Ethnopharmacology, phytochemistry, and biological activities of Cymbopogon citratus (DC.) Stapf extracts

Christopher E Ekpenyong¹*, Ernest Akpan¹, Azah Nyoh²

¹Department of Physiology, Faculty of Basic Medical Sciences, University of Uyo, Uyo Nigeria; ²Department of Physiology, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria

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[ABSTRACT] Cymbopogon citratus is a widely distributed perennial herb belonging to the Poaceae family and has been extensively consumed for its medicinal, cosmetic, and nutritional effects for centuries. A large number of reports have been published describing the pharmacological, biological, and therapeutic actions of this herb. In this review, we summarized the literatures on related studies (up to January, 2014) that highlighted the pharmacologic and biological effects of the major phytochemicals isolated from C. citratus extracts and its essential oil. The components of the essential oils found in C. citratus have a similar pharmacokinetic properties, including absorption, distribution, metabolism, and excretion. They are quickly absorbed following oral, pulmonary, and dermal administration. Based on the published reports, it can also be inferred that, after absorption from the small intestine, some phytochemicals in C. citratus can undergo oxidation, glucuronidation, sulfation, and/or O-methylation. Excretion is through urine, feces and/or expired volatiles. The biotransformation reactions of C. citratus bioactive constituents are essential for its relatively safe consumption and therapeutic applications. The data available so far warrant further studies evaluating C. citratus pharmacokinetics. Reliable pharmacokinetic data in humans would be critical for a better understanding of the the systemic handling of C. citratus.

[KEY WORDS] Cymbopogon citratus stapf; Phytochemistry; Pharmacological effects

[Introduction] Cymbopogon citratus is an aromatic perennial plant of the Poaceae family, with long slender green leaves. It is widely distributed and extensively used worldwide¹-⁴. The consumption of infusions and decoctions made from C. citratus has been a common practice in various countries since the discovery of the medicinal value of the plant throughout recorded history¹-³. It is most frequently consumed for recreational and medical/therapeutic purposes², much like green, black, and red (rooibos) teas, herbal tea blends, and coffee. Many consumers prefer C. citratus tea to other beverages, because of its physicochemical characteristics, including taste, distinctive lemony smell, color, strength, and intensity¹, while many others consumes C. citratus tea or decoction for physiological reasons²-³. For example, Dangupen et al¹ have reported a study performed among residents of the Benguet province, Northern Philippines, indicating that the participants believe that C. citratus has healing properties and is helpful in alleviating indigestion and stomach problems, soothing stress, relieving colds, fevers, and pain, and managing arthritis. In Nigeria and some other African countries, some people consume ethanolic decoctions made from C. citratus leaves under the belief that it can cure variety of diseases¹-³. Various studies have shown that C. citratus is used in herbal medicine worldwide for a wide range of applications, including antibacterial, antifungal, antiprotozoal, anti-carcinogenic, anti-inflammatory, antioxidant, cardioprotective, antitussive, antiseptic, and anti-rheumatic activities²-⁴. It has also been used in the prevention of platelet aggregation⁹ in the treatment of diabetes¹⁰, dyslipidemia, gastrointestinal disturbances¹¹-¹², anxiety¹³, malaria¹⁴, flu, fever, and pneumonia¹¹; in aromatherapy, and in cosmetology. In addition to its therapeutic uses, C. citratus is also added to non-alcoholic beverages and baked foods as a flavoring and a preservative in confections and cuisines¹¹,¹⁵. Several studies evaluating the phytochemical composition of C. citratus have shown the presence of saponins, tannins, anthraquinones, flavonoids, phenols, and alkaloids, in addition to terpenes, aldehydes, alcohols, and esters¹⁵,¹⁶-¹⁸. Furthermore, trace
amounts of other components have been detected, including myrcene, geraniol, geranial, limonene, burnanol, citronellol, nerol, α-terpineol, elemicin, catechol, luteolin, 6-C and 7-C-glycosides, caffeic acid, apigenin, luteolin, kaempferol, quercetin, chlorogenic acid, and geranyl acetate [11, 16-18]. Fumonosol, furfural, isopulegol, isovaleric aldehyde, L-linalool, methylheptenone, n-decyclic aldehyde, nerol, terpineone, p-coumaric acid, and valeric esters have also been isolated in some studies [11, 16-18]. Cheel et al [19] have reported the presence of isoscoparin, swertiajaponin, and orientin in C. citratus, along with numerous other phytochemicals reported recently by Bharti et al [20]. C. citratus also contains electrolytes and minerals (including sodium, potassium, calcium, copper, magnesium, manganese, selenium, phosphorus, iron, and zinc), vitamins (including folate, niacin, pyridoxine, riboflavin, and vitamins A, C, and E), and macronutrients (carbohydrates, proteins), and a small amount of fat [21].

Growing evidence suggests that these phytochemical components are responsible for the wide range of biological and therapeutic actions of C. citratus. The major phytochemicals and their relative biological activities of C. citratus are presented in Tables 1 and 2.

To date, a number of studies have been conducted to assess the therapeutic activities of C. citratus. However, as with other teas, it’s pharmacokinetic and pharmacodynamic properties remain to be described and discussed, thereby warranting this review.

Pharmacokinetics of Phytochemicals in C. citratus Extracts

For any chemical moiety to exert a biological effect, it needs to be bioavailable and to have the potential to exert its effects in vivo [22]. There is a dearth of information on the disposition of C. citratus from either human or animal studies. However, the disposition of its essential oils, component phytochemicals, and other essential bioactive constituents has been reported in a number of separate studies [23-29].

Essential oils: Essential oils are mixtures of lipophilic, volatile, and compounds (often terpenoids) present in plants. Components of essential oils, such as those found in C. citratus, often share similar profiles in terms of their absorption, metabolism, and excretion [23]. They are quickly absorbed following oral, pulmonary, and dermal administration [21]. Most are metabolized and eliminated by the kidney in the form of glucuronides, or exhaled as CO2 [23]. Their accumulation in the body is unlikely, owing to rapid clearance and short biological half-lives [23].

The essential oils from C. citratus contain various monoterpenes, with citral being the most abundant (65%-85%) and the most pharmacologically and physiologically important constituent. The disposition of citral (an aldehyde) is studied in male Fischer rats after intravenous (i.v.), oral (p.o.), and dermal administrations [24]. The patterns of distribution and elimination are the same following i.v. or p.o. administration, with urine being the major route of elimination of citral-derived radioactivity, followed by feces, 14CO2, and

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**Table 1** Bioactive natural constituents reported in C. citratus extracts

<table>
<thead>
<tr>
<th>Phytonutrients</th>
<th>Mineral contents</th>
<th>Vitamins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>Sodium</td>
<td>Vitamin A</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Potassium</td>
<td>Vitamin C</td>
</tr>
<tr>
<td>Vitamins</td>
<td>Calcium</td>
<td>Vitamin E</td>
</tr>
<tr>
<td>Tannins</td>
<td>Iron</td>
<td>Folate</td>
</tr>
<tr>
<td>Phenols</td>
<td>Phosphorus</td>
<td>Thiamine</td>
</tr>
<tr>
<td>Saponins</td>
<td>Selenium</td>
<td>Niacin</td>
</tr>
<tr>
<td>Essential oils</td>
<td>Zinc</td>
<td>Pyridoxine</td>
</tr>
<tr>
<td>Steroids</td>
<td>Magnesium</td>
<td>Riboflavin</td>
</tr>
</tbody>
</table>

**Essential oil constituents**

<table>
<thead>
<tr>
<th>Essential oil constituents</th>
<th>Citral</th>
<th>α-Terpineol</th>
<th>β-Myrcene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural oils</td>
<td>β-O-Cimene</td>
<td>Allo-o-cimene</td>
<td></td>
</tr>
<tr>
<td>α-Pinene oxide</td>
<td>Myrcenol</td>
<td>t-Myroolol</td>
<td></td>
</tr>
<tr>
<td>Linalool</td>
<td>1-Octyn-3-ol</td>
<td>3-Undecylen</td>
<td>3-carvomethenone</td>
</tr>
<tr>
<td>Citronellal</td>
<td>Neral</td>
<td>Geraniol</td>
<td></td>
</tr>
<tr>
<td>Nerol</td>
<td>trans-(−)-Carveol</td>
<td>Geraniol</td>
<td></td>
</tr>
<tr>
<td>Methyl-n-nonyl-keitone</td>
<td>Dextro-carvone</td>
<td>Geranic-acid</td>
<td></td>
</tr>
<tr>
<td>α-Bergamotene</td>
<td>Isolongifolene-4-5-9-10-dehydro</td>
<td>y-Murolene</td>
<td></td>
</tr>
<tr>
<td>α-Murolene</td>
<td>α-Amorphone</td>
<td>β-Sesquiphellandrene</td>
<td></td>
</tr>
<tr>
<td>α-Farnesene</td>
<td>α-Elemol</td>
<td>α-Gurjunene</td>
<td></td>
</tr>
<tr>
<td>α-Cadinene</td>
<td>Germacrene-D</td>
<td>Valencene</td>
<td></td>
</tr>
<tr>
<td>α-Cadinene</td>
<td>Germacrene-D</td>
<td>Valencene</td>
<td></td>
</tr>
<tr>
<td>(E, E)-Farnesal pinemyl dihydrazide</td>
<td>Di-n-octylphthalate</td>
<td>Geranyl-acetate</td>
<td></td>
</tr>
</tbody>
</table>

* Phytoconstituents documented hitherto from an extract of C. citratus plant leaf [26, 181, 190-192]

**Table 2** Phytoconstituents of C. citratus and their biological activities

<table>
<thead>
<tr>
<th>Essential oils</th>
<th>Aromatherapy, food preservative, hypolipidemic, antioxidant, antiallergic, antimicrobial, diuretic, antiglycemic, hypoestrogenic, anti-inflammatory, neuropharmacological actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>Antimicrobial, antioxidant, astrincent, hypolipidemic, antiallergic, antimicrobial actions</td>
</tr>
<tr>
<td>Saponins</td>
<td>Antioxidant, antiproteolytic, antihelminthic actions</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Antioxidant, antiproteolytic, antihelminthic actions</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Astringent, neuropharmacological actions</td>
</tr>
<tr>
<td>Vitamins</td>
<td>Antioxidant, cardioprotective actions</td>
</tr>
<tr>
<td>Minerals</td>
<td>Cell signaling, and transport, hemodynamic, neuropharmacological actions</td>
</tr>
</tbody>
</table>

Ref [13, 19, 40, 65, 66, 70-71, 75, 84-86, 157,181]
expired volatiles [24]. However, urine elimination is decreased in favor of the fecal route following dermal exposure, suggesting that the first-pass metabolism plays a role in citral metabolism following transdermal absorption [24]. Citral is almost completely absorbed following oral administration, while much of the dermal dose is lost owing to its extreme volatility [24]. The citral remaining on the skin is fairly well absorbed. Although feces are a minor route of elimination, approximately 25% of the administered citral dose is eliminated via the bile within 4 h of intravenous administration [24]. No difference in citral disposition is detected with oral doses ranging from 5 to 500 mg kg\(^{-1}\). The metabolism of citral is both rapid and extensive, with no unmetabolized compound being detected in the blood within 5 min of an i.v. dose. Repeat exposure to citral results in increased biliary elimination, without any significant changes in the pattern of excretion through urinary and fecal routes, or exhalation. The rapid metabolism and excretion of citral does not allow any significant bioaccumulation of citral to occur.

In a follow-up study by Dilberto et al. [25], citral is shown to be rapidly metabolized and excreted as metabolites, including several acids and a biliary glucuronide conjugate. Seven different urinary metabolites are isolated and identified: 3-hydroxy-3,7-dimethyl-6-octenoic acid, 3, 8-dihydroxy-3, 7-dimethyl-6-octenoic acid, 3, 9-dihydroxy-3, 7-dimethyl-6-octenoic acid, E- and Z-3, 7-dimethyl-2, 6-octadienoic acid, 3, 7-dimethyl-6-octenoic acid, and E-3, 7-dimethyl-2, 6-octadienoic acid. Although citral is an α, β-unsaturated aldehyde and therefore has the potential of being highly reactive, urinary metabolites of citral appear to arise from metabolic pathways that do not involve a nucleophilic addition to the double bond [25].

In studies with rats and mice, a mixture of geraniol and nerol (commonly recognized as citral) is shown to undergo rapid absorption from the gastrointestinal tract and is distributed throughout the body [26]. The finding that citral enters entero-hepatic circulation is consistent with the observation that it induces hepatic cytochrome P-450, glucuronyltransferase, and alcohol dehydrogenase [27-29]. Additionally, citral is shown to increase glutathione S-transferase and aminopyrine dimethylase activities [30]. In rats, citral is metabolized to a mixture of dicarids and hydroxyl acids through omega-oxidation, reduction, hydration of the unsaturated C-2 bond, and oxidation of the aldehyde functional group [23]. Although the only metabolites observed in the urine are those derived from the oxidation of the aldehyde functional group, hepatic reduction of aldehyde may precede the oxidation pathways [25, 28]. Citral is rapidly reduced to the corresponding alcohol by alcohol dehydrogenase in the hepatic cytosolic fraction [28]. It is not oxidized by the mitochondrial aldehyde dehydrogenase and is a potent inhibitor of aldehyde dehydrogenase-mediated acetaldehyde oxidation [28].

Conversely, the ester geranyl acetate is hypothesized to undergo hydrolysis to geraniol and acetic acid [31]. In animals, including fish, hydrolysis of the aliphatic esters is catalyzed by carboxylesterases or esterase enzymes [31].

Following hydrolysis, geraniol, nerol, and citronnellol undergo a complex pattern of alcohol oxidation, omega-oxidation, hydration, and selective hydrogenation [31]. Subsequent conjugation results in the formation of oxygenated polar metabolites that are rapidly excreted in animals, primarily in urine [31]. Alternatively, the corresponding carboxylic acids formed by the oxidation of the alcohol function may enter the beta-oxidation pathway and eventually undergo cleavage to yield shorter-chain carboxylic acids that are completely metabolized to CO\(_2\) [32]. Geraniol, related terpenoid alcohols (citronellol and nerol, 1-octyn-3-ol), and related aldehydes (geranial and neral) could undergo similar metabolic detoxification pathways in animals [31-32]. Such reactions could be possible for essential oils such as humulene, β-Eudesmol, α-Bergamotene, α-Farnesene, γ-Muurolene, α-Gurjunene, δ-Cadinene, and many others that belong to the sequiterpenes group [31], suggesting that they may have similar pharmacokinetic profiles. Other studies have shown that other bioactive components, such as 1, 8-cineole, limonene, D-carvone, and α-pinene, could hypothetically undergo similar disposition fates as other monoterpenes and phenylpropanoid compounds [33-38].

**Phenolics, flavonoids, and glycosides:** While most of the polyphenols are known to be readily absorbed [37-38], these compounds tend to undergo modifications into other forms in the biological systems [22]. One of the common chemical modifications is conjugation [22]. Three major flavonoids (kaempferol, quercetin, and myricetin) and two major flavones (luteolin and apigenin) have been isolated from *C. citratus* [22].

Flavonoids tend to have poor bioavailability, because they are largely decomposed by the bowel flora [39]. Studies have shown that flavonoid-O-glycosides are converted into aglycones by the intestinal flora [39]. The decomposition can proceed further, with the aglycones undergoing further breakdown by the fission of the C-ring, the central ring in the flavonoid structure, to yield two different phenolic products [39]. The ring fission products formed from several common flavonoids, flavonoid glycosides, and related products are as follows: quercetin, and rutin: 3,4-dihydroxyphenylactic acid, 3-methoxy-4-hydroxyphenylactic acid, and m-hydroxyphenylactic acid; kaempferol: 4-(p-hydroxyphenyl)-gamma-valerolactone and p-hydroxyphenylactic acid; and myricetin and myricitrin: 3, 5-dihydroxyphenylactic acid [39]. In a study by LIU et al. [40], the permeability of rat intestine to aglycones such as quercetin and apigenin \( \text{P} = \text{eff} \geq 2\), is found to be comparable to that of compounds such as propranolol, which exhibit 100% intestinal absorption. However, a significant portion of the absorbed aglycones is conjugated and the metabolites are excreted into the intestinal lumen. Flavonoid glycosides, such as isoquercitrin and apigenin-7-O-glucoside,
also exhibit high apparent permeability in the upper small intestine because of their rapid hydrolysis \[39\]. However, isoorchitrin absorption is much slower when hydrolysis is absent or inhibited by 20-mmol glucuronolactone \[39\]. Absorption of other intact glycosides is similar to that of the intact isoorchitrin, and is much slower than the absorption of the corresponding aglycones \[39\]. Therefore, glycosidase-catalyzed hydrolysis is a critical first step in the intestinal and microbial disposition of the flavonoid glycosides. Aglycones are not only rapidly absorbed, but also rapidly metabolized to phase II conjugates, which are then excreted back into the lumen. The intestinal and microbial glycosidases and intestinal phase II enzymes significantly contribute to the disposition of flavonoids via the proposed enteric and entero-hepatic recycling scheme \[39\].

The findings summarized by Bone and Mills \[39\] add important new concepts to our understanding of the pharmacokinetics of flavonoids. Their major findings include the following:

- Aglycones (such as quercetin) have poor, or even zero, bioavailability.
- Active uptake and metabolism of flavonoid glycosides by enterocytes exist and are determined by the nature of the sugar (with a preference for glucose).
- Before the flavonoid is taken up by the enterocyte, it is usually hydrolyzed to an aglycone by a membrane-bound \(\beta\)-glucosidase.
- Quercetin-4-O-glucoside is absorbed in the small intestine, unlike rutin (which contains a molecule of glucose and a mannose, referred to in combination as a rutinoside). Once absorbed, the flavonoid aglycones are subject to three main types of conjugation: methylation, sulfation, and glucuronidation \[40\]. The extent of conjugation is high, with only a small percentage of free flavonoid aglycones being found in the plasma \[41-42\]. The presence of conjugated metabolites in the portal blood of rats suggests that conjugation first occurs in the enterocytes, before further metabolism in the liver \[41, 43\].

Due to its caffeic acid (3, 4-dihydroxycinnamic acid) component and putative salicin content, \textit{C. citratus} has been used as an anti-pyretic agent and for relief of headaches and pains \[2, 11, 14\]. Salicin derivatives are first converted into salicin in the stomach or small intestine \[44-45\]. The salicin may then be absorbed in the small intestine, but in humans, it is mainly carried to the distal ileum or the colon, where gut flora convert the glycoside into its corresponding salicyl alcohol aglycone \[44-45\]. The salicyl alcohol is absorbed and oxidized in the blood, tissues, and liver to yield salicylic acid, a pharmacologically active form \[44-45\].

Caffeic acid and chlorogenic acid undergo similar processes and are metabolized extensively into other compounds following absorption \[44-49\]. These two compounds are absorbed through different absorption mechanisms; while chlorogenic acid may be absorbed as an intact molecule, it can undergo hydrolysis in the stomach and the small intestine to caffeic and quinic acids before absorption \[44-49\]. Caffeic acid is likely to be absorbed through a mechanism that involves passive absorption across the stomach lining and active absorption across the mucosal lining of the small intestine \[46-49\].

Further studies by Rathabai and Kanimozhi \[50\] have found anthraquinone glycosides pass through the digestive tract, a significant portion undergoes polymerization to form inactive polymers. Any remaining glycosides remain unchanged and unabsorbed until they reach large intestine. The action of intestinal flora converts the glycosides into their corresponding active anthrone aglycones, which then exert a laxative action \textit{in situ} in the colon \[39, 51\].

Lipid-soluble saponins can be absorbed unchanged in significant quantities through the lining of the small intestine \[39\], in a manner similar to the absorption of cardiac glycosides, a related chemical group. If saponins are not absorbed, they pass to the large intestine, where the gut flora convert them to aglycone sapogenin \[39\]. Sapogenin tends to be more lipid-soluble and will therefore be absorbed to some extent \[39\]. In these cases, saponin acts as a prodrug. Since the bioactivity of saponins may be owing to their aglycones, the extrapolation of \textit{in vitro} results from evaluation of saponins to the \textit{in vivo} context is potentially unreliable \[39\].

Because of their large size, high affinity for protein binding, and poor lipid solubility, tannins have negligible bioavailability \[39\]. The biological activity of tannins, and the herbs that contain them, should therefore be explained in terms of local effects \[39\]. This poor bioavailability of intact tannins is a key factor in their safety, since hydrolyzable tannins absorbed into the bloodstream can cause hepatotoxicity \[39\]. Many common herbs would be poisonous if tannins had high bioavailability. Additionally, subcutaneously injected tannins were shown to be carcinogenic, making it fortunate that they do not penetrate the skin \[59\]. Products of tannin breakdown in the colon by the gut flora and possibly the spontaneous breakdown in the small intestine, in the case of hydrolyzable tannins, are absorbed \[39\].

The data available so far warrant further studies evaluating the pharmacokinetic profile of \textit{C. citratus} in humans. Reliable pharmacokinetic data in humans would be important for the evaluation of whether bioactive and volatile components of \textit{C. citratus}, and their metabolites, have a therapeutically relevant effect in a number of diseases. Detailed information about the absorption, metabolism, distribution, and elimination may also be important in the context of safety evaluation of \textit{C. citratus}. Of note, however, although some basic pharmacokinetic parameters of isolated compounds have been reported, they cannot be conclusively linked or compared to the
Pharmacodynamic and Biological Activities

Neuropharmacodynamic effects

After ingestion, systemic handling, and cerebral reuptake, the active constituents of *C. citratus* affect behavior, pain sensitivity, neurotransmitter signaling, and hormone release [52-71]. While some studies have shown that extracts of *C. citratus* leaves exhibit sedative, anxiolytic, and hypnotic effects on the central nervous system, these effects are not consistently demonstrated across all studies [52]. As reported by Noguiera [53], *C. citratus* was used by 201 out of 479 women that visited health centers in Sao Paulo, Brazil, for its neuropharmacological effects. In Parana, Brazil, *C. citratus* stands out as a preferentially used sedative in a number of ethnomedical studies [11, 54-56]. As an illustrative example, although Peigen [13] has observed hypnotic and anxiolytic effects among participants after ingestion of the decoctions prepared from *C. citratus* leaf extracts, such effects are absent in the study conducted by Leite et al. [57]. Additionally, Seth and colleagues [59] have observed that *C. citratus* essential oil produces marked depression of the central nervous system in mice, while a separate study shows that *C. citratus* essential oil is three times as potent in prolonging sleep in mice as sodium thiopeptide, a common anesthetic [59]. In a similar study by Blanco et al. [60], the essential oil of *C. citratus* extract is effective in increasing the sleeping time, as well as the percentage of entries and time spent in open arms of the elevated plus maze, and the time spent in the light compartment of the light/dark box. The essential oil also delays phenylenetetrazole-induced clonic seizures and blocks tonic extensions induced by electroshocks, indicating an elevation in seizure threshold and suppression of the spread of seizure activity. These effects are observed in the absence of any motor impairment, as evaluated using the rotarod and the open field tests [60]. Therefore, *C. citratus* essential oil has the potential to alter the course of convulsive episodes, interfering in the seizure threshold and/or blocking seizure propagation. However, this finding is in conflict with those reported by Carlini et al. [12], who have found that fresh and dry leaves of *C. citratus* lack any sedative effect in the test animals. The specific finding of this study is that an oral ingestion of up to 208 times the common human dose of *C. citratus* and oral citral (up to 200 mg kg⁻¹) shows no effect on the central nervous system of rats. This discrepancy in experimental findings could reflect the chemical differences in the composition of the variants of *C. citratus* analyzed in these studies, in terms of their citral and myrcene contents. Since *C. citratus*, which has a high citral content and elicited anticonvulsant activity, it may be a potential candidate for further investigation of development of novel treatments for epilepsy management.

Isolated citral, limonene, and myrcene are found in a study of *Lippia alba* (Mill.) N.E. Brown to elicit potent central nervous effect [61]. Flavonoids, such as 5, 7-dihydroxyflavone, are known to displace (³H)-flunitrazepam binding to the benzodiazepine receptors, suggesting that flavone derivatives possess anxiolytic properties, without inducing sedation and muscle relaxation [62]. Anxiolytic properties have also been demonstrated for some isolated flavonoids that selectively bind with high affinity to central benzodiazepine receptors, including apigenin, which are present in *C. citratus* [63-64].

In aroma and massage therapy, essential oils have been used to promote psychological and physical well-being via inhalation or massage. Aromatherapy is defined as the use of aroma delivered through inhalation for the purpose of inducing psychological or physiologic effects [65] excluding massage therapy from consideration. Empirical studies have shown that the inhalation of the essential oils or the individual terpenes of aromatic plants (including *C. citratus*) plays a significant role in the modulation of the central nervous system [65-66]. These essential oils tend to exert an inhibitory effect on the central nervous system by affecting the gamma-aminobutyric acid (GABA)-ergic neurotransmitter system [65], with evidence supporting an increase in the brain GABA levels [66]. It could, therefore, also be suggested that the anxiolytic effect of the *C. citratus* essential oils may be mediated through the action on the GABA-ergic-benzodiazepine interaction complex.

Among their actions on the brain neurotransmitters and hormones, R- and S-limonene, citral, and Y-terpinene have been shown to inhibit the elevation of serum corticosterone level and reduce cerebral monoamine levels [67]. Since the levels of monoamines and corticosterone are integral to the stress responses [68], the consumption of foods (such as *C. citratus*) rich in these micronutrients could be recommended for the alleviation of physical and psychological stress.

Other constituents of *C. citratus*, such as zinc, magnesium, and folates, have been shown to elicit neuroprotective effects and may be pharmacologically relevant, despite being detected in trace amounts by some studies [69-70]. Folates and zinc, for example, have been linked to the regulation of neuronal development and signaling in animals [69-70]. These trace elements help improve concentration, memory, and the capacity to adequately process information, thus contributing to the health benefits of *C. citratus* tea.

Many of the CNS diseases can lead to a release of large amount of glutamate, and raised cerebral glutamate induces neuronal cell damage and death [71]. It has been reported that essential oil from *C. citratus* extract displays neuroprotective effects in glutamate induced neurotoxicity [71]. The antiapoptotic activity of the extract in cerebellar granule leading to cell cycle arrest in G2/G0 phase, has also reported. Evidently, these beneficial effects of *C. citratus* essential oil inform its use as therapy for neurological disorders.

In summary, significant literatures have provided respective data in complex mixed essential oils.
increasing evidence supporting that the neuropharmaco-
dynamic action of *C. citratus* can be traced to the
synergistic actions of all its phytochemical, nutritional, and
essential oil constituents.

**Antimicrobial effects**

Previous studies have shown that *C. citratus* is
therapeutically effective as an antibacterial [14, 23], antifungal
[77], and antiprotozoal agent [80]. In Nigeria, it is used as an
antipyretic and antiprotozoan agent in the treatment of
malaria and associated symptoms [14, 74]. These effects are
attributed, in part, to the geraniol (α-citral) and neral (β-citral),
1, 8-cineole, α- and β-pinene, p-cymene, α-terpineol,
camphene and limonene constituents of the essential oil
obtained from *C. citratus* leaves and other aromatic plant
parts by steam distillation [75]. The oil is shown to exhibit
antimicrobial activity when tested against 42 microorganisms
(20 bacterial, 7 yeast and 15 fungi) [11]. Additionally, *C.
citratus* antibacterial activity has been tested against a variety
of gram-positive and gram-negative bacteria. *C. citratus* is
shown to be effective against *Escherichia coli*, *Proteus
vulgaris*, and *Klebsiella pneumonia* [76], among others. *C.
citratus* has also been demonstrated to have activity against
common respiratory tract pathogens, such as *Aspergillus spp*,
*Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Streptococcus
aureus*, *Streptococcus pyogenes*, and *Staphylococcus aureus*
[75-79]. Additionally, studies have suggested its activity against
*Candida albicans*, *Enterobacter faecalis*, *Bacillus subtilis*,
*Neisseria gonorrhoea*, *Salmonella enterica*, *Salmonella typhi*,
and *Shigella sonnei* [77, 80-82]. In a study by Inouye et al [77]
essential oil containing aldehydes or phenols as major
components shows the highest antibacterial activity and those
with terpenes alcohols show weak activity while terpene
alcohol or ether containing oils show inactive antibacterial
activities.

Antifungal activities of the *C. citratus* oil can be
attributed to the presence of a number of constituents,
including citral, β-myrcene, linalool, and geraniol [83-100].
These compounds have been previously tested individually
for their antifungal activity. The effects of linalool on the cell
biology of *Candida albicans* have been evaluated by electron
microscopy, which shows that linalool induced a reduction in
cell size and abnormal germination [83], confirming the
postulated antifungal activity of the extract. It is also observed
that citral has inhibitory effects on both mycelial and
yeast-form growth of *Candida albicans*. These effects could
lead to an inhibition of *Candida* biofilm formation and activity
against pre-formed biofilm. However, the specific
mechanisms responsible for the inhibition of *Candida
albicans* adherence by the *C. citratus* oils and its major
constituents need further investigation.

Studies by Kishore et al [84], Mishra et al [85], and Abe et
al [86] have shown antifungal activities against keratinophilic
fungi, ring worm, and common food-storage fungi. Other
studies [84-88] have also found that *C. citratus* extract could
serve as a food preservative, as it prevents the growth of
common food-storage fungi. The antifungal activity of *C.
citratus* has also been observed against dermatophytes, such as
*Trichophyton mentagrophytes*, *Trichophyton rubrum*,
*Aspergillus fumigatus*, *Epidermophyton floccosum*, and
*Microsporum gypseum* [72, 84-85, 89].

Importantly, a study has shown that *C. citratus*
completely inhibits the growth of *Helicobacter pylori* without
any development of resistance, whereas the bacteria develops
a resistance to clarithromycin used under the same conditions
[90]. *C. citratus* has also been reported to increase the spectrum
of action and reduce the necessary therapeutic dosage of
phenoxethanol against *Escherichia coli*, *Staphylococcus aureus*,
and *Pseudomonas aeruginosa* [91].

*C. citratus* has been found to elicit morphological
changes and inhibit septum formation, spheroblast formation,
production of blisters or mesosomes, development of
abnormally shaped cells, and cell lysis [92]. It has also been
found to permeabilize the cytoplasmic membrane with the
leakage of carboxy fluorescein [70]. One study shows that *C.
citratus* might exert its antibacterial activity by influencing
the bacterial targets involved in cytoplasmic metabolism and
cell wall formation [93]. Another study has found the
essential oil of *C. martini* to exhibit broad-spectrum
inhibiting properties with strong activity (MIC between 100
and 500 mg mL⁻¹) against 10 out of 13 *Escherichia coli*
serotypes, three enterogenic, two enteropathogenic, one
enteroinvasive, and Shiga toxin-producing serotypes [94].
Adesegun et al [82] have found that *C. citratus* inhibits the
activity of the extracellular protease of *Shigella sonnei*.

Based on the findings of the aforementioned studies,
attempts have been made to clarify the mechanism
responsible for the antifungal and antibacterial activities of
the essential oils and to identify the key components.
Cinnamic aldehydes, citral, geraniol, eugenol, and menthol
have been found to possess detectable antibacterial activity,
with the cinnamic aldehydes being the most active
antimicrobial components [95].

Additionally, linalool has been found to be the most
active antibacterial agent, while citral and geranol are the
most active antifungal agents, with myrcene reinforcing this
antifungal effect when mixed with these compounds [96-97].
Conversely, the presence of alkaloids and phenols is inferred
as being responsible for the antibacterial properties of the *C.
citratus* extracts in a study by Hindumathy [78]. Regardless of
the specific compounds involved, the antibiotic activity of *C.
citratus* can be inferred to result from a synergy of action of
its antimicrobial constituents, rather than a single component.

Antimicrobial activity may be parallel to the cytotoxic
activity, suggesting a common mode of action likely exerted
by the membrane-associated reactions [98]. In simple terms, it
appears that the mobile and lipophilic nature of the
components of the essential oil, especially the monoterpenes,
enables them to penetrate and disrupt cell membranes.
Essential oils do not appear to have specific cellular targets, as indicated by the variety of their active constituents \[99\]. This suggests a very low risk of the development of microbial resistance against essential oils. Being lipophilic (fat-soluble), they pass through the cell wall and membranes, disrupting their structures. In bacteria, permeabilization of the membranes is associated with the loss of ions and a reduction in membrane potential, collapse of the proton pump, and depletion of the adenosine-triphosphate pool \[39, 100\].

Essential oils can also cause coagulation of the cytoplasm and damage lipids and proteins \[99-100\]. Damage to the cell wall and membrane can lead to a leakage of macromolecules and result in lysis, necrosis, and apoptosis \[39, 101-102\].

Aside from cytotoxicity, Dijoux et al \[103\] have also posited that essential oils of \textit{C. citratus} and \textit{Citrus aurantium dulcis} are phototoxic. In the postulated mechanism of phototoxicity, essential oils penetrate the target cells without damaging its membrane, proteins, or DNA. Radical reaction is elicited by the excitation of certain molecules when cells are exposed to the activating light, and an energy transfer results in the production of an oxygen singlet. This causes cellular macromolecular damage and, in some cases, the formation of covalent adducts to DNA, proteins, and membrane lipids.

Due to the putative pro-oxidant basis of the cytotoxic and phototoxic properties of its essential oil, \textit{C. citratus} could be an excellent antiseptic and antimicrobial agent for topical application, oral consumption (as tea), food preservation, and management of microbial infection and resistance.

The anti-plasmodic effect of \textit{C. citratus} has been tested against \textit{Plasmodium berghei} in mice in a study by Tchoumboungnang \textit{et al} \[14\]. It is found that, at concentrations of 200, 300, and 500 mg\(\text{kg}^{-1}\text{d}^{-1}\), the essential oil of \textit{C. citratus} effectively suppresses parasitemia. Though commonly consumed in some countries under the belief that it relieves pleurisy \[119\], the mechanism of action of myrcene is proposed to involve the stimulation of the arginine nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) pathway. As reported by Hans \textit{et al} \[114\], cGMP directly modulates the ion channels and acts indirectly by stimulating G protein signaling and opening the \textit{K}_{ATP} channels. However, the mechanism of myrcene-induced anti-nociception is proposed not to necessarily involve the potassium channels \[117-118\]. The peripheral analgesic effect of myrcene has also been confirmed in mice and rats by a separate study by Lorenzetti \[13\]. In these experiments, myrcene, unlike other centrally acting analgesics such as morphine, do not cause tolerance after repeated administration. According to these authors, this finding is a breakthrough, allowing researchers to use myrcene in the development of novel peripheral analgesics whose profile of action would differ from current drugs, such as aspirin. However, Moron \textit{et al} \[119\] have reported an absence of analgesic effect in the extract of \textit{C. citratus} leaves orally administered to rodents in their study. These discordant results could be due in part to the phytochemical variations between the \textit{C. citratus} species used in various studies.

Myrcene is also found to inhibit the lipopolysaccharides (LPS)-induced inflammatory processes, including cell migration and NO production, along with the inhibition of c-interferon and interleukin-4 (IL-4) in a mouse model of pleurisy \[119\].

In a similar study by Quintans-Junior \textit{et al} \[120\], citral
component of the essential oil of C. citratus is found to possess significant central and peripheral anti-nociceptive properties, reducing the sensitivity to acetic acid and formalin. Further studies have shown that citral suppresses the cyclooxygenase-2 (COX-2) expression and activates peroxisome proliferator-activated receptors (PPAR) α and γ, which are activated by a number of non-steroidal anti-inflammatory drugs, such as indomethacin [122]. Lee et al [122] report that citral inhibits NO generation through the suppression of inducible NO synthase (iNOS) expression via inhibition of nuclear factor-kappa β (NF-κB) activation. It could therefore be said that the mechanism of the anti-nociceptive and anti-inflammatory properties of citral are mediated through the inhibition of NO production [121] or could be related to the arachidonic acid cascade and modulation of pro-inflammatory molecules [120,123].

In addition to myrcene and citral, citronellal, citronellol, and linalool are also found to exhibit anti-nociceptive activity [123-124]. Furthermore, it has been shown that polyphenolic components of C. citratus, such as flavonoids, tannins, and constituents of the phenolic acid-rich fractions, can contribute to its anti-inflammatory action. Fequeirinha et al [125] report the effects of the phenolic fraction of C. citratus on LPS-induced NO production in a skin-derived dendritic cell line, showing that C. citratus infusion significantly inhibits LPS-induced NO production and iNOS protein expression. All polyphenolic fractions tested (flavonoid-, tannin-, and phenolic acid-rich fractions) also reduce the iNOS protein levels and LPS-stimulated NO production in the experimental cells, without affecting cell viability. The strongest effects are observed with the fractions containing mono- and polymeric flavonoids (flavonoids and tannins, respectively). Additionally, the results of this study also suggest that the anti-inflammatory properties of flavonoids are primarily due to the luteolin glycosides. Additionally, a study by Francisco et al [126] have found that C. citratus extract inhibits iNOS expression, NO production, and various components of LPS-induced signaling pathways, including p38 mitogen-activated protein kinase (MAPK), c-jun N-terminal kinase (JNK) 1/2, and NF-κB. The activation of extracellular signal-regulated kinase (ERK) 1/2 and the phosphatidylinositol-3-kinase (PI3K)/Akt are not affected by the C. citratus extract. Both phenolic acid- and tannin-rich fractions significantly inhibit NF-κB activation, iNOS expression, and NO production, but none of the polyphenol-rich fractions modulate MAPK or PI3K/Akt activation. Neither C. citratus extract nor polyphenol-rich fractions have any effect on LPS-induced COX-2 expression. Finally, LPS-induced PGE2 production is inhibited by the C. citratus extract and phenolic acid-rich fraction.

Based on these reports, it would be appropriate to infer that the analgesic (anti-nociceptive) and anti-inflammatory effects of C. citratus can be attributed to its component alkaloids, tannins, terpenoids, and flavonoids, since anti-nociceptive and anti-inflammatory activities are observed in studies evaluating these compounds [127-128].

Anti-oxidant properties and free radical scavenging action
C. citratus oil has also been evaluated for its antioxidant properties [7,19,125]. In addition to its antimicrobial properties, its antioxidant and free radical scavenging effects could provide the justification of its use as a mouth wash and food preservative [129-130].

Based on this rationale, methanol/water extracts, infusion, and decoction of C. citratus are shown in a study by Cheel et al [129] to elicit free radical scavenging effects by measuring the bleaching of the 1, 1-diphenyl-2-picryl-hydrazal (DPPH) radical, scavenging of the superoxide anion, inhibition of the enzyme xanthine oxidase, and lipid peroxidation in human erythrocytes. Isoorientin, isoscoparin, swertiajaponin, isoorientin 2′′-O-rhamnoside, orientin, chlorogenic acid, and caffeic acid isolated and identified by spectroscopic methods are found to exhibit free radical scavenging properties. A study by Rahim et al [131] has demonstrated the protective effect of C. citratus on hydrogen-peroxide-induced oxidative stress in the reproductive system. In another related study, C. citratus is also found to have potent protective effect against H2O2-induced liver injury. C. citratus treatment significantly reduces the increase in liver enzymes activities and attenuates oxidative stress-induced pathological changes [132].

According to Liu et al [40], a glutamic acid derivative is found to exhibit antioxidant and free radical scavenging activity with an IC50 value being 48 μmol·L−1 in the DPPH assay. Caffeic acid has been reported to be a potent antioxidant [133], while chlorogenic acid and its isomers have long been recognized to exhibit free radical/antioxidant properties [134-135]. Olthof et al [136] have shown that chlorogenic and caffeic acid are effectively absorbed from the lumen of the intestinal tract and can be detected in blood. According to the findings of Tapia et al [137], caffeic acid, chlorogenic acid, neochlorogenic acid, and the flavonoid rutin elicit free radical scavengers, as shown in a DPPH discoloration assay. Other flavonoids extracted from C. citratus with potent antioxidant effects are licochalcone A and B, which have antioxidant activity equal to that of vitamin E and glabrene, and have been shown to be three times as potent as vitamin E [138].

Phytochemical and nutritional compositions of C. citratus extract indicate that vitamins A, C, and E are also present, in addition to the flavonoids [21]. These vitamins have been proven to be potent antioxidants.

In a study evaluating its antioxidant properties, citral is given to rats orally at a 60 mg·kg−1 dose for a week, followed by an i.p. administration of nickel chloride, a known mutagen, to induce nuclear damage [139]. It significantly inhibits the adverse effects of nickel chloride when the antioxidant activity is tested in vitro. With a slightly different focus, Nakamura et al [73] have found that citral isolated from C. citratus induces the activity of the phase II enzyme.
glutathione S-transferase, which plays important detoxification and anticancer roles in vivo. This enzyme is found to detoxify polycyclic aromatic hydrocarbons, as demonstrated in rat liver epithelial cell lines. Topical application of citral is found to elicit antioxidant effects in an animal skin cancer model [140]. C. citratus shows free radical scavenging effects [90] and is anti-genotoxic against γ radiation, suggesting that its cytoprotective properties are based on the free radical scavenging mechanisms [141].

Previous studies have demonstrated significant biological effects of tannins, including antioxidant and radical scavenging activity, as well as inhibition of lipid peroxidation and lipoxygenase activity in vitro [142]. The antioxidant activity of tannins results from their free radical and reactive oxygen species-scavenging properties, as well as the chelation of the transition metal ions that modify the oxidation process [143].

Although a number of other phytochemicals could play a role in the anti-oxidative properties of C. citratus, growing evidence suggests that constituent polyphenols and vitamins could be the major factors [142,144].

Hematologic effects

The essential oil of C. citratus has been tested for antiplatelet activity in guinea pigs and rats [9], showing the highest antiplatelet activity relative to adenosine diphosphate (ADP), arachidonic acid and a thromboxane A2 agonist U46619 (IC50, 4–132 μg·mL−1). Additionally, it exhibits a notable ability to destabilize clot retraction (IC50, 19–180 μg·mL−1). There is a significant correlation between antiplatelet potency and the phenylpropanoid content (54%–86%) of this oil, suggesting a key role for this moiety in the prevention of clot formation.

Additionally, Tarkang et al [3] have reported an increase in white blood cells, red blood cells, and platelets in rats given extracts of C. citratus. These findings may provide a rationale for the use of C. citratus extract in traditional medicine as a “blood booster,” the treatment of jaundice, and the used in stopping bleeding among some tribes in Cameroun, as reported by Mpondo and Dibong [145].

Hypoglycemic/hypolipidemic effects

An aqueous extract of fresh C. citratus leaves administered to normal rats lowers the fasting plasma glucose concentration and dose-dependently decreases the levels of total cholesterol (T-chol), triglycerides (TG), low-density lipoproteins (LDL), and very low-density lipoprotein (VLDL), while simultaneously increasing the plasma high-density lipoprotein (HDL) levels [146]. Additionally, Elson et al [147] have reported the hypocholesterolemic effects of the C. citratus oil, which is rich in geraniol and citral, in human subjects. The pure component of essential oils inhibits the hepatic 3-hydroxy-3- methyl-glutaryl-coenzyme A (HMG-CoA) reductase, which is a key regulatory enzyme in cholesterol synthesis. The hypocholesterolemic effect of the essential oil is mediated by the post-transcriptional down-regulation of the regulatory enzyme, HMG-CoA reductase, without changing the mRNA levels of the enzyme [147]. A variety of essential oils (some of which have also been isolated from C. citratus), including borneol, cineole, citral, geraniol, methanone, menthol, fenchone, fenchyl alcohol, and β-ionone, have been shown to suppress hepatic HMG-CoA reductase activity [147]. Middleton and Hu [148] have proposed that the inhibitory action of essential oils on the hepatic HMG-CoA reductase is independent of the diurnal cycle of the enzyme and of hormones such as insulin, glucagon, glucocorticoids, and triiodothyronine.

In a study by Ewenighi et al [149], four weeks of treatment with the C. citratus extract elicited significant reductions in body weight, and blood glucose, TG, T-chol, and LDL levels in diabetic rats, as compared with the untreated animals. The treated diabetic rats also exhibit significantly higher HDL levels, as compared to the untreated controls. These findings indicate that the C. citratus extract elicits both hypoglycemic and hypolipidemic effects.

A similar study by Agbafor et al [150] has substantiated the anti-hypercholesterolemic potential of the ethanolic extracts of fresh C. citratus leaves. The cholesterol-lowering potential of the extract may also be ascribed to the modification of the intestinal cholesterol uptake, increased conversion of cholesterol to bile acids, and increased excretion of the formed bile acids, elicited by the extracts of C. citratus [151]. These actions are traceable to its rich saponin, tannin, and flavonoid content, or to the action of the essential oil of C. citratus on cholesterol metabolism and oxidation.

Hemodynamic effects

Aqueous extract of C. citratus reduces cardiac rate without altering the contractile force in isolated rat hearts [8]. Conversely, Cymbopogon goenngii volatile oil has been shown to inhibit the contraction and prolong the functional refractory period in isolated guinea pig papillary muscles and atrium, suggesting the antiarrhythmic action of this species [152].

C. citratus oil causes vasorelaxation in isolated and perfused mesenteric artery preparation through an effect apparently mediated by the NO-independent non-prostanoid mechanism [153]. C. citratus elicits weak diuretic and anti-inflammatory effects in rats [108] and hypertensive humans [154]. The diuretic action of the twice daily administration of a C. citratus decoction decreases the mean arterial blood pressure. It induces urination, increasing the frequency of micturition and thereby decreasing the blood volume and eliciting a decrease in cardiac workload, resulting in a decrease in blood pressure [154].

Infusions prepared by steaming fresh or dried C. citratus leaves are commonly used for the prevention and treatment of cardiovascular disorders in folklore, due to its vasodilatory and diuretic properties [106]. In Brazil and Cuba, many hypertensive patients drink C. citratus tea daily for its hypotensive effects [156], and in some communities in
India, a large number of hypertensive individuals consume infusions from C. citratus to lower their blood pressure, particularly when experiencing symptoms presumed to be associated with elevated blood pressure [154]. Anecdotal evidence suggests that this use of C. citratus has been the folkloric practice among the residents of these communities [154]. Additionally, a recent study by Moreira et al. [155] has detected a transient hypotension and bradycardia in rats treated with a citral-rich essential oil obtained from C. citratus. Similarly, Shina et al. [157] have evaluated the cardiovascular effects of C. citratus essential oil in humans, noting an improvement in coronary flow, hypotension, and bradycardia.

Citronellol, another essential oil constituent of C. citratus, Cymbopogon winterianus, and Lippia alba has been shown to lower blood pressure in rats by a direct effect on the vascular smooth muscle, leading to a vasodilation [158].

The presence of several bioactive constituents such as tannins, saponins, anthraquinones, flavonoids, alkaloids, and phenolics has been posited to contribute to the hemodynamic activities of C. citratus [11]. Growing body of evidence, however, suggests that these bioactive constituents could induce diuresis, natriuresis, and saponins, thereby causing hypotension [159] by interfering either individually or synergistically with the re-absorption of electrolytes (sodium and chloride) and water through the walls of the kidney tubules [160]. Previous studies have shown that these substances induce diuresis and natriuresis through the inhibition of Na⁻ and Na⁺-K⁺ATPase activities at the site of sodium tubular re-absorption in the kidney [160-162] by either binding directly to the enzymes and impairing their function, or by altering the membrane fluidity [163]. Other researchers have postulated that these substances could cause changes in the interactions between membrane phospholipids and proteins [162]. Additionally, inhibition of the renin-angiotensin-aldosterone system (RAAS) has been identified as another plausible mechanism through which some of the bioactive constituents of C. citratus reduce the blood pressure. In studies by Hiywataishi et al. [163] and Chen et al. [164], saponins have been found to inhibit circulating and tissue RAAS, thereby modulating a key regulator of blood pressure and body volume in humans.

The presence of some antioxidants in the C. citratus extract, such as flavonoids, tannins, saponins, alkaloids, and vitamins A, C, and E, could also contribute to its hemodynamic effects, given the role oxidative stress plays in the pathogenesis of hypertension.

Moline et al. [165] have proposed that flavonoids may elicit hypotensive effects, but few studies have evaluated this hypothesis. For example, flavonoids such as kaempferol and rutin are observed to induce endothelium-dependent and endothelium-independent relaxation in rat aortic ring tissue in a study by Padilla et al. [166]. This relaxation is not inhibited by the estrogen receptor α antagonist ICI 182,760. Kaempferol also potentiates the endothelium-dependent relaxation induced by acetylcholine, which is reversed by Nω-nitro-L-arginine-methyl ester (L-NAME). Hagiwara et al. [167] have posited that flavonoids, including kaempferol, are potent inhibitors of the myosin light chain kinase in vascular smooth muscles cells, which could explain at least some of the observed endothelium-independent relaxation effects.

It is opined that the essential oil of C. citratus induces hypotension, possibly through a reduction in vascular resistance caused by the inhibition of the Ca²⁺ influx, and bradycardia, due to an activation of cardiac muscarinic receptors [159].

In summary, the hypotensive effects of C. citratus may involve a decrease in heart rate, decrease in blood volume via diuresis, and/or a reduction in vascular resistance via effects on ion (sodium, calcium, and potassium) transport. Additionally, as discussed in the previous sections, its hypoglycemic, hypolipidemic, antioxidant and free radical scavenging actions [131-132, 147-150] could reduce oxidative stress, ameliorate vascular pathology/atherogenesis, and hence improve endothelial functions and cardiovascular health. All of these effects are orchestrated by the wide array of phytochemicals present in C. citratus.

Anti-tumor and anti-carcinogenic activities

Manosroi et al. [168] have shown a suppression of the proliferation of murine and human mouth epidermal carcinoma cells lines by C. citratus, among other herbs. Several previous studies have demonstrated the anti-tumor and anti-carcinogenic activities of the extracts of C. citratus leaves and its bioactive constituents [169-170]. For instance, α-myrcene, α-limonene, and geraniol have been shown to possess anti-tumor activities against the mammary cell, liver, and intestinal mucous membrane cancers in mouse models [169]. It is posited that the extracts from the C. citratus leaves contain inhibitors of the development phase of the cutaneous tumors. Additionally, experiments in mice have found that an extract from C. citratus leaves inhibits colorectal carcinoma [170]. Inhibition of the early phase of hepato-carcinogenesis has also been reported in rats following initiation with diethylnitrosamine [171].

Anti-mutagenic effects

C. citratus is found to have anti-mutagenic properties in a salmonella mutation assay [30]. Citral extracted from C. citratus induces cell death, apoptosis, DNA fragmentation, and caspase-catalytic activity in several hematopoietic cancer cell lines in another study [172]. C. citratus extract administered to rats before the induction of DNA adducts and aberrant crypt foci significantly attenuate these manifestations in the colon [173]. Conversely, in a study performed in rats with induced hepatic tumorigenesis, C. citratus extract exhibits an inhibitory effect only on the early phase of carcinogenesis [171].

Anti-obesity and anti-diabetic effects

Extensive research has been carried out worldwide on
the molecular targets suitable for intervention in type 2 diabetes mellitus (T2DM) that would allow the development of newer anti-diabetic agents. Among other targets, nuclear receptor PPAR-γ [20, 172], human incretin-degrading enzyme dipeptidyl peptidase IV (DPP-IV) [172], protein tyrosine phosphatase 1B (PTP1B) [173], retinaldehyde hydrogenase [174], and others have been intensively studied. Selective effects on these therapeutic targets may improve the efficacy-to-safety ratio of anti-diabetic agents, resulting in a durable maintenance of glycemic control in a majority of people with diabetes. Numerous structurally unrelated natural products, including alkaloids, flavonoids, and organic acids, have also been described to interact with these diabetes-related proteins in micromolar concentrations [175].

PPAR-γ is encoded by the PPARG gene in humans, and is in turn a key regulator of the expression of genes involved in glucose metabolism, inflammation, and other metabolic pathways, such as fatty acid storage [176]. For this reason, PPAR-γ agonists have been used in the treatment of hyperglycemia [20]. DPP-IV, encoded by the DPP-IV gene in humans, is a membrane-bound, serine protease ectoenzyme responsible for the degradation and inactivation of a number of glucose-regulating incretin hormones. Among these hormones, glucagon likepeptide-1 (GLP-1) causes an increase in the amount of insulin released from the pancreatic β-cells. GLP-1 is found in a number of sites, including the kidney, intestine, and capillary wall [177]. DPP-IV has been reported to have clinical significance, since DPP-IV inhibitors are currently undergoing development for use as a new class of oral anti-hyperglycemic agents [178]. PTP-1B, encoded by the PTPN1 gene in humans, is a negative regulator of the insulin signaling pathway and regulates the lipogenesis and hypertriglyceridemia associated with T2DM [173]. It has also emerged as a promising potential therapeutic target for the management of T2DM [179-180].

A study by Bharti et al [181] has evaluated the anti-diabetic activities of the essential oil of the C. citratus leaf sheath in poloxamer-407-induced T2DM model in wistar rats. When compared to the diabetic control rats, the diabetic rats treated with the C. citratus essential oil show a significant amelioration of glycemia, insulinemia, and lipid metabolism dysfunction, accompanied by increased GLP-1 content in the caecum and a remarkable reduction in markers of oxidative stress. Histopathological analysis of the pancreas has shown an increase in β-cell mass, islet number, and severity of insulinitis. Phytocomponents like myrcenol, linalool, α-elemol, and β-eudesmol are found to interact with PPAR-γ and DPP-IV while only pimelyl dihydrazide is shown to interact with PTP-1B. The results provide pharmacological evidence for the C. citratus essential oil as a potential anti-diabetic agent, with the effect mediated by the interaction of various phytocomponents with multiple targets operating in diabetes mellitus.

In the management of diabetes and insulin resistance, enzymes such as retinaldehyde dehydrogenase serve as targets of anti-adipogenic substances [182-183]. Citral is an illustrative example of enzyme-inhibiting agents, since it has been shown to competitively inhibit E1, E2, and E3 isozymes of retinaldehyde dehydrogenase, thereby increasing retinaldehyde concentrations in the adipose tissue [182]. Retinaldehyde, in turn, inhibits adipogenesis, increases the metabolic rate, reduces weight gain, and improves tolerance to a glucose load. The role of retinoids in the adipose tissue is complex, involving a number of functions and effects. Retinol-binding protein 4 (RBP4) is an adipokine implicated in the development of obesity-linked T2DM [183]. Retinaldehyde suppresses the differentiation of fibroblasts into adipocytes and slows down their maturation [184]. Retinoic acid, in turn, promotes maturation, consequently increasing glucose intolerance and insulin resistance [184].

In a study performed by Modak and Mukhopadhyaya [176], citral-treated rats show a dose-dependent reduction in body weight gain. Treated animals exhibit lower fasting glucose levels, improved glucose tolerance, lower fasting plasma glucose, higher metabolic rate, and smaller adipocytes after drug administration. These findings suggest that citral increases the energy dissipation and reduces lipid accumulation, consequently preventing and ameliorating diet-induced obesity. Additionally, it improves insulin sensitivity and glucose tolerance. In the current scenario of rapidly increasing prevalence of obesity and diabetes, citral may prove to be valuable as a novel agent in the management of metabolic disorders.

Citral administration significantly decreases serum insulin levels with an accompanying improvement in glucose tolerance and reduction in fasting plasma glucose levels, suggesting that citral exhibits insulin-sensitizing properties [176]. Hyperinsulinemia generally results from an elevated caloric intake. It has been postulated that this hyperinsulinemic state can ultimately lead to the development of insulin resistance in adipocytes [185]. At the same time, insulin resistance exacerbates the abnormalities in hepatic fat metabolism [185]. Citral could, therefore, slow down or halt the pathological progression from impaired glucose tolerance to frank diabetes by correcting the initial insulin resistance. The exact site of insulin sensitization is yet to be determined. It has been conclusively proven that citral leads to an accumulation in retinaldehyde [184]. Retinaldehyde activates PPAR receptors, leading to an increase in serum adiponectin levels, which sensitizes both the liver and the muscle [177, 180]. Retinaldehyde regulates adipocyte metabolism by regulating the retinoic X receptor α (RXR-α) and PPAR-γ, in turn suppressing the adipogenic gene expression and adipocyte maturation, while increasing fat metabolism and secretion of adiponectin [184]. Adiponectin is a multifunctional protein that exerts pleiotropic insulin-sensitizing effects. It decreases hepatic glucose production and increases glucose uptake and fatty acid oxidation in the skeletal muscle [186]. It
is not known at this time if citral acts, either directly or indirectly, on any tissues other than the adipose tissue to modulate the whole-body insulin resistance \[176\].

The postulated effects could underlie the mechanism of action of \textit{C. citratus} as an herbal anti-diabetic and anti-obesity agent. Yuliana \textit{et al} \[187\], in their study of Asian herbs with anti-obesity activity, have reported that the extract of \textit{C. citratus} has no effect on adenosine A1 receptor binding, CB1 receptor binding, or the induction of lipolysis in 3T3-L1 adipocytes. Additionally, they have detected a low inhibitory activity on LPS-induced accumulation of TNF-\textalpha.  

\textbf{Genito-urinary tract effects}

Infusions prepared from dry or fresh leaves of \textit{C. citratus} are extensively used in traditional medicine in many parts of the world, including Cuba, Brazil, India, and Indonesia, for the treatment of bladder disorders (including the inflammatory conditions of the urinary duct), renal stones \[11, 188\], and urinary incontinence \[189\].

The antifungal actions of \textit{C. citratus} tested against \textit{Candida albicans}, as reported by Abe \textit{et al} \[80\], could provide the rationale for its use in the management of genitourinary tract infections, along with its other antibiotic effects.

\textbf{Miscellaneous effects}

In folk medicine, \textit{C. citratus} is used as a mild astringent and to reduce constipation \[190\]. While tannins are potent astringents, cinnamic acid derivatives, such as chlorogenic and caffeic acids, are potent astringents, despite being pseudotannins. It is therefore reasonable to infer that the astringent property of \textit{C. citratus} may be due to its pseudotannin and tannin constituents.

In folk medicine, \textit{C. citratus} is employed as an antitussive and for relief of cough, management of asthma, and some gastrointestinal disturbances \[11, 190\]. While the rationale for this use is still not scientifically proven, it may be based on its myrcene and citral constituents. The two compounds are recognized to be potent phlegm relievers, antidepressants, and analgesics \[10\]. Additionally, the use of \textit{C. citratus} in aromatherapy and as a mood enhancer could be traced to the effects of its essential oil \[11, 190\].

\textbf{Conclusions}

We believe that this comprehensive review may help in better understanding the existing body of knowledge on the beneficial effects of \textit{C. citratus}. The pharmacokinetics of its constituents and the mechanism of action of its isolated compounds may substantiate the rationale behind its use in traditional and Ayurvedic medicine in many part of the world. The data available so far warrant further pharmacokinetic studies evaluating \textit{C. citratus} disposition in humans. Reliable pharmacokinetic data in humans would provide key information that would allow us to ascertain whether bioactive, potentially therapeutically relevant volatile compounds present in \textit{C. citratus} or their metabolites, have a potential effect on a number of diseases. Detailed information about the absorption, metabolism, distribution, and elimination may also be important in the context of the evaluation of the safety of \textit{C. citratus} use. Although some basic pharmacokinetic parameters of the isolated compounds have been reported, they cannot conclusively be linked or compared to respective data available on the complex mixed essential oils. Therefore, much remains to be learned about this promising herb possibly through isolating more bioactive constituents and elucidating their mechanisms of action, especially in humans. More clinical trials on isolated components should be conducted in order to establish a concrete scientific basis for the use of \textit{C. citratus} as therapeutics in various human diseases.

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