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Review on Morphology, Distribution, Phytochemical, and Pharmacological Properties of European Privet: *Ligustrum vulgare* [L] [(Oleaceae)]

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ABSTRACT: Most of the plants in the genus *Ligustrum* (privet) are native to Asia, some of them from Australia, Europe, and North Africa. Many species in this genus have become weed in places where they were originated. The invasive nature of the plant results in displacing native vegetation, alternative ecosystem services and genetic diversity. It shows wide range of tolerance to temperatures; salinity and soil conditions are responsible for its abundance and dominance in the areas once they were found. Due to the aggressive and troublesome nature of the many species in this genus and lack of information, several research studies have focused on it to reveal the positive effects particularly the pharmacological uses of the plant material. The present paper studies the present knowledge on species *Ligustrum vulgare* with particular focus on its taxonomy and morphology, range of distribution, phytochemical and pharmacological properties. The review's most important elements underline its potential to keep widening its distribution and its positive aspects such as anti-inflammatory, antimutagenic, cytotoxic, antioxidant, anti-lipoxygenase and anti-proliferative activities.

Keywords: Invasive species, Pollen allergy, Flavonoids, Antimutagenic, Anti-lipoxygenase, Environmental Impacts

INTRODUCTION

Numerous plant species were brought in by the humans into the urban area and personal gardens to provide services and goods like landscaping, shade, edible fruits, pulp for paper manufacturing and timber for construction (Dickie *et al.,* 2014; Castro Diez *et al.,* 2019). The non-native species may dominate the ecosystem which has a huge effect at the ecosystem services and biodiversity (Richardson *et al.,* 2011). The invasive species are considered as a crucial ecological disturbance which disturbs the biodiversity and reduces the native species richness (Mollot *et al.,* 2017).

The genus *Ligustrum* (Privet) belongs to the family Oleaceae, consisting of about 200 types of variety distributed widely globally. Majority of them are native of Asia and few of the types come from North Africa, Australia and Europe. Most of them were weed in places where they were planted. Once introduced, some of them modify the native species and habitats by dominance, which in turn have serious consequences on ecosystem services and functioning (Vila *et al.,* 2011; Simberloff *et al.,* 2013). In traditional Chinese and Japanese medicine, *Ligustrum* species have been widely used mostly because of their anti-diabetic potential (Andrade-Cetto *et al.,* 2005), anti-cancerogenic, immunomodulatory effects, cardio protective (Yim *et al.,* 2001) or antibacterial activity (Jantova *et al.,* 2000). The European and Chinese privets (*L.sinense* and *L.vulgare* respectively) are shade-tolerant as well as rapid growing shrubs and aggressive invasive species in forestlands of southern USA. The broad range of distribution with pharmacological properties, allergenic cross reactivity detected by IgE antibody and associated nasal and bronchial symptomatology are more prominent in *L.vulgare* commonly called 'European privet'. The dominance of privet has resulted in changes to community structure, loss of native biodiversity, and alterations to ecosystem processes and services. These changes have manifested through the decrease in fine herbaceous fuel concurrent with increasing coarse woody fuels in the understory of forest. The forest fires are the effect of these alterations in fuel structure and disturbs the important resources of forests during extreme weather. The invasion is positively facilitated by the parameters such as mean daily maximum temperature, elevation, productivity, adjacent to water body (approx. 300m) and ownership of private land and is impeded negatively by the parameters such as artificial regeneration, slope, stand age and fire disturbance (Wang *et al.,* 2016). The objective of this paper is to document positive and negative impacts such as taxonomy and morphology, native and global distribution range, environmental impacts, phytochemistry and pharmacological potentials of the privet *L. vulgare*.

MATERIALS AND METHODS

The literature was retrieved through a search on the electronic databases like Scopus, PubMed, and Google Scholar. The keywords and phrases used during the search were *Ligustrum vulgare*, Medicinal plants, Antiviral activity, Animal models, *in vitro* activity and *in vivo* activity. The number of relevant articles finalized through the combination of the above keywords/phrases was 100. The inclusion was based on mainly on therapeutic potential activity of *Ligustrum* and its mechanism of action.

RESULTS AND DISCUSSIONS

A. Taxonomy and Morphology

Ligustrum, shrub or trees; lenticellate branchlets. Leaves: simple, opposite and entire leaf margins. Flowers white, in terminal panicles, the lower nodes often bracteates, small, shortly 4 lobed truncate calyxes, funnel shaped corolla, stamens 2 on the corolla tube; 2 ovules in every cell, fruit a 1-3 seeded drupe with pendulous seeds (Gamble *et al.,* 1921). *L. vulgare* (Oleaceae) a wild privet is a woody and semi-evergreen deciduous shrub which grows up to 5m. Its stems are erect, stiff, with spotted greyish-brown bark, broadly elliptical or lanceolate leaves, 0.5-2cm broad and 2–7cm long. It flowers are fragrant and white, pedicelled, in dense pyramidal panicle, long leaves are oblong-ovate to lanceolate; Calyx 4-toothed, persistent; four-lobed corolla and corolla tube as long as spreading lobes; stigma 2, style single, ovary superior, stamens 2, attached with corolla tube. Its fruit is small, is a gloccy black berry of diameter 6-8mm, with 1 or 4 seeds. World Checklist of Vascular Plants cites about 11 synonyms of *L. vulgare* (World Checklist of Vascular Plants).

B. Habitat

L.vulgare invades the forest edges and riparian habitats frequently and can also grow into dense thickets. It can tolerate almost all soil type, shade and drought and its growth is well in sunlight, and along the banks of the stream says that in North America. It is considered as an invader in riparian regions, floodplains, old fields, forest edges, and woodland distribution. It possesses wide habitat distribution like bottomlands, old fields, riparian forests, primary woodlands, closed canopy forests, grass and barren land, calcareous glades, fence rows, roadsides, windbreaks deciduous forests, and other regions with soil. It develops well in when exposed to proper and direct light, soils with low nutrients and can tolerate limey or chalky soils, drought and extremely tolerant to atmospheric pollution (Irish Gardeners 2015). The drought tolerance acclimation of *L. vulgare* is attributed to development of stomatal regulation and drought-tolerant xylem; physiological and structural response to drought is specific to species and relies on the hydraulic strategy of a plant (Beikircher *et al.,* 2009). The mesophyll conductance is maximum in response to changes in light intensity (Fini *et al.,* 2016).

C. Distribution

The name "European privet" depicts its native range. *L. vulgare* is native to Asia: Turkey, Iran, Armenia, Georgia and Azerbaijan (USDA-ARS 2015); Africa : native to Morocco but introduced in South Africa (Henderson 2001); Europe : introduced in Azores and native to Bulgaria, Belgium, Albania, Austria, Czechoslovakia, Federal Republic of Yugoslavia, Germany, Greece, Ireland, Italy, France, Moldova, Netherland, Poland, Portugal, Norway, Spain, Romania, Sweden, Switzerland, Ukraine, United kingdom, Russia, Hungary (Vajna *et al.,* 2002); South America : Introduced and invasive to Argentina, Brazil, Parana, Rio Grande do sol, Santa Catharina North America :Native and Invasive to Canada 1 (Stobbs *et al.,* 2009), Delaware, Indiana, Louisiana, Maryland, New jersey, Pennsylvania, Tennessee, Arkansas, Montana, Nebraska, New Hampshire, New York, Rhode island, North Carolina, Washington, Texas, Missouri, Georgia, Illinois, Maine, West Virginia, Wisconsin, Virginia, Utah ,Vermont and introduced to United States Ontario, New Mexico, British Columbia, Colorado, Missouri; Oceania: introduced and invasive to Australia, "Victoria, Tasmania, South Australia, New South Wales," along with New Zealand (McGregor *et al.,* 2000). But now, it is naturalized widely only in Southern Africa, South-East Australia, New Zealand, the USA, the Azores, and Canada (southern). Fig.1. and Fig.2. represents the summary distributional map and distributional percentage of *L. vulgare*.

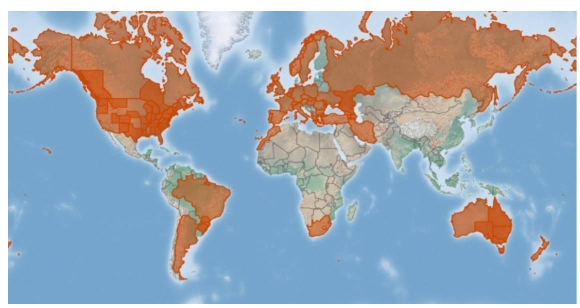

Fig. 1. Summary distributional map of *L. vulgare.*

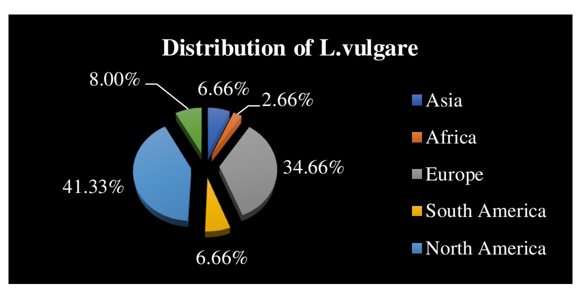

Fig. 2. Percentage distribution map of *L. vulgare.*

D. Environmental Impact

The *L. vulgare* commonly known as privet, which forms dense thickets, therefore displacing native vegetation15. It might reduce tree recruitment and excludes native understory species. The allelopathic effect of *L. vulgare* contributes to a significant decline of susceptible native species in Indiana's Eastern Deciduous Forest (Shannon-Firestone *et al.,* 2015). The investigations demonstrated that *L. vulgare* inhibits the colonisation of native forest underlying vegetation in Eastern Deciduous Forest by arbuscular mycorrhizal fungi (Shannon *et al.,* 2014). Pollens and berries had negative impacts on human such as pollen may cause allergic responses in people, although they are consumed by birds, berries can be poisonous to humans. The adsorption, blotting, inhibition and elution studies concludes the allergenic cross-reactivity (cross-reactivity of antigenic by IgE antibody detection) between the privet (*L. vulgare*) pollen components. The three new identified and purified allergen from *L. vulgare* and two other plant species of Oleaceae has the ability to bind with human IgE antibody from serum of people with olive allergy same as that of the Ole e 1 allergen. The silver enhancement techniques were used for electron and light microscope localization of Ole e 1 in different Oleaceae pollen. Similarly, the allergens from *L. vulgare* shows the possibility of developing nasal and bronchial symptomatology after inhalation of pollen grains. The heavy metal especially lead and magnesium (and Mg Pb) accumulating research on landscape plants in the form of shrubs including *L. vulgare* in regions with no, heavy or low dense traffic, revealing that concentration of Pb was high in branches as compared to the leaves of these species. Here, the tested plants were used as the bio-monitors for monitoring heavy pollution by metal (Sevik *et al.,* 2020).

E. Phytochemistry

The main phytoconstituents identified in the genus *Ligustrum* were triterpenes, iridoids, monoterpenoids and phenylpropanoids. The iridoids, ursolic acid (triterpenes) and flavonoids, in *L. vulgare*, were identified as the taxonomic marker of the Oleaceae family (Pieroni *et al.,* 2000; Romani *et al.,* 2000; Pieroni *et al.,* 2000; Jensen *et al.,* 2002). A common privet leaf was found to contain constituents such as ligstroside, oleuropein, ligustaloside B with smaller ligustaloside A quantity. Higher Nuzhenide quantity with small amount of ligstroside and oleuropein has been found in fruit. Quantification and identification of secoiridoids and flavonol glycosides was conducted on *L. vulgare* (Oleaceae) leaves by "High-Performance Liquid Chromatography with Diode-Array Detection" (HPLC-DAD) and "HPLC with Mass Spectroscopy" (HPLC-MS) method. Along with the previously reported secoiridoids (ligstroside, "ligustaloside A, B, and oleuropein), 4 kaempferol glycosides (kaempferol 3-O-glucoside, kaempferol 3-O-rhamnoside, kaempferol 3, 7-O-dirhamnoside, and kaempferol 3-O-glucoside 7-O-rhamnoside) and 2 quercetin Glycosides (quercetin 3, 7-O-dirhamnoside and quercetin 3-O-glucoside 7-O-rhamnoside") is present in *L. vulgare* leaves. Whereas ligustaloside A, the primary component of secoiridoids, made up almost 76 percent of the total leaf polyphenol content, kaempferol glycosides were also accumulated to a significant degree (23%) in the leaves of *L. vulgare* L. Wax samples from *L. vulgare* leaves' intra-cuticular and epicuticular layers were measured with gas chromatography, flame ionization detection, and their identities were determined by mass spectrometry. As a result, the intra-cuticular layer was dominated (80 percent) by two oleanolic acid: ursolic acid and cyclic triterpenoids, while the outside wax layer is totally composed of homologous series of very-long-chain aliphatic chemical classes (Buschhaus *et al.,* 2007).

The quantitative determination of phenylpropanoids (echinacoside) and secoiridoids (oleuropein, oleacein) in varying amount of extracts such as aqueous, methanolic and decoction made from the *L. vulgare* leaves using HPTLC-photo-densitometry method revealed that oleacein were detected in aqueous extracts, where in particular oleuropein was present, specifically in ethanolic extracts but negligible in infusions and decoctions. All of the produced extracts included secoiridoids and echinacoside derivatives varied during various phases of plant development from May to September (Czerwinska *et al.,* 2015). The aqueous leaf extract of *L. vulgare* revealed the presence of constituents such as phenylpropanoids: verbascoside and echinacoside; Secoiridoids- oleacein, oleocanthal and oleuropein and by "Ultra-High Performance Liquid Chromatography-Diode Array Detector-tandem Mass Spectrometry" (UHPLC-DAD-MS) technique. The quantitative phytochemical analysis using HPLC-DAD method showed oleacein is most abundant (23.48±0.87mg/g), oleocanthal with 8.44±0.08mg/g. The echinacoside content (6.46±0.07mg/g) is high as compared toverbascoside (4.03±0.04mg/g) and oleuropein, the lowest out of all estimated compounds (1.50±0.01mg/g) in the dried aqueous extract of privet leave (Czerwinska *et al.,* 2018).

F. Pharmacological Activity

1. Anti-diabetic activity

Aqueous leaf extract (50-200mg/kg) were administered for 21 days always between 10-12 am resulted in the decreasing of diabetes-induced allodynia and hyperalgesia in streptozotocin (STZ) induced rat model of diabetes. There is no significant alteration in blood glucose levels when the concentration of 50,100,200 mg/kg were injected in treated rats and water intake and body weight decreased significantly (Czerwinska *et al.,* 2018).

2. ACE inhibitory activity

The inhibitory activities of numerous types of extracts such as water, ethyl acetate and n- butanol from flowers, fruits and leaves on "Neutral Endopeptidase" (NEP) and "Angiotensin Converting Enzyme" (ACE) were determined using in vitro fluorimetric assays showed that ethyl extracts have highest activity at 100g/ml concentration. Compounds fractioned through NMR spectroscopy act as dual ACE/NEP inhibitors having IC50 values of 20M and 25M for ACE along with half maximal inhibitory conc. (IC50) of 35M and 75M for

NEP. The phytoconstituents tyrosol and hydroxytyrosol, secoiridoid glycosides (Tanahashi *et al.,* 2009) and flavonoids in ethyl extracts showed no or little inhibitory activities (Kiss *et al.,* 2008).

3. Anti-inflammatory Activity

Aqueous extract of *L. vulgare* leaves on neutrophil function especially in inflammation, revealed that by using the method luminol or lucigenin-dependent chemiluminescence, the inhibition of reactive species of oxygen produced by phorbol-12-myristate, 13-acetate-stimulated neutrophils or formyl-met-leu-phenylalanine were determined. The impact of neutrophils on metalloproteinase, myeloperoxidase, and interleukin production were analyzed using enzyme-linked immunosorbent assay. Inhibition effect of concentration extract in 5-50μg/ml range on interleukin production and metalloproteinase and was around 20 %; elastase and myeloperoxidase were 23.9-34.1 %, 24.2-37.4 % respectively. Similarly, the inhibition of oxidative burst in both models of stimuli like IC50=19.8 ± 3.0μg/ml phorbol-12-myristate 13-acetate and IC50=18.2±4.0μg/ml formyl-met-leu-phenylalanine were observed. Extract of highest conc. modulated the expression of β2 integrin and L-selectin suggests using *L. vulgare* on as the anti-inflammatory agent (Czerwinska *et al.,* 2013).

The flavonones isolated from methanolic leaf extract of the plants like the apigenin-7-O-glucoside, luteolin-4'-O-glucoside, luteolin-7-O-glucoside apigenin-7-O-rutinoside, and ligustroflavone were accountable for vital anti-inflammatory activity (Pieroni *et al.,* 2000). The flavonoidic fractions, luteolin and apigenin derivatives depicted a crucial complement inhibition effect on the complement system pathway (Pieroni *et al.,* 2000). *L. vulgare* leaf extracts were tested to determine anti-inflammatory activity using organic solvents like DCM (dichloromethane), ethyl acetate, n-butanol, using the liquid-liquid partition. These extracts were estimated for the in-vitro cyclooxygenase-1/2 inhibitory activity using assays with COX-1 and COX-2 purified enzymes and investigated its LeukotrieneB4 (LTB4) forming of inhibitory activity with activated human neutrophil granulocytes, where NS-398 and indomethacin used as synthetic inhibitors. The dichloromethane extract depicted a significant inhibition effect against COX-1 and COX-2 enzyme activity compared to other extracts and shows 2.7 times high inhibitory activity against LTB4 formation compared to the known LT inhibitor zileuton with IC50 value = 5.0μM (Mackova *et al.,* 2013).

4. Antioxidant Activity

For methanol, chloroform and petrol free radical scavenging activity along with butanol and hot water infusion of *L. vulgare* leaf extract were assessed with the help of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). The sample concentration is required to 50% scavenge (SC50) of DPPH radical were estimated from a regression curve indicates that the flavonoid aglycones, the most scavenging active constituent were present in chloroform extracts compared to other fractions (Nagy *et al.,* 2006). The antioxidant activities of infusing water in *L. vulgare* leaves were estimated using the "2,2-diphenyl-1-picrylhydrazyl (DPPH); 2, 2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS);" and "Ferric Reducing Antioxidant Power Assay" (FRAP) method and the concentration of Oleuropein and echinacoside were determined using HPLC. These compounds were investigated for its anti-lipoxygenase activity assay using Lipoxygenase (LOX) isolated from rat lung cytosol fraction. The infusion of water shows high activity against all lipoxygenases such as 8-, 12-, 15-LOX, and were monitored by 8-hydroxyeicosatetraenoic acid (8-, 12- and 15-HETE, respectively) and followed by oleuropein. The presence of echinacoside shows negligible activity (Mucaji *et al.,* 2011).

5. Antiproliferative Activity

"The antiproliferative activity of fruit extracts and methanolic leaf of *L. vulgare* alone or in combination with the Palladium (Pd (apox)) complex on human colon cancer cells (HCT-116)" were estimated using the viability assay of MTT cell, where IC50 value was the cytotoxicity parameter. The findings demonstrate that *L. vulgare* extracts have antiproliferative activities with lowering IC50 values and increasing effects with increased exposure duration. But there is an exception for seventy-two hours where the methanolic fruit extract IC50 values were higher than leaf extract. The Pd (apox) complex or plant extract had negligible anti-proliferative impact, but the plant extract combination showed stronger and higher effects with lower values of IC50. Similarly, treatments of plant extract in combination with Pd complex causing high levels of apoptotic cells than just the plant extracts which showed typical apoptotic morphological changes in human cancer cell lines (HCT-116) where the cell death were observed using fluorescence microscopy following the ethidium bromide/ acridin orange method. The results proved that *L. vulgare* possess a natural bio-active substance with anti-proliferative activities on HCT-116 cells which depicted synergistic impact in combination with Pd complex (apox) (Curcic *et al.,* 2012). Cytotoxic effect of plant extract from oleaceae (*L. vulgare* and *L. elavayanum*) on human transformed line of HeLa cells. *L. vulgare* and *L. delavayanum* extracts have effective cytotoxicity on HeLa cells. Cytotoxicity and anti-tumour activity are attributed to flavonoid derivatives especially oleanoic and ursolic acid (Jantova *et al.,* 2000).

6. Antimutagenic Activity

Ligustrum leaves water infusion and the eight phenolic constituents isolated from it such as esculetin, tyrosol, apigenin-7-rutinoside, quercetin-3-rutinoside, luteolin-7-rutinoside, luteolin-7-glucoside, quercetin, and luteolin were tested in-vitro on genotoxicity induced ofloxacin in Euglena gracilis. The applications of phenolic compounds do not show any mutagenic effect, but the ofloxacin application resulted in incedent of mutation in E. gracilis. The phenolics compounds inhibited ofloxacin-induced bleaching at a concentration of 43μM. The water infusions of sample at 86μM ofloxacin concentration, the infusion of *L. delavayanum* showed notable anti-mutagenic activities (41.8%, pt<0.01). Effect of apigenin-7-rutinoside, luteolin-7-rutinoside, quercetin was insignificant. Its anti-mutagenic impact of most phenolics particularly apigenin-7-rutinoside, luteolin-7-rutinoside, quercetin, were significant and could be attributed to their DPPH scavenging activity (1, 1-diphenyl-2- picrylhydrazyl (DPPH) assay), lipophilicity and substitution pattern (Nagy *et al.,* 2009).

CONCLUSION

The *L. vulgare*, common privet is a popularly decorative and invasive plant of Europe which are widespread among subtropical and temperate regions of the world. These plants are tolerant to wide range of temperatures, drought and almost all soil types. The aggressive and troublesome invasive colonized with root sprots and wisely spread by animals and birds dispersed seeds. All these contribute to its abundance and invasiveness which in turn may alter the native species, ecosystem functions and services. Traditionally, *L. vulgare* is still used for its anti-lipoxygenase, anti-inflammatory, hypotensive, diuretic, and anti-rheumatic features. Privet, when screened phytochemically revealed the presence of phenylethanoid glycosides, iridoids and flavonoids especially oleacein, oleuropein and echinacoside. There are many reports which mentioned the pharmacological characteristics like antioxidant, anti-inflammatory, anti-proliferative, antimutagenic, and inhibitory effect. The privet would continue to expand its distribution range with negative effect on ecosystem and biodiversity, but has positive effects by virtue of its pharmacological properties in traditional medicine.

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