qualities of the plant that make it a potential element in the research and defining of cosmetics with natural ingredients. The biological antibacterial and antifungal activities of the species *Cymbopogon citratus* (DC) Stapf, have been identified by several authors by highlighting a potential action on a large number of pathogens, some of them related to dermal problems. Various other activities have been evaluated, such as antioxidant and insecticide; allowing the extract to be incorporated in cosmetic and insecticide formulations. Several studies consider the species for patented formulations that include microencapsulation as a viable option. Non-invasive *in-vivo* studies have been reviewed in this work to analyse any potential toxicology and to define the best concentrations of use.

**Keywords:** Biological activity, Cymbopogon citratus (DC), efficiency cosmetics.

## Introduction

The species *Cymbopogon citratus* (DC) or traditionally known as "Hierba Luisa" in Ecuador, corresponds to an identified group of 175 plant species, popularly sold in the markets of some provinces of the Ecuadorian Andes. These species are valued for their medicinal uses and were traditionally used to treat conditions such as blood pressure, nerves, inflammation, and jaundice.[1] The species is native to the tropical region of Southeast Asia, India, and Sri Lanka. It is cultivated in tropical and subtropical regions for medicine, as a spice and to control erosion.[2]

*Cymbopogon citratus* (DC.) Stapf is a perennial grass that grows spontaneously throughout the world, mainly in tropical regions and savannas. The infusions of its leaves are used in traditional medicine in several countries. The effects generally observed are antimicrobial, anti-inflammatory and sedative, while the essential oil of leaves is used in food industries, perfumery, soaps, cosmetics, pharmaceuticals, and insecticides.

A review conducted to examine its uses in different regions of the world, identified that in China, it is used as an anxiolytic. [3] In the Malaysian peninsula it is commonly used against the flu, fever, pneumonia and as a sudorific for gastric problems.[4] In Nigeria, is used as an antipyretic, for its antispasmodic effects.[5] In Indonesia, the plant is prescribed to help digestion, to promote diuresis, sweating and as an emmenagogue.[6] It is widely used in traditional medicine in Cuba and in many other countries of the Caribbean region. Among the properties attributed popularly, the most common are those related to its' analgesic and anti-inflammatory actions.[7]

As already mentioned its use not only is medicinal, *Cimbopogon citratus* is also used for flavoring and spicery for food and the essential oil is used in the perfumery and cosmetics industries.[8] This background review indicates the extensive uses and the importance give by communities to the properties and actions of the plant.

In regard to the biological activity, the studies are numerous and have shown that it has a great antimicrobial and antifungal capacity. The action was mainly observed over certain pathogens such as *Vibrio cholerae* and *Salmonella paratyphi*, different dermatophytes: *Epidermophyton floceosum, Microsporum canis*, *Trichophyton mentagrophytes* and *T. rubrum*. The action observed is the inhibition of the development of bacteria such as *Escherichia coli, Bacillus subtilus*, and *Staphylococcus aureus*. It was also found that it acts on A*spergillus flavus*, being more powerful than the synthetic fungicides. [9]

The species also shows important antioxidant and repellent properties that can be incorporated in formulations for pharmaceutical or cosmetic products. This review, however, also touches upon the constraints such as toxicity of the extracts, the inability to incorporate them, due to their volatility, to the alternative of using various techniques such as microencapsulation. There are also tests of cosmetic efficacy performed *in vivo* that support the incorporation of the species as a natural active as an alternative in skin problems.

The review of the species *Cymbopogon citratus* considers a number of qualities of the species that could be considered in the research and development of cosmetics with natural ingredients, to achieve a secure, stable and effective formulation.

## Biological Activity of *Cymbopogon citratus* (DC) Stapf

A number of components have been identified in the essential oil extract of the plant. Although there is variability in the percentages found, there is consistency in its main components, some of which include citral, (the name given to the mixture of stereoisomers geranial and neral). As Negrelle and Gomes have pointed out, irrespective of their origin, the essential oils of *Cymbopogon citratus* are composed of citral (30 to 93.74%).[10] Tests have been carried out to identify the metabolites responsible for the antibacterial activity. These activities are shown in two of the three main components of oil, identified through chromatography and mass spectrometry methods. While the components (neral) alpha-citral (geranial) and Î²-citral cause individually antibacterial action on gram-negative and gram-positive strains, the third component, myrcene on its own shows no antibacterial activity. However, myrcene shows a marked improvement when mixed with either of the other two main components identified.[11]

In regard to the antibacterial and antifungal activity of the species, the study conducted by Guerra in 2004, used microorganisms composed of eight bacteria: *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 7001, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 10033, *Serratia sp*, and *Salmonella typhimurium*, 4 dermatophytic fungi: *Microsporum canis*, *Trichophyton mentagrophytes*, *Trichophyton rubrum* and *Epidermophyton floccosun* and a yeast: *Candida albicans*.[12]

The antibacterial activity was greater for the gram-positive bacteria with CMI of 0.3 to 0.6 mg/ml, for gram-negative bacteria the activity was lower with CMI values of 2 to 5 mg/ml even against *Pseudomonas aeruginosa* ATCC 27853. Inhibition did not take place until the concentration was increased to 10mg/ml (the highest concentration of the test). The results in fungi showed antifungal activity in concentrations from 0.04 to 1.25mg/ml. [12]

There are contrasts in regards to the microbiological activity tested in different studies. In the case of the studies of Guerra and Lemon, the gram positive bacteria presented greater sensitivity to oil[13] but in the case of Alzamora, the study determined greater activity in gram-negative bacteria tan in gram positive. In all studies, however, activity was evidenced for bacteria tested to a greater or lesser degree except for *Pseudomona aeruginosa*, which was resistant to the tested concentrations.[14]

The biological variables in which the species has been developed, such as differences in soil, temperature, humidity, habitat in general, could influence the chemical composition of the species and, consequently, influence the antibacterial activity of the essential oil. The reviewed studies on the antimicrobial activity of the essential oil of *Cymbopogon citratus* represent a good foundation for the antibacterial potential of natural ingredients that could be used in different food products, medicines or cosmetics. Although the results are not replicable by the variables handled in each investigation, it is unquestionable the antibacterial activity of the essential oil. The biological activity has also been tested with strains of *Aspergillus ochraceus*, *Penicillium expansum* and *P. verrucosum*. The fungicidal activity was determined and expressed as a decimal number of the reduction of the colony forming units per ml (cfu NDR). The influence of pH variation was studied. *C. citratus* to 8000 ppm presents activity against the strains, the study concludes that a change in pH enhances the activity of the plant material: at pH3 against the strain of *Aspergillus* not so for the species of *Penisiliun* which at pH 9 it presented greater activity. [15]

In relation to the antifungal activity, some in vitro tests indicate that an 80 % of the strains of dermatophytes tested have been inhibited by the essential oil. The essential oil also showed activity over *M. canis*, a rather resistant strain.[16] Some reports mention that the antifungal action is the result of a mixture of compounds present in the oil, rather than citral as an independent compound.[17]

If these antimicrobial results of *Cymbopogon citratus* are oriented, a potential use could be identified in the area of cosmetics, depending on their action against gram positive and negative bacteria, and could be used in products such as disinfectants or liquid antibacterial soaps, considering that most studies define activity on bacteria that are responsible for cutaneous conditions such as *Staphylococcus aureus, Staphylococcus epidermidis* and even *Propionobacterium acnes* ATCC 1951, according to research in Thailand in which it concludes an action of essential oil on the pathogen with one (IMC and one CMB of 0.6 µl/ml).[18]

According to research in Thailand demonstrating the action of the essential oil on the pathogen with one (IMC and one BMC of 0.6 µl/ml.[18] This information becomes the basis for considering *Cymbopogon citratus* as a natural active to treat skin pathologies such as acne, which occur by an infection where most of the agents mentioned are the cause.

The antioxidant activity of extracts of *Cymbopogon citratus* demonstrates a high DPPH uptake activity

The following IC 50 values (µg/ml) for aqueous extract 1615.7 +/- 302.2; Ethanolic extract 97.7 +/- 0.2; and Methanolic extract 85.7 +/- 12.2, show that the aqueous extract presents the best antioxidant activity. Consequently, this plant could be used as a potential agent for the prevention of various dermal diseases and pathologies that are the result of oxidative stress.[19]

Although it is known that aging "per se" does not generate greater oxidative stress, studies such as that of Beristain, assert that oxidative stress is linked to chronic degenerative diseases, which are usually associated with an early aging. [20] This would allow the identification of active principles that control oxidative stress and the release of free radicals, which could be used as anti-aging products.

### Application of Biological Activities in Phyto-Pharmaceutical or Cosmetic Products

After reviewing the biological activity of the species, it became important to identify how much of the active principles have already been used in the manufacture of natural products. In the work done by Mosquera we identified the "CMI of the oil of *Cymbopogon citratus* on Propionibacterium acnes by determining effective concentrations of inhibition from 1 to 5%, concentrations which are used to make a cosmetic formulation tested *in vivo* by a non-invasive analogous chamber Visiopor PP34N which determines the presence of porphyrins on the surface of the skin, directly related to the population density of Propionobacterium acnes, a lotion made with 5% of essential oil of *Cymbopogon citratus* shows a percentage of decrease from 44.4 to 100% ".[21]

In regards to the cosmetic value of the plant: studies have been carried out where the natural actives have been applied to creams to test their effectiveness. The results not only showed the effectiveness, but also the low irritation of the compounds on the skin. The cream formulation obtained from *Cymbopogon citratus* possesses antifungal activity. When tested on *Trichophyton* and *Epidermophyton* the CMF values ranged from 0.08 to 2.50 mg/mL. The action proved to be fungicidal, except for yeast *C. albicans* with a value of 5 mg/ml, for which it was fungistatic, the same study determines its' irritating effect. The reactions on the skin for erythema and edema were established according to the system described for the evaluation of skin irritation. To determine the primary irritation index (PII), only the observations performed at 24, 48 and 72 hours were taken into account, with a PII value equal to zero classifying it as non-irritant. [22]

An interesting study that generates a great potential in the manufacture of cosmetic products took place in Lima. It assessed the essential oil of *Cymbopogon citratus* leaves on *Brevicoryne brassicae* and aphids, demonstrating a repellent effect with concentrations oscillating between (0.1 to 0.5%).[23] This is not the only study that evaluates the insecticide capacity, the plant has been tested on a variety of insects, and comparative tests that have been carried out with the genus Cymbopogon (Cymbopogon: *Cymbopogon martinii*, *Cymbopogon nardus*[24] conclude that plants of this genus can play an important role as insecticides. The use of this plant reduces the risks of synthetic insecticides, with a repellent activity CR50 (ability to repel the 50% of insects) 0.03 and 0.04 μL/cm2, tested in *Oryzaephilus surinamensis* (L.) and S*itophilus Zea* mays Motsch.

In the field of herbal medicines, Melgarejo in 2004 establishes a practical guide for the use of the species as a phytotherapeutic in Primary Health Centres. Based on the pharmacological benefits of the plant, he recommended the plant to be used: antibacterial, antifungal, analgesic, diuretic antihypertensive, antispasmodic, expectorant, antiasthmatic. The guide also mentions the methods and most effective ways in which the apply and use the plant such: plant material, tincture 20%, cream with concentrations of 2% to 5%, syrup and aqueous extract. Dosages, contraindications, and warnings are summarized as well, which all together offer a practical and efficient tool for the prescription of products in their natural form or as pharmaceuticals obtained from medicinal plants.[25]

One of the activities tested in food, but should not be limited only to this field, but interpolate in different products such as cosmetics or phytopharmaceuticals is the preservative action exerted by the species due to its action against fungi and mycotoxins. The Prakash study identifies that the essential oil of *Cymbopogon citratus* can be used in stored foods that are frequently contaminated by fungal infestations, or mycotoxins secreted by toxigenic fungi, responsible for the generation of free radicals. The results define the activity of the essential oil on the fungi and its action as antioxidant; the work concludes that complementing the activity studies with residual toxicity studies of the oil. The species, in fact, could be included in the category "GRAS"by the Food and Drug Administration (FDA) and adopted as flavoring or food additive. With studies like these, it can be determined that the essential oil of Cymbopogon citratus can be developed as a safe alternative of preservatives for different products. Their eco-chemical biorrational mode of action would be useful in achieving the goal of "green consumption" and "sustainable food security". [26]

Another study confirming its use as a preservative is that of Nguefack, in which determination of the conservative activity of *Cymbopogon citratus* is made by combining it with other essential oils of *Ocimum gratissimum* and *Thymus vulgaris* against two strains producing *P. expansum* mycotoxins. The antifungal activity was determined by the dilution method expressed as a decimal reduction number of colony units per ml (cfu NDR). The study showed that the essential oils extracted from *O. gratissimum* were significantly ($P < 0.05$) more active against *P. expansum* than those extracted from *C. citratus* and *T. vulgaris*. The fungicidal activity of mixtures of fractions of the same essential oils or of two different essential oils showed that there are synergistic effects, additives against both fungal strains.The observed synergistic effects could be exploited in order to maximize the antimicrobial activity of essential oils and to minimize the concentrations of essential oil required to produce an antimicrobial effect without any alteration in the tested product.[27]

These effects tested in the essential oil of *Cymbopogon citratus* in the cosmetic field could present interesting results, since it would be a polyfunctional active ingredient that not only has activity against some cutaneous pathogen, or that controls the generation of free radicals, but also would be responsible for the stability of the formulation.

Within a cosmetic formulation, selecting and correctly using the preservative system in a product means not only defining it or the combination of actives with the preservative role, but also the ideal concentration of action, interaction with the other components of the formula and the incidence In the irritating capacity that can generate the product. The European directive for cosmetics (76/768 EEC) in Annex VI has more than 60 active substances, which can be used for the preservation of cosmetics, however only about a dozen are actually part of the market. Among these, the most important are: parabens, isothiazolinones, formaldehyde donors (DMDM), hydantoin, imidazolidinylurea and diazolidinylurea, organic alcohols, aromatic alcohols, phenoxyethanol, methyl dibromo glutaronitrile and bronopol.[28]

Within this list, no natural ingredients are found which, as previously indicated, have a high potential. The use of natural preservatives, far from being novel, is a technique used since antiquity, and in fact, new research is focused on new substances that, besides being natural, do not compromise the health and safety of the users. Some research supports the relationship of the chemical components of essential oils with a certain chemical and/or biological activity, so it is important to know the functional groups and possible synergistic interactions between the components. A correlation of the antimicrobial activity concludes that the components with the phenolic structures, present great in-vitro activity against some microorganisms, granting the bactericidal or bacteriostatic characteristic to the vegetal species, that possess these components.[29]

One of the activities that must be tested in the field of cosmetics, is the antioxidant capacity, an activity that could be corroborated in *in vivo* studies; Although they have already been carried out using an animal model, as in the Alvis research that determined the antioxidant activity of the hydro-alcohol extract of *Cymbopogon citratus*, by two methods: I) 2, 2'-azino-bis (3-ethylbenzthiazoline-6 (ABTS), and II) ferric reducer of antioxidant power (FRAP). Phenolic content was determined through Folin Ciocalteu using the Rancimat method, and the *in vivo* effectiveness of this extract was determined using grease as an oxidizing matrix. Ethanol 50% v/v, proved to be the best solvent to extract the bioactive constituents capable of stabilizing the lipid material from the pig fat and protect them against oxidation. According to the results, the extract exhibits a high content of phenolic compounds with anti-radical and high reducing effects. In regards to the antioxidant effectiveness, it was found that the extract in a concentration of 250 ppm it manages to double the time of induction with respect to the control grease.[30]

These data will allow cosmetic formulations in the concentrations tested to determine the antioxidant capacity *in vivo* in human subjects, through non-invasive methods. Studies of this type have already been carried out with natural ingredients such as the one made by Mosquera, in which ingredients with proven antioxidant action were added to cosmetic emulsions (creams). The study was carried out with 30 women with some degree of photoaging. A dermatological clinical evaluation and an instrumental evaluation were performed using the Cutometer MPA580, a device that allows visualization of changes in elasticity and skin firmness. The evaluation was done at the beginning and after four weeks of using the product and the data were subjected to analysis of variance. At 28 days of treatment the clinical evaluation showed the creams had significant improvement in skin brightness and softness, and the instrumental evaluation indicates that an improvement in skin firmness and elasticity was achieved.[31]

In the cosmetics area are identified certain guidelines already given for this species, the essential oil of the leaves of *Cymbopogon citratus*, is registered in the page of the CosIng database of the European Commission that contains information on the substances and ingredients used in cosmetics, with very limited activity only with application in perfumery or with masking effect of aromas,[32] but efficiency studies cosmetics would endorse the Commission other properties and can extend the application of the ingredient.

Regarding the regulations, there are considerations that must be taken into account in the production of the oil, one of them corresponds to the shelf-life of the species. Studies indicate that the leaves of *Cymbopogon citratus* gradually lose their color and fragrance under the action of the sun when stored outdoors. Solar drying is, unfortunately, the only current method of conservation. Likewise, the lack of control in the distillation process has shown a reduction of its main components.[33]

The establishment of the collection, drying and distillation procedures will allow the standardization of extracts of *Cymbopogon citratus*; Guaranteeing the efficiency attributed to the product. Countries like Brazil have these specifications in Pharmacopoeias that determine that the plant material used in phytotherapeutic preparations must have at least 60% of its components identified with pharmacological activity.[34]

Within these specifications, the toxicity of the ingredient should also be considered. There are toxicity determinations presented in oral forms as an acute toxicological evaluation of the fluid extracts at 30 and 80% of *Cymbopogon citratus (*DC) Stapf, demonstrating that the manifestations of toxicity are more evident, at higher fluid concentration, which became more evident in animals treated with the 80% fluid extract. The observed damages were centered in the stomach with light vascular congestion and focal hemorrhagic infiltrate in liver and kidney, where the histological findings evidence the hepatotoxic and nephrotoxic effect. [35]

Another toxicity study with an internationally validated method (fixed dose procedure), in Prague Dawl rats that received the plant extract orally with a dose limit of 2000 mg/kg body mass and a negative control of sodium chloride 0.9% showed no mortality or symptoms indicative of toxicity in the animals. Macroscopic anatomopathological studies showed no alteration in the organs studied. The LD50 of the test extracts is above 2000 mg/kg of body mass, classifying it under the Global Harmonized System as "Unclassified" ("Non-toxic").[36]

The inability to incorporate natural ingredients into formulations can be overcome with some revised application alternatives in order to extend the applications of volatile compounds (essential oils) and to reduce their to obtain a longer shelf life. To overcome these drawbacks, encapsulation processes are suggested, whereby a liquid essential oil is enclosed in a special carrier or matrix, which achieves continuous release and at the same time prolongs the effect of the volatile components with techniques using a polymer matrix.[37]

Some techniques have been evaluated, microencapsulation is considered as an alternative, offering a solution to modify the physical state and present them in a solid form for administration by different routes, this alternative allows to mask the unpleasant smell and taste of the products as well as protecting the fatty acids present from oxidation. Spray drying is one of the most widely used industrial scale methods for obtaining microcapsules. Due to its increasing use in the pharmaceutical industry, it is a technique that could also be considered in the field of cosmetics.

An interesting study took place with essential oils, in which a microencapsulation was carried out using yam starch (*D. rotundata*), which was submitted to hydrolysis and lyophilization processes, using dodecenylsuccinic anhydride (DDSA). This significantly increased the capacities of oil and emulsifier (surfactant) of the starch, which was used to microencapsulate essential oil of thyme, which in previous studies demonstrated potent antibacterial activity on the strains involved in the development of acne. Microencapsulation was carried out by solid lipid microparticulating, followed by emulsification. This achieved an efficiency higher than 98%, and the product obtained, challenged in various tests, showed the capacity to retain more than 90% of the essential oil under conditions of evaporation, avoiding its oxidation and the change in its composition profile. Finally, the microcapsules of essential oils of thyme, on coming into contact with the acne bacteria, maintained their bactericidal activity. The results of this work contribute to the development of stable and functional pharmaceutical, cosmetic and food formulations of essential oils, by protecting them from evaporation and degradation, [38] and can also be applied to the essential oil of *Cymbopogon citratus.*

A similar microencapsulation process has also been performed with *Cymbopogon citratus*. The essential oil was obtained by steam stripping, and 10DE maltodextrin (Proquimposa) and gum arabic (GA) (Hycel) were employed as encapsulating agents. The essential oil concentrations evaluated were 1%, 3% and 5% v/v. A pre-emulsion was formed in a homogenizer (Ika-Ultra-Turrax® T25 Basic) and then an ultrasound treatment was applied. The encapsulation was performed in a laboratory sprinkler (Pulvis GB 22 YAMATO). The quantification of the α-β citral and β-myrcene present in the microcapsules was performed by GC.

In the chromatographic analysis of the essential oil it was found that the major component was citral with 65.03%, which in turn is composed of a 26.07% of β-citral and 38.96% of α-citral, and the second major component was β-myrcene with 19.32%. The retention percentage for β-myrene varied from 25 to 99%, for β-citral from 22 to 99% and for α-citral from 27 to 99%. The highest retention percentages were obtained with the highest concentration of the encapsulating agent (30%) and with the lowest concentration of oil in the emulsion (1%), respectively, using gum arabic. The encapsulation allows for the controlled release of the aromatic compounds present in the solid matrix. The best retentions for β-myrcene, and for α- and β-citral were obtained with 30% gum arabic and a maximum of 3% essential oil.[39]

All these techniques can be applied in the manufacture of cosmetic products, a number of products already exist many with patented formulas that use both extract and essential oil, in combination with other oils such as lemon and grape, plus other natural raw materials.[40] The patent has as innovation the combination of natural raw materials in a simple process.

Another very interesting patent in the species is the identification of the bacterium Pantoea sp endophytic. CCSH-1 preservation number is CGMCCNo.8715 which generates a volatile fragrant substance. The bacteria strain comes from the mature pod of *Cymbopogon citratus,* and is capable of generating a refreshing and pleasant fragrance. The aromatic substance is identified as citral, thus the bacterium provides a new source for the substitution of vegetable raw materials that produce perfumes. [41]

## Conclusions

This bibliographic review, it is clear that the species *Cymbopogon citratus* can be considered an active potential for efficient cosmetic formulations, acne control lotions, antibacterial soaps, repellent lotions, anti-aging creams, dermal fungal creams, among others. Scientific support exists, evaluations that have been carried out in vitro on some pathogens, identification of an effective concentration in their growth, may allow defining effective formulations

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