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AN INTEGRATED REVIEW ON PHYTOCHEMICAL DIVERSITY AND PHARMACOLOGICAL POTENTIAL OF CITRUS PSEUDOLIMON (L.) TANAKA: CURRENT EVIDENCE & FUTURE PERSPECTIVES

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ABSTRACT

In terms of pharmacological and economic relevance, the Citrus genus (Rutaceae family) is one of the most important families of fruit-bearing plants worldwide. Among the lesser explored of its members, a citrus plant which is often considered a wild or pseudo-lemon, Citrus pseudolimon, because of its rich phytochemical content is traditionally used in different indigenous medicine to treat metabolic, infectious, inflammatory and gastrointestinal disorders and it is currently being tested through the modern phytochemical and pharmacological systems. The plant is reported to have various groups of bioactive compounds, such as flavonoids, phenolic acids, limonoids, alkaloids, terpenoids, essential oils, coumarins, and vitamins, which occur unevenly in its fruits, peels, leaves, seeds, and bark. Solvents of various polarities have been used to prepare extracts that have antioxidant, anti-inflammatory, anti-microbial, anti-diabetic, hepatoprotective, anti-obesity, anti-cancer and cardioprotective properties in in-vitro and in-vivo experimental models. Despite these encouraging data, there is no systematic review of literature that consolidates already obtained information on the phytochemistry and pharmacology of C. pseudolimon. This review critically summarizes and analyzes the published data on the qualitative and quantitative phytochemical profile of various parts of C. pseudolimon and compares them with known pharmacological activities. The focus is made on the methods of extraction, phytochemical yields of specific solvents, traditional and ethnomedicinal applications, and biological effects confirmed experimentally. This review will form the basis of a reference framework for additional drug discovery, nutraceutical development and functional food applications based on C. pseudolimon can be supported by combining traditional knowledge with present scientific evidence.

KEYWORDS: Citrus pseudolimon; Phytochemical profiling; Citrus flavonoids; Medicinal plants; ethnomedicine; Nutraceuticals, citrus fruits.

INTRODUCTION & BACKGROUND

Medicinal plants are at the core of the healthcare systems of most countries and traditional medicine known as Ayurveda, Unani, Siddha, and Traditional Chinese Medicine have utilized plant-based medicine to prevent and treat disease over centuries.[1,2,3] The world healthcare system has indicated that about 80 percent of the global population depends on plant-based medicines as a primary source of healthcare, especially in developing nations.[4] The emergence of chronic diseases such as obesity, diabetes mellitus, cardiovascular disorders, cancer, neurodegenerative conditions has increased scientific interest in phytochemicals as alternatives to other drug categories due to their relative safety, multimodality, and low cost of development.[5,6]

Many studies on citrus species indicate that different parts of citrus fruits like peel, juice and seeds are rich in bioactive compounds such as flavonoids, phenolics, limonoids, essential oils and vitamins.[7,8] Even though commercially useful species like Citrus limon, Citrus sinensis, and Citrus aurentium have been the subject of intensive research, little-known or wild citrus species have received less attention even their usage in traditional medicine for a very long time. One of these underutilized species is Citrus pseudolimon (L.) Tanaka, also known as wild lemon.[9] C. pseudolimon is well known in different traditional medicine: in Ayurveda, to balance pitta and digestion and inflammation; in traditional Chinese medicine, to improve qi, digestion and phlegm, and in Kampo to treat respiratory health and digestion and increase immunity because it contains lots of vitamin C.[5,10]

Common flavonoids in Citrus species include hesperidin, naringin, naringenin, rutin, and quercetin, renowned for their anti-inflammatory and antioxidant properties. Limonoids such as limonin and nomilin contribute to the fruit's bitter taste and exhibit anticancer and antimicrobial activities. Phenolic acids like ferulic, caffeic, gallic, and p-coumaric acids are also present. Citrus peel contains essential oils like limonene, pinene, terpinene, and linalool, while the juice is rich in vitamin C and micronutrients. Extraction of these compounds is carried out using traditional methods like steam distillation as well as advanced techniques including ultrasonic-, microwave-, and supercritical fluid-assisted extraction.[11,12,13]

Despite some experimental studies having reported individual phytochemical and pharmacological results of C. pseudolimon, a thorough and critical review of the literature is yet to be formulated. The available reports tend to be very fragmented, targeting individual extracts, individual compounds, or individual biological activities without data aggregation across plant segments and extract methodologies.[14]

The present review aims to bridge this knowledge gap by compiling and critically reviewing published data on the phytochemical composition of C. pseudolimon and pharmacological activity in a systematic way. The aims of this review are to:

- Give an elaborate botanical and ethnopharmacological description of C. pseudolimon and substantiated by scientific works.
- Concisely present a qualitative and quantitative phytochemical analysis of various extracts of the various parts of the plant.
- Correlate traditional and medicinal applications with experimental pharmacological actions.
- Determine gaps in the research, methodology, and inconsistency in literature.
- Indicate research opportunities in the future such as mechanistic research, bioavailability research, toxicity profiling research, and clinical validity research.

By integrating the traditional information with the current scientific evidence, this review aims to prove that C. pseudolimon is an underused, but promising, medicinal plant and to arouse interest in further research needed to rationalize its use into the therapeutic and nutraceutical products.[15]

Table 1: Major compounds reported in Citrus pseudolimon fruits[12,16]

S. No.	Phytochemical class	Compounds identified
1.	Flavonoids	Hesperidin, Naringin, Naringenin
2.	Limonoids	Limonin, Nomilin
3.	Organic acids	Polyphenols, Phenolic acids
4.	Phenolic compounds	Citric acid, Malic acid
5.	Essential oil terpenes	D-Limonene, β-Pinene, γ-Terpinene, Citronellal, Citronellol, Linalool, Geranial, Neral
6.	Other constituents	Pectin, Carotenoids, Ascorbic acid (Vitamin C)

Taxonomical and Botanical Overview

Citrus pseudolimon is a species of the genus Citrus, family Rutaceae, a taxonomic family with a combination of aromatic shrubs and trees that have gland-dotted leaves and fatty tissues.[17] Citrus is taxonomically complicated because of a high prevalence of hybridization, apomixis, and phenotypic plasticity, something that has in past made it difficult to delimit the species.[18] However, C. pseudolimon is also identified as a distinct taxon regarding morphological, anatomical and phytochemical features. The accepted taxonomic classification of C. pseudolimon is as follows:

Kingdom : Plantae
Clade : Angiosperms
Clade : Eudicots
Order : Sapindales
Family : Rutaceae
Genus : Citrus
Species : Citrus pseudolimon (L.) Tanaka
Common Names : Khatta, Nibuwa, Galgal, Kumaon, Chukh, Chinara, Pahadi nibbu, Bada nimbu, and Jamir.

The plant is an evergreen shrub or small tree with botanically thorny branches, and aromatic leaves. The leaves are elliptic to ovate, leathery and glabrous with noticeable oil glands which give off a distinct citrus smell when crushed.[19] Flowers are bisexual, white to pale pink and very fragrant in nature which is because of volatile terpenoids and essential oils.[20] The fruit is a hesperidium which is similar to a lemon but which has a thicker rind, greater acidity, and which has a lower pulp-to-peel ratio. The seeds are usually polyembryonic and this is a characteristic that most citrus species possess, making them genetically stable and efficient at propagation.[21] C. pseudolimon is accustomed to the tropical and subtropical climate and is highly tolerant to the environmental stress, such as drought and some phytopathogens. The adaptive characteristics highlight its ecological strength and possible utility in citizenry enhancement initiatives.[22]

Geographical Distribution

C. pseudolimon is grown at an average elevation of 1100 meters above sea level in the plains and good sub-mountainous regions in the North-Western Himalayas (Shivalik mountain ranges). Harvested between October and December, it is likewise a seasonal crop. It is primarily found in India's mountainous northwest states, including Uttarakhand, Jammu

Kashmir, Himachal Pradesh, and the northeastern provinces of Assam and Manipur. It also flourishes in other states, including Uttar Pradesh (Lucknow) and Punjab. In several Himachal Pradesh districts, including Sirmour (Poanta valley), Shimla, Hamirpur, Una, Kangra, Bilaspur, and Mandi, it is farmed both conventionally and organically. Beyond India, C. pseudolimon has been found in other neighboring countries of South Asia like Nepal and Pakistan that also have agro-climate that is similar to the Himalayan region. Nevertheless, it is not commercially important (and has very narrow world distribution) compared to commercially important citrus species, and is hardly grown outside South Asia, and is very little represented in major citrus-producing areas of Europe, Africa, and the Americas. The general distribution pattern of Citrus pseudolimon indicates that it is a local, native citrus species with little global distribution, but high genetic as well as ecological values to the Indo-Himalayan biodiversity hot spot.[23,24]

Botanical and Commercial Importance.

In botanical terms, C. pseudolimon is a significant source of genetic and phytochemical diversity in the Citrus genus. The wild and semi-domesticated citrus species are currently being identified as having contributed to resistance to diseases, tolerance to abiotic and biotic stresses, and improved nutritional value in the cultivated citrus by breeding and biotechnological modification.[25] C. pseudolimon is underexploited commercially even though it has a promising biochemical profile. The fruits are also eaten fresh or turned into juices, pickles and condiments, the peel and foliage are also utilized in folk medicine, and home preparations.[26] The peel have been singled out as the most promising source of flavanones, polymethoxylated flavones, and limonoids, which have demonstrated antioxidant, anti-inflammatory, anticancer as well as cardioprotective effects. Citrus polymethoxyflavones have been widely investigated for anti-inflammatory and cytoprotective effects, including modulation of oxidative and inflammatory mediators.[27,28] The agro-industrial waste, citrus peels, is currently being viewed as high-value raw materials to extract bioactive compounds, essential oils and natural preservatives.[29] Antimicrobial and aromatic properties of the essential oils derived by evaporation of C. pseudolimon peel and leaves have proven them suitable in the food preservation, cosmetics, perfumery, and alternative medicine applications.[30] As the world moves towards plant-based medicines and clean label foods, C. pseudolimon has not been utilized to its full marketing potential as a source of functional ingredient and phytopharmaceutical leads.

Phytochemical Examination of Various Extracts of the Citrus pseudolimon.

Phytochemical studies of Citrus pseudolimon have demonstrated that the plant is a good source of structurally diverse and highly varied secondary metabolites, which are distributed and differ greatly based on plant part, geographical origin, maturity stage, and the extraction methodology. Like other citrus species, the phytochemical composition of C. pseudolimon is dominated by flavonoids, phenolic acids, limonoids, terpenoids, essential oils, alkaloids, coumarins, and vitamins, especially ascorbic acid.[31-33] Traditional phytochemical screening as well as spectrophotometric screening tests, chromatographic and hyphenated analytical systems have been employed in both qualitative screening and quantitative estimates.

Preliminary qualitative phytochemical screening is an initial procedure in the discovery of major classes of bioactive compounds that may be found in plant extracts. The researchers have used solvents of different polarity levels including petroleum ether, hexane, chloroform, ethyl acetate, acetone, methanol, ethanol and water to ascertain exhaustive extractions of phytoconstituents.[34] Fruits peel extracts, especially methanol and ethanol extracts, always produce a positive reaction with flavonoids, phenolics, tannin, terpenoids, and glycosides. Citrus extracts have demonstrated substantial antioxidant activity in radical scavenging and reducing power assays, which correlate strongly with their phenolic content.[35] The fruit pulp contains aqueous extracts rich in carbohydrates, organic acids, vitamin C and water-soluble phenolics, which are relevant in traditional applications in the treatment of digestive and febrile problems. Citrus peel extracts rich in flavonoids such as hesperidin have demonstrated significant gastroprotective effects against ethanol-induced gastric ulceration in experimental models.[36] Leaf extracts exhibit the existence of alkaloids, flavonoids, saponins, and essential oils, which could be responsible in their antimicrobial and anti-inflammatory characteristics.[37] Seed extracts, which are less investigated, are reported to contain limonoids, fixed oils, and sterols, whereas the extracts of the bark contain moderate levels of tannins and phenolic compounds.[38] The value of qualitative variability across extracts demonstrates that solvent polarity and the selection of plant parts are significant parameters during the correlation of phytochemical composition and pharmacological activity. [39]

C. pseudolimon fruit peel methanolic extracts have been documented to record very high total phenolic content values, and these luxuriously surpass the value of 150-250 mg Gallic Acid Equivalent /g extract, which is the densely packed concentration of phenolics in citrus peels.[40] These extracts contain 60-120 mg Quercitin Equivalent /g extract of flavonoid and hesperidin, naringin, eriocitrin, and diosmin were determined as the major constituents by High Performance Liquid Chromatography (HPLC) and Liquid chromatography – Mass Spectroscopy. (LC-MS)[41-42] Nobiletin and tangeretin are also polymethoxylated flavones that have been identified in peel extracts, which are considered to have increased bioavailability and strong anti-inflammatory properties.[43]

Leaf extracts exhibit moderate phenols and flavonoids, but are notably on the volatile terpenoids as evidenced by the gas chromatography-mass spectrometry (GC-MS) profiling of essential oils.[44] The predominant constituents include limonene, b-pinene, g-terpinene, linalool, and citral which attribute the typical aroma and antimicrobial properties of the leaves.[45] Seed extracts present respectable amounts of limonoids, such as limonin and nomilin, which are widely researched due to their anticancer and hypolipidemic activity.[46]

The quantification of the fruit pulp ascorbic acid has been done through titrimetric and HPLC techniques and the reported values have been found to be similar or a little lower when compared with the reported values of commercial lemon varieties, which has supported the nutritional relevance of C. pseudolimon fruits.[47]
In addition to spectrophotometric methods, sophisticated chromatographic methods have been used to profile single phytochemicals in C. pseudolimon. Liquid chromatography, High-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC), LC-MS/MS and GC-MS analyses have facilitated accurate identification and quantification of flavonoids, phenolic acids, limonoids and volatile constituents.[48-50]
Essential oils and other volatile and semi-volatile phytoconstituents are mostly identified using gas chromatography (GC) and GC-MS. The temperature of the injector and detector was set at 250˚C and 275°C, respectively. The oven temperature was set at 40°C for one minute, then gradually increased to 240°C at a rate of 8°C/ minute, remaining there for two minutes, and finally reaching 300°C at a rate of 10°C/ minute for one minute. As a carrier gas, helium was utilized at a flow rate of 0.7 mL/min. 1 mL of n-hexane was used to dilute 10 µL of oil sample, which was then injected in split mode. An electron ionization device with an ionization energy of 70 eV was used in MS for detection. Citrus pseudolimon essential oils were found to have more than 36 components. Limonene (47.07%), cuminal (11.41%), eugenol (10.17%), β-pinene (5.23%), styrene glycol (3.49%), γ-terpinene (3.67%), 3-isobutyl-1-cyclohexene (2.57%), caryophyllene (2.04%), menthene (1.53%), α-pinene (1.56%), p-cumic aldehyde (1.47%), αisopropylbenzyl alcohol (1.56%), and eugenyl acetate (1.29%). HPLC and UPLC techniques were also used to identify several non-volatile components, such as phenylpropanoid glycosides (citrusin-B, citrusin-C, citrusin-F, citrusin-II, citrusin-III, citrusin-IV, citrusinine-I, and citrusinine-II).[51,52]
The major qualitative and quantitative phytochemicals reported from different parts of Citrus pseudolimon extracted using various solvents are summarized in Table 2.

Table 2: Qualitative and quantitative phytochemical profile of different extracts of Citrus pseudolimon

Plant part	Solvent used	Major phytochemical classes detected	Quantitative findings (reported range)
Fruit peel [35, 40-43]	Methanol / Ethanol	Flavonoids, phenolics, limonoids, terpenoids, tannins	TPC: 150–250 mg GAE/g; TFC: 60–120 mg QE/g
Fruit pulp[36,47]	Aqueous / Ethanol	Phenolics, organic acids, vitamin C, sugars	Ascorbic acid: 30–55 mg/100 g fresh weight
Leaves[37,44,47]	Methanol/ Hydro-distilled oil	Flavonoids, alkaloids, terpenoids, essential oils	Essential oil yield: 0.3–0.8% (w/w)
Seeds[38,46]	Hexane / Methanol	Limonoids, fixed oils, sterols	Limonin: 0.2–0.6% of dry weight
Bark[38,49]	Methanol /Aqueous	Tannins, phenolics, glycosides	TPC: 40–90 mg GAE/g

Traditional Uses of Citrus pseudolimon
The C. pseudolimon has been used in traditional medicine as a digestive, antimicrobial, febrifuge and tonic. Ethnobotanical surveys conducted in South and Southeast Asia in which fresh fruit juice was used to treat indigestion, nausea and loss of appetite, and the leaf and peel decoction was used to treat fever, cough, and inflammatory conditions.[53,54] The peel is usually sun dried and powdered to be used in gastrointestinal disorders and carminative agent.[55] Considering the antimicrobial and anti-inflammatory possibilities, leaf infusions are used in the treatment of skin infections, wound and respiratory illnesses.[56] Seeds are sometimes used as an anthelmintic agent and in the treatment of intestinal discomfort, but in other places, a decoction of the bark is used as an astringent and for remedy of diarrhea.[57]
Citrus pseudolimon and related citrus species are widely used in AYUSH systems because of their digestive, antibacterial, and medicinal qualities. The fruit is referred to in Ayurveda as deepana and pachana, which help with gastrointestinal function, indigestion, and hunger stimulation; the peel and rind serve as stomachic and carminative agents. While Unani medicine recognizes citrus as a stomachic, deobstruent, and general tonic used in appetite loss, respiratory illnesses, and parasite infections, Siddha medicine uses citrus juice and peel formulations to treat fever, digestive disorders, and inflammatory conditions. Ethnomedicinal data supports the traditional uses of fruit juice, peel, and seeds to treat intestinal parasites, nausea, and gastrointestinal disorders, demonstrating C. pseudolimon as a multipurpose medicinal plant with proven pharmacological significance.[21,22,24]

Table 3. Traditional Uses of Different Parts Citrus pseudolimon Plant

Plant Part	Traditional Preparation	Ethnomedicinal Use	Region Reported
Fruit[53,54]	Fresh juice	Indigestion, fever, appetite stimulant	India, Sri Lanka

Fruit peel[55]	Dried powder/ Decoction	Carminative, antiemetic, digestive tonic	India, Nepal
Leaves[56]	Infusion/Poultice	Fever, cough, wound healing	India, Thailand
Seeds[57]	Crushed paste	Anthelmintic, abdominal discomfort	India
Bark[57]	Decoction	Diarrhea, astringent	South Asia

Medical Uses of the Different Plant Parts

In addition to the traditional uses, C. pseudolimon has been used in complementary and alternative medicine as a source of nutraceuticals, and functional foods ingredients. Fruit pulp is taken as a source of vitamin C and antioxidants, which helps to modulate immunity and maintain health in general.[47] The use of peel extracts in herbal formulations to address metabolic disorders is because of the presence of flavonoid and limonoid compounds in them.[25,59] Topical and oral use of herbs using leaf extracts and essential oils are being considered due to their anti-inflammatory and antimicrobial properties.[28,45] Seed extracts, which are less popular, are being investigated as lipid-lowering and hepatoprotective preparation, because of their limonoid content.[46] These medical uses are similar to experimentally measured biological activities and may have a strong translational significance.

Table 4: Medicinal Uses of Citrus pseudolimon

Plant part	Formulation/Extract	Medicinal application	Supporting evidence
Fruit pulp[47,58]	Juice/Aqueous extract	Antioxidant, immune support	Nutritional and biochemical studies
Fruit peel[25,58]	Methanolic/Ethanolic extract	Metabolic disorders, antioxidant	In vitro and in vivo models
Leaves[28,45]	Essential oil / Extract	Antimicrobial, anti-inflammatory	GC–MS and bioassays
Seeds[46,59]	Methanolic extract	Hypolipidemic, hepatoprotective	Animal studies
Bark[38,57]	Aqueous extract	Antidiarrheal, astringent	Preliminary pharmacological screening

The pharmacological potential of C. pseudolimon is supported by increasing amounts of experimental evidence. Most of investigations have been carried out on fruit peel and leaf extracts, yet there is also new information available on seeds and bark.

Figure 1: Different Pharmacological Activities of Citrus pseudolimon

Antioxidant Activity

The antioxidant activity of different parts of C. pseudolimon including peel, pomace, seed, juice, and leaves and essential oil has been extensively evaluated using assays such as 2,2- Diphenyl-1-Picryl Hydrazyl (DPPH), Fluorescence Recovery After Photobleaching (FRAP), Trolox Equivalent Antioxidant Capacity (TEAC), 2,2' Azeno-Bis(3-ethylbenzoThiazoline- 6- sulphonic acid) (ABTS), and metal chelating activity. The results showed by (Sajid A et.al 2016) that the essential oil obtained from the fruit peel of C. pseudolimon had the greater antioxidant activity (IC50 – 37.23 µg/ml). Similarly, (Janoti D S et.al 2014) showed that essential oils of leaves of C. pseudolimon had antioxidant activity (IC50 – 18.43 µg/ml)[51,60]

Anticancer and Anti-inflammatory Activity

Polyphenols C. pseudolimon have been found to have anticancer properties. Results of Sajid et al. C. pseudolimon essential oil (CPEO) suppressed cancer and inflammation via downregulating nuclear factor kappa B (NF-kB) activation pathways. They also discovered that CPEO dose-dependently inhibited cell proliferation up to 83%–98% for 100 µg/ml oil. Moreover, it decreased the expression of X1AP, ICAM-1, and Cyclin E1, all of which are controlled by the inflammatory transcription factor NF-kB. The inhibition of C. pseudolimon is higher (81.25%) than that of the positive control Vincristine (78.69%). [51]

Antimicrobial Activity

C. pseudolimon leaf solvent extracts and essential oils have exhibit broad spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria, and selected strains of fungi.27,44 Monoterpenes like limonene and citral have been proposed to break the microbial cell membranes causing bactericidal effects.[61]

The antibacterial activity of C. pseudolimon was evaluated by Thakur N et.al by using the agar well diffusion method. The presence or absence of a zone of inhibition and zone diameter were used to qualitatively evaluate their potency. Peel extract had no effect on any of the two bacterial strains, whereas juice had the strongest antibacterial action against Escherichia coli (24 mm) and Staphylococcus aureus (18 mm). The susceptibility levels of gram-negative bacteria were greater than those of gram-negative bacteria. The positive control exhibited distinct zones of inhibition against Staphylococcus aureus and Escherichia coli, measuring 20 mm and 19 mm, respectively. There was no zone of inhibition against S. aureus and E. coli in the negative control.[62]

Antidiabetic Activity

Kumar et al. used C. pseudolimon methanolic extract (CPMLE) and ethyl acetate fraction (CPEALF) to study the antidiabetic efficacy of C. pseudolimon. In this work, rats were given streptozotocin (45 mg/kg) to develop diabetes, and their fasting blood glucose levels were tracked for seven and twenty-eight days. Blood sugar levels for CPMLE (200 mg/kg) were 304.08 on day seven and 245.66 on day twenty-eight, whereas blood sugar levels for CPMLE (400 mg/kg) were 306.92 on day seven and 206.50 on day twenty-eight. The blood sugar levels for CPEALF (100 mg/kg), on day seven and day twenty-eight were 303.62 and 216.33 respectively. They used gliclazide (10 mg/kg)as a standard drug, which resulted in blood sugar levels of 302 on day seven and 204.83 on day twenty-eight. HDL levels for CPMLE (200 mg/kg) were 34.14 on day seven and 43.55 on day twenty-eight, whereas HDL levels for CPMLE (400 mg/kg) were 32.11 on day seven and 46.7 on day twenty-eight. On day seven and day twenty-eight, the HDL values for CPEALF (100 mg/kg) were 34.91 and 46.7, respectively. HDL levels for the usual medication gliclazide (10 mg/kg) were 33.12 on day seven and 46.27 on day twenty-eight.[63]

Correlation Between Traditional Knowledge and Pharmacological Evidence

The union of ethnomedicinal information with modern pharmacological discoveries has a strong indicator of the therapeutic significance of C. pseudolimon. The antioxidant, anti-inflammatory and gastroprotective effects support the traditional digestive and febrifuge effects, whereas the antimicrobial effects are consistent with the essential oil bioactivity.[53,56,64] This is an important concordance that emphasizes the importance of ethnopharmacology as a systematic drug discovery guide, and it confirms C. pseudolimon as a scientifically credible medicinal plant.

DISCUSSION

The evidence that has been presented in the above sections demonstrates that Citrus pseudolimon is a phytochemically capable, pharmacologically diverse plant species with a high therapeutic potential. The qualitative and quantitative phytochemical methods demonstrated that various parts of the plant, especially the fruit peel, leaves, and seeds contain high levels of bioactive secondary metabolites, such as flavonoids, phenolic acids, limonoids, and volatile terpenoids.[31,40,46] The presence of flavanones like hesperidin and naringin, polymethoxylated flavones in peel extracts gives a good biochemical explanation to the high antioxidant, anti-inflammatory, and metabolic regulatory effects of peel extracts in experimental models.[41,43,65] Suitability of the available literature is that there is uniformity between the traditional ethnomedicinal applications and the experimentally validated pharmacological effects. Modern researches confirm that C. pseudolimon has gastroprotective, antimicrobial, and anti-inflammatory properties, which support traditional use of C. pseudolimon in digestive diseases, fever, infections, and inflammatory states.[36,64,66,67] In the same vein, the mounting evidence on the antidiabetic, hypolipidemic and hepatoprotective properties supports well with the growing popularity of citrus-based phytochemicals in the management of metabolic syndrome.[46,58,59] Nevertheless, with these encouraging results, the majority of the pharmacological studies are limited to in vitro tests and short term in vivo testing with crude extracts. Differences in extraction regimes, solvent systems, authentication of plant material and experimental models make it hard to compare studies across studies and restricts reproducibility.[29,49] Moreover, most of the research is based on fruit peel extracts, whereas other parts of plants like the bark and roots are not well researched. Currently, no well-documented human clinical trials specifically evaluating Citrus pseudolimon (hill lemon or galgal) have been reported in scientific literature. Most of the pharmacological evidence available for this plant comes from in-vitro experiments and animal studies, including antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and cytotoxic investigations.

Unexplored Areas in Citrus pseudolimon Research

There are some promising but not developed or not studied well enough areas that should be considered:

- **Neuroprotective**: Due to established neuroprotective effects of citrus flavonoids, studies are warranted on a cognitive neurodegenerative disease, anxiolytic neurodegenerative disease, and neurodegenerative disease models.
- **Immunomodulatory Activity**: Organized screening of immune-modulatory effects would reveal new uses in autoimmune and inflammatory diseases.
- **Gut Microbiota Interactions**: The effects of C. pseudolimon polyphenols on the composition and metabolic well-being of gut microbiota have not been studied so far.
- **Synergistic and Polyherbal Formulations**: C. pseudolimon extracts interact with other plants or with conventional drugs to produce synergy or to have herb-drug interactions which need to be investigated systematically.
- **Agronomic and Genetic Investigations**: The investigation of the cultivation methods, genetic variability, and chemotype selection may also increase phytochemical production and uniformity.[20,23]

Investigation of these regions has the potential to expand the potential of C. pseudolimon greatly with regard to its therapeutic and commercial applications.

Summary and Outlook

The present all-inclusive review of literature presents Citrus pseudolimon (L.) Tanaka as a scientifically plausible yet under-utilised medicinal plant possessing a considerable amount of phytochemical richness and pharmacological diversity. Ethnobotanical records, phytochemical studies and experiments in pharmacology have shown that various parts of plants especially fruit peels, leaves, seeds and pulp contain a wide range of classes of bioactive compounds, such as flavonoids, phenolic acids, limonoids, terpenoids, essential oils, and vitamins. All of these constituents have a wide range of biological activities, including antioxidant, anti-inflammatory, antimicrobial, antidiabetic, hypolipidemic, hepatoprotective, gastroprotective, and anticancer activity. Owned by the review is the high degree of concordance between conventional applications of C. pseudolimon and contemporary pharmacological discoveries, which confirms the usefulness of ethnomedicinal knowledge as a basis of drug finding in modern times. Meanwhile, there still exist critical gaps, such as the lack of mechanistic research, the absence of standardized extracts, toxicological profiling, and the lack of clinical validation. These limitations should be addressed by conducting rigorous and multidisciplinary studies that will see C. pseudolimon be translated out of experimental potential to clinical practice. In general, C. pseudolimon is a useful source in the design of phytopharmaceuticals, nutraceuticals, and functional food. The current study on bioactive compound isolation, molecular mechanism, safety assessment, and clinical translation will be decisive in unlocking its complete medicinal and commercial potential in the future.

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