Loranthus ferrugineus: a Mistletoe from Traditional Uses to Laboratory Bench

Omar Z. Ameer[1,2*], Ibrahim M. Salman[1,2], Ko Jin Quek[1], Mohd. Z. Asmawi[2]

[1] The Australian School of Advanced Medicine, Macquarie University, Sydney, Australia
[2] Department of Physiology & Pharmacology, School of Pharmaceutical Sciences, Universiti Sains Malaysia, Minden, Malaysia

Abstract

Objectives: *Loranthus ferrugineus (L. ferrugineus)* from *Loranthaceae*, a mistletoe, is a medicinal herb used for a variety of human ailments. Traditionally, decoctions of this parasitic shrub have been mainly used to treat high blood pressure (BP) and gastrointestinal complaints; usage which is supported by experimental based pharmacological investigations. Nonetheless, there is still limited data available evaluating this plant's traditions, and few studies have been scientifically translated toward evidence based phytomedicine. We therefore provide a concise review of the currently available *L. ferrugineus* literature and discuss potential directions for future areas of investigation.

Methods: We surveyed available literature covering ethnopharmacological usage of *L. ferrugineus* and discussed relevant findings, including important future directions and shortcomings for the medicinal values of this parasitic shrub.

Results: Evidence based pharmacological approaches significantly covered the medicinal application of *L. ferrugineus* for hypertension and gastrointestinal complaint management, with a particular focus on the active hydrophilic extract of this herb.

Conclusion: Understanding the sites of action of this plant and its beneficial effects will provide justification for its use in old traditional treatments, and potentially lead to the development of therapies. Other medicinal applicative areas of this parasitic shrub, such as wound healing, gerontological effects, and antiviral and anticancer activities, are yet to be researched.

1. Introduction

Medicinal herbs constitute the cornerstone of traditional medicinal practice worldwide. These herbs are relatively cheap and available, and their use depends on ancestral experience [1-3]. Medicinal plants represent a great deal of untapped reservoirs of drugs, and the structural diversity of their components make a valuable source of novel compounds [3, 4]. Thus, there is a growing interest in the utilization of phytoceuticals and natural product scientists are intensifying efforts towards evaluation of these valuable medicinal plants.

One of the various families of herbs that has long been used worldwide in traditional medicine is *Loranthaceae* [5, 6], which is formed by three distinct families: *Loranthaceae* (sensu strictu), *Viscaceae*, and E*remolepidaceae* [7, 8]. The members of *Loranthaceae* are about 75 genera, and most of them are globally known as mistletoes [9, 10].

Historically, the mistletoe, whose name is believed to be derived from the Celtic word for "all-heal", was used for a variety of treatments. Among the recognized therapeutic properties of *Loranthaceae* members include antitumor actions [11, 12], cough treatment [13], headache treatment [14], uterus tightening following childbirth [14], and immunomodulatory [15, 16], an-

*Corresponding Author

Omar Ziad Ameer. Australian School of Advanced Medicine, Macquarie University, 2 Technology Place, North Ryde, 2109 New South Wales, Australia.
Tel: +61-4-2629-2791   Fax: +61-2-9812-3600
E-mail: omar_3m@yahoo.com

tioxidant [16-19], antiinflammatory [20, 21], antimicrobial [22-24], antiviral [25], antidiarrhoeal [16, 26], anticancer [23, 27], antinociceptive [21], antipyretic [21], antihyperglycemic, and antidiabetic [16, 18, 28, 29] activities and antihypertensive effects [30-32]. However, despite its wide applicability, mistletoe is mainly used for its blood pressure (BP) lowering and gastrointestinal treatment properties.

Chemical and pharmacological studies of some species of the *Loranthaceae* family have identified different kinds of constituents, such as flavonoids [15, 16, 21, 28-30, 33-35], alkaloids, lectins, and polypeptides [11, 12, 16, 36, 37], arginine [38], histamine [39], polysaccharides [40, 41], tannins [16, 28, 29], terpenoids and/or steroids [28, 29], acidic compounds [16, 27], glycosides [16, 28], gallic acid [16], and recently discovered, a new flavocoumarin named Loranthin [42].

Loranthus ferrugineus (*L. ferrugineus*) Roxb., with the synonym *Scurrula ferruginea* Danser, is a member of the *Loranthaceae* family. This herb has a unique way of living, being a hemi parasitic shrub that never grows on land but can partially survive on photosynthesis, and is more dependent on the nutrients and water that can access its roots [9]. These roots have modified structure that attaches the herb onto a host plant which is usually a dicotyledonous tree.

The members of the *Loranthaceae* are widely distributed in many countries like Malaysia, Sumatra, India, Singapore, Australia and New Zealand [9]. Particularly in Malaysia, *L. ferrugineus* is locally known as "dedalu", "dalu-dalu", or "dedalu-api" where "api" means "fire" in the Malay language, and probably reflects the rusty appearance of the herb leaves.

L. ferrugineus has been proven to possess antiviral and cytotoxic activities [25]. Other reports have demonstrated that an aqueous extract obtained from *L. ferrugineus* possesses remarkable capabilities to lower BP *in vivo* [43]. Moreover, phytochemical investigation of *L. ferrugineus* has indicated the presence of components like flavonoids and a high concentration of condensed tannins [25]. Three natural flavonol compounds have been isolated from the ethyl acetate fraction of *L. ferrugineus*: in addition to quercetin and quercitrin, an unusual flavonol glycoside 4"-O-acetylquercitrin was also isolated [44].

In addition to its reputable use for treatment of hypertension and gastrointestinal complaints [43], a decoction of *L. ferrugineus* is also used as a home remedy for general health maintenance and its gerontological effects, which include the enhancement of memory and well-being in the elderly people. Leaves, fruits and flowers are the most common parts of *L. ferrugineus* used to treat high BP, while the unique roots attaching the herb to the host plant are employed for other therapeutic uses, such as ulcer and cancer treatment [43]. Other deep rooted uses for the leaves of this plant are snakebites, wounds, fever, malarial infection or women pouch for after childbirth [45, 46].

L. ferrugineus potential antioxidant and anticancer effects represent a relatively unexplored facet of this herb's knowledge database. Much research has focused on mistletoe's efficacy as an anticancer treatment [47-50], and thus spurred a variety of pharmaceutical medicines, such as Isorel®, Eurixor®, Helixor®, and Lektinol® [51]. However,

though there seems to be strong evidence suggesting certain constituents, especially the lectins, exhibit anticancer effects, the value of the whole plant in cancer treatment is not fully accepted due to significant discrepancies in research findings [49, 50, 52]. Nonetheless, because *L. ferrugineus* has been shown to contain some chemical constituents which are believed to have anticancer effects [25, 43, 44], there is a possibility that *L. ferrugineus* itself, in addition to its supposed antiviral and cytotoxic activities [25], may have anticancer effects too.

Despite *L. ferrugineus* wide range of medicinal applicability, to date there is little data and limited literature review available. Thus, there is a need to review information that can describe this plant and evaluate its traditions, and survey how it has been scientifically and experimentally translated toward evidence based phytomedicine. Therefore, this poses a necessity to review and analyze all the relevant scientific publications of *L. ferrugineus* that can add up to our knowledge and understanding of this least explored herb. Furthermore, we aimed to describe the points that remain to be addressed, and thus from this we propose critical future research directions of this herb that could enhance awareness toward evidence based ethnopharmacology.

2. Taxonomy and plant description

The taxonomical classification of *L. ferrugineus* was first described by Bengal [53] as in Table 1.

The herb's (Fig. 1) physical and structural appearance can vary slightly, based on the plant's tropical natural habitat and age. However, the general description and characterization is more or less the same [2].

Slenders of the bush, young parts, are described to be inflorescences [54]. Branches and twigs of the herb are long, pendulous, and densely clothed with *L. ferrugineus* down when young, in addition to the underside of the leaves, pedicels, calyxes, and corollas. The rusty colored leaves are opposite in direction, positioned on short petioles, elliptic, obtuse, coriaceous, and glabrous above [55]. These leaves are elliotic, with 4 – 8 cm long inder surfaces densely cov-

Figure 1 *Loranthus ferrugineus* Roxb.

Table 1 Taxonomical hierarchy of *Loranthus ferrugineus* Roxb.

Kingdom	*Plantae*
Subkingdom	*Viridaeplantae*
Phylum	*Tracheophyta*
Subphylum	*Euphyllophytina*
Infraphylum	*Radiatopses*
Class	*Magnoliopsida*
Subclass	*Rosidae*
Superorder	*Santalanae*
Order	*Santalales*
Family	*Loranthaceae*
Genus	*Loranthus*
Specific epithet	*ferrugineus* Roxb
Botanical name	*Loranthus ferrugineus* Roxb

ered with reddish scurf [54]. Usually, peduncles present as 1 – 4 together in the axils of the leaves. The flowers of *L. ferrugineus* are: tetrandrous, 2 – 6 most commonly, in axillary cymes; have perianth 1.5 – 2 cm long; are drupe club-shaped; and densely rusty [54]. The flowers are bracteas small, adpressed to the ovaria, and one to each [55], with a tubular and deeply 4 parted flower corolla. For herbs that are native of the East Indies, Pulau-Penang, Singapore and Sumatra, the flowers corolla are densely clothed with rusty hairs, 7 lines long [54]. *L. ferrugineus* berries are yellowish and ovate in shape, and are an orchard pest that is often parasitic on Melastoma and many other trees [54].

3. Commonly used chemical extraction methods

Most traditional productions of *L. ferrugineus* extract involve a simple water decoction or herbal tea preparation in households, intended for human consumption to treat and alleviate various symptoms. However, this preparation method is not necessarily always implicated for scientific research investigations, because chemically characterizing and isolating constituents from water extracts is laborious. Therefore, as in with all natural product chemists, it is necessary to break down herbal material into many different polarity components to ease their identification, enhance yield or mass product, and to occasionally ensure compound stability [56].

Based on previous published observations, we summarized a general scheme illustrating how *L. ferrugineus* extractives are usually produced (Fig. 2). We believe that this method can help separate different elements within a plant based on their polarity, potentially purify and optimize yield of active molecules, and most importantly be applied to various bioactive plant constituents.

As per the tradition of the most commonly used portions of the herb, fresh aerial parts of *L. ferrugineus*, including leaves, young stems, twigs, flowers and berries are usually used. It is important that the plant is collected from the

same single host plant to reduce variation between different batches of collection, since chemical composition of the parasitic shrub can be affected by the host plant [57]. Only healthy parts of the plant must be taken, while the diseased portions are discarded. This is because, when contaminated by severe microbial infection, diseased portions may affect healthy portions, and thus eventually alter metabolism of the plant: an effect which may contribute to the formation of large amounts of unexpected products [56, 58]. The plant material is then cleaned of adulterants, chopped into small pieces, dried in an oven at 42℃ for 5 days, ground into a fine powder using a milling machine, and the resultant powder successively treated as per Fig. 2.

As mentioned earlier, various classes of organic compounds described elsewhere have been shown to be present in this herb. These phytochemicals include phenolics, flavonoids, terpenoids, saponins and condensed tannins. The chemical profiling and assays performed thus far on *L. ferrugineu*s and its extractives are summarized in Table 2. On the other hand, constituents such as amino acids, peptides, secondary amines, alkaloids, anthraquinones, sterols and coumarins have been reported to be absent at least in part in *L. ferrugineus* methanol extract (LFME) [2, 59].
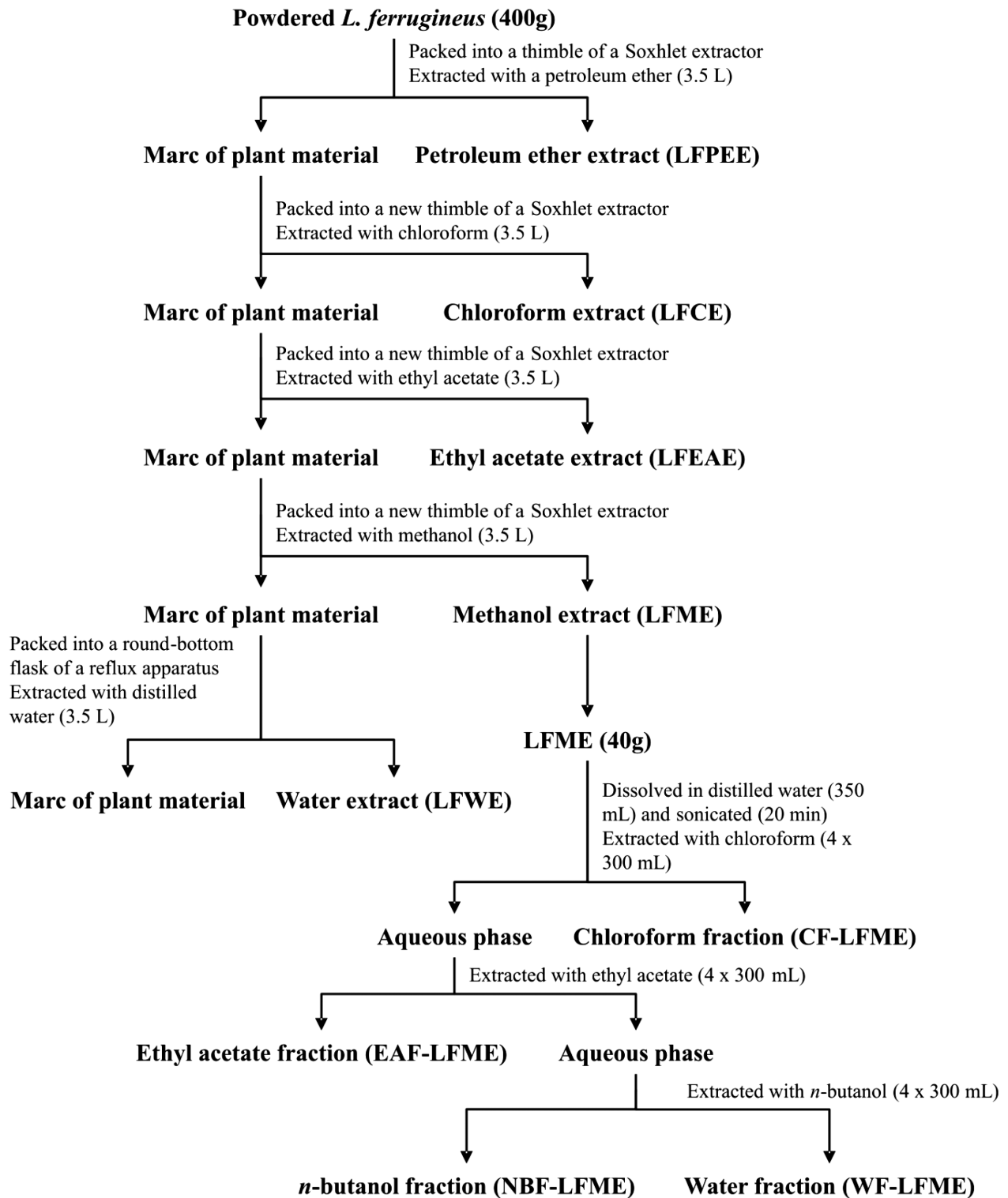
Preliminary thin layer chromatography analysis of LFME and its n-butanol fraction of LFME (NBF-LFME) showed the presence of terpenoids and flavonoids [2], while ultraviolet visible and fourier transform infrared peak spectroscopy analyses of these two extractives were mostly in favoring terpenoids as the major constituents [60].

Few research groups have taken the opportunity to characterize the components of *L. ferrugineus*; hence there is limited chemical investigation that would possibly identify or isolate the active components in this plant, and thus elucidation of the mechanism by which its bioactive components may exert its cardiovascular effects is largely unexplored. In addition, there have been no investigations of potential chemical composition changes across seasons. Continuing research in this area would considerably enhance our understanding of the natural diversity of this herb, and would definitely open the door for a new era of nutraceuticals. Perhaps future studies may employ DNA barcoding, which allows the identification of species in samples with short reliable DNA regions, or highly degraded DNA. DNA barcoding has been used in many studies to test regions in plant groups, and provides species identification by using standardized DNA regions as tags [61]. In addition, there have been previous studies that have used this method to help identify possible usable DNA sequences in plants for the application of DNA barcoding [51].

4. Pharmacological assessments and animal experimentation

4.1. Cardiovascular effects
Cardiovascular disease is a major public health problem contributing to 17.3 million deaths worldwide, and with over 80% of cardiovascular deaths taking place in the low and middle income countries [62, 63]. By 2030, it has been estimated that 23 million people will die annually from

Powdered *L. ferrugineus* (400g)

Packed into a thimble of a Soxhlet extractor
Extracted with a petroleum ether (3.5 L)

Marc of plant material   **Petroleum ether extract (LFPEE)**

Packed into a new thimble of a Soxhlet extractor
Extracted with chloroform (3.5 L)

Marc of plant material   **Chloroform extract (LFCE)**

Packed into a new thimble of a Soxhlet extractor
Extracted with ethyl acetate (3.5 L)

Marc of plant material   **Ethyl acetate extract (LFEAE)**

Packed into a new thimble of a Soxhlet extractor
Extracted with methanol (3.5 L)

Marc of plant material   **Methanol extract (LFME)**

Packed into a round-bottom
flask of a reflux apparatus
Extracted with distilled
water (3.5 L)

LFME (40g)

Marc of plant material   **Water extract (LFWE)**

Dissolved in distilled water (350
mL) and sonicated (20 min)
Extracted with chloroform (4 x
300 mL)

Aqueous phase   **Chloroform fraction (CF-LFME)**

Extracted with ethyl acetate (4 x 300 mL)

Ethyl acetate fraction (EAF-LFME)   **Aqueous phase**

Extracted with *n*-butanol (4 x 300 mL)

***n*-butanol fraction (NBF-LFME)**   **Water fraction (WF-LFME)**

Figure 2 Schematic diagram for preparation of *L. ferrugineus* crude extracts and fractionation of its methanolic extract (LFME).

Table 2 Chemical characterization and assays on *Loranthus ferrugineus* extracts and fractions

Chemical class / antioxidant capacity	Plant/extractives				
	L. ferrugineus	CF-LFME	EAF-LFME	NBF-LFME	WF-LFME
Total phenolics (% w/w)	–	21.2 [2]	40.9 [2]	6.3 [2]	2 [2]
Flavonoids (% w/w)	+ [25], quercetin and quercitrin, glycoside 4''-O acetylquercitrin in the ethyl acetate fraction [44]	3.7 [2]	12.9 [2]	0.4 [2]	0.6 [2]
Terpenoids	–	–	–	+ [60]	–
Saponins	–	–	–	–	–
Tannins	+ [25]	–	–	+ [2]	–
Free radical scavenging activity (mg/mL)	–	0.06 [2]	0.09 [2]	0.04 [2]	0.08 [2]
Total antioxidant activity (mM) TEAC	–	1.3 [2]	1.9 [2]	0.3 [2]	0.1 [2]

+, present; LFME, *Loranthus ferrugineus* methanol extract; CF-LFME, chloroform fraction of *Loranthus ferrugineus* methanol extract; EAF-LFME, ethyl acetate fraction of *Loranthus ferrugineus* methanol extract; NBF-LFME, n-butanol fraction of *Loranthus ferrugineus* methanol extract; WF-LFME, water fraction of *Loranthus ferrugineus* methanol extract; TEAC, trolox equivalent antioxidant capacity.

cardiovascular complications [63, 64]. Hypertension is a powerful risk factor for cardiovascular disease and is perhaps one of the most prevalent underlying etiologies of cardiovascular risks [63]. A number of synthetic antihypertensive medications are available in clinical practice; however, these agents do not always provide optimal control of BP, and in many cases are highly associated with adverse effects. This therefore poses a need for alternative therapies that provide better BP control with minimal side effects. Herbal treatments have been successfully utilized to treat patients with congestive heart failure, systolic hypertension, angina pectoris, atherosclerosis, cerebral insufficiency, and arrhythmia [65, 66]. Specific to hypertension, previous and current evidence suggests that *L. ferrugineus* possesses promising BP lowering activity.

Earlier preliminary investigations conducted by Othman [43] revealed that *L. ferrugineus* has a remarkable BP lowering activity. Here, it was reported that the water extract of *L. ferrugineus*, along with other water based herbal extracts, produce BP lowering effects in the anesthetized rat when given as intravenous (i.v.) boluses. Though Othman's investigation only involved the use of water based extract, subsequent work involved a series of both *in vitro* and *in vivo* ethnopharmacological investigations to explain how *L. ferrugineus* exerts its effects in hypertension management. It is noteworthy mentioning that characterization of *L. ferrugineus* potential cardiovascular effects can pave the way to a more useful understanding of natural herb remedies. For example, evidence based research has enabled us to obtain fair knowledge of the antihypertensive therapeutic properties of *Viscum album* [67, 68], a mistletoe herb from *Santalaceae* which is of close proximity to *L. ferrugineus*.

Early experiments conducted by our laboratory group [69] involved utilization of essential extracts of the herb, as shown in Fig. 2. These experiments were conducted by incubation of isolated Sprague-Dawley (SD) rat aortic ring preparations with increasing concentrations of 0.05% – 0.20% (w/v) of petroleum ether extract (LFPEE), chloroform extract (LFCE), ethyl acetate extract (LFEAE), methanol extract (LFME) and water extract (LFWE) in an organ bath chamber, then separately challenging these extracts by cumulative additions of the α-adrenergic agonist norepinephrine. The results showed that LFME possesses a remarkable effect in concentration dependently inhibiting norepinephrine induced vasoconstriction. In addition, further examination showed that LFME can modulate norepinephrine induced aortic ring contraction through a reversible noncompetitive antagonism mechanism [70], suggesting that chemical constituents within LFME modulate vasomotor tone via a non α-receptor mediated action and may likely possess BP lowering properties. To this end, a different set of investigations involved the use of i.v. bolus administration of increasing doses of 25 – 200 mg/kg of *L. ferrugineus* extracts in *in vivo* whole animal experimentations. In accordance with *in vitro* findings, LFME was found to produce the most significant dose dependent yet brief reduction in BP. It was also noted that LFWE had some hypotensive actions; however, these effects were weaker relative to LFME. Although this finding contradicted Othman's [43] earlier results, where LFWE was purported to be the most potent extract, this discrepancy between findings may be explained by the fact that the earlier study involved only water based extraction of *L. ferrugineus*, while subsequent investigations, as illustrated in Fig. 2, used increasing polarity solvents for extraction: hence, the

active components may be maximally presented in LFME rather than LFWE. Collectively, these findings suggest that relative to the other *L. ferrugineus* extracts, LFME possesses the most significant vasorelaxant and hypotensive activities in rat models. Additionally, LFME's promising active constituent(s) is/are of a relatively high polarity, and therefore can justify the herb's use in the management of hypertension in a form of water based decoction. Thus, perhaps modification of *L. ferrugineus* chemical structure may yield compounds that present with more sustained antihypertensive effects.

It is well established that BP is a function of cardiac output and total peripheral resistance; two variables that are regulated by two major branches of the autonomic nervous system (sympathetic and parasympathetic) [71]. The autonomic pathways that control the heart and vasculature are primarily sympathetic, targeting both α- and β-adrenoceptors located within these target organs, and hence altering cardiac output and systemic vascular resistance. Parasympathetic autonomic pathways, on the other hand, play an important cardiac effect, modulating both myocardial contractility and heart rate via M2 muscarinic receptors stimulation [72, 73]. Blood vessels mostly lack parasympathetic innervation, yet M3 muscarinic cholinergic receptor subtype is still expressed within the vasculature and responds to circulating acetylcholine (ACh) and other cholinomimetics [73, 74]. Subsequent studies on *L. ferrugineus* have therefore focused on investigating the role of active constituents contained within its methanol extract in modulating these key BP regulatory pathways *in vivo*. Thus, the hypotensive mechanisms of *L. ferrugineus* were characterized by monitoring the extract's effect on mean arterial pressure, following i.v. bolus injections of several antagonists of the adrenergic and cholinergic pathways, using anesthetized normotensive SD rat preparations [59]. Possible effects on α- and β-adrenergic cardiovascular receptors were investigated using respective agonists and antagonists of these receptors. Here, the ability of prazosin, an α_1-adrenergic blocker, and propranolol, a non selective β-adrenoceptor blocker, to alter *L. ferrugineus* hypotensive effects was investigated with reference to both norepinephrine and isoprenaline, a β-adrenoceptor agonist, as positive controls [69]. Interestingly, the BP lowering activity of the extract was unaffected by either antagonist, suggesting that *L. ferrugineus*, unlike extracts obtained from *Pseuderanthemum palatiferum* [75], *Artocarpus altilis* [76], *Solanum torvum* [77] and *Platycapnos spicata* [78], does not exert its hypotensive action via antagonism of cardiovascular α- or β-adrenergic receptors. Indeed, the lack of differences in the hypotensive response to *L. ferrugineus* following prazosin administration confirmed our previous assertions of a reversible noncompetitive antagonism of norepinephrine mediated vasoconstriction in isolated rat aorta, driven by a non α-adrenoceptor mediated action [2].

The possible action of *L. ferrugineus* on the cholinergic pathways was subsequently studied, with a particular focus on LFME's effects on cardiovascular muscarinic receptors and ACh esterase (AChE), an enzyme which metabolizes ACh. In these experiments, *L. ferrugineus* mean arterial pressur effects were recorded before and after systemic blockade of muscarinic receptors with atropine or

AChE inhibition with neostigmine. Data driven from these experiments showed that *L. ferrugineus* retained a behavior almost similar to the positive control ACh, as *L. ferrugineus* hypotensive activity was significantly abolished in the presence of atropine. Together, this indicates that *L. ferrugineus* extract, like extracts derived from *Tulbaghia violacea* [79], *Bidens pilosa* [80], *Moringa oleifera* [80], *Cinnamomum zeylanicum* [81] and *Chenopodium ambrosioides* [82], possibly possesses some cholinergic properties, and that its BP lowering effect was most likely driven by stimulation of cardiac and/or vascular muscarinic receptors.

Further exploring the role of the cholinergic pathway, we were able to show that pretreatment with i.v. neostigmine tended to augment the BP lowering effect of *L. ferrugineus* and the duration of its action: yet, the effect was not as notably significant when compared to the enhancement of ACh action on BP and the action duration. This data suggested that AChE may not be the primary enzyme responsible for termination of the LFME effect in plasma and that other esterases, including butyrylcholinesterase or pseudocholinesterase, which is found in great abundance in plasma as compared to AChE, is likely to hydrolyze esters of plant sources [83]. Importantly, as neostigmine did not block *L. ferrugineus* action on BP, our study concluded that LFME does not produce its cholinomimetic action through the antagonism of AChE, but rather through direct muscarinic receptor stimulation [2].

The cholinergic pathway exerts a powerful antagonistic influence on the heart by modulating cardiac rate (chronotropy), conduction velocity (dromotropy), contraction (inotropy), and relaxation (lusitropy) [73]. For instance, the cholinergic hypotensive properties of *Echinodorus grandiflorus* ethanolic extract are associated with powerful reductions in cardiac output and heart rate in spontaneously hypertensive rats [84]. Likewise, *Raphanus sativus* (radish) seed crude extract produces muscarinic receptor dependent inhibition of cardiac force and rate of contractions in isolated guinea pig atria [85]; however, whether *L. ferrugineus* exhibits similar direct cardiac effects remains unexplored. Future investigations should therefore consider exploring cardiac activities of *L. ferrugineus*, investigating not only chronotropic, inotropic and dromotropic responsiveness of the heart but also changes in ventricular pressure, end diastolic volume and coronary blood flow. These experiments will potentially further our understanding of *L. ferrugineus* cardiovascular properties and provide comprehensive characterization of its therapeutic benefits.

The activity of *L. ferrugineus* on the autonomic ganglia, which controls cholinergic and preferentially adrenergic cardiovascular targets, were previously studied by monitoring the ability of hexamethonium, a ganglionic blocker, to modulate the hypotensive response evoked by *L. ferrugineus* extract. It was found that ganglionic blockade did not markedly alter the BP lowering effects of the extract, suggesting *L. ferrugineus*, unlike dietary soy [86] and *Gossypium barbadense* [87], does not promote hypotension via ganglionic blockade mechanisms.

Nitric oxide (NO) is probably one of the most important molecules produced by the endothelium and is synthesized by the enzyme NO synthase (NOS) from the precur-

sor amino acid L-arginine (L-Arg) [88]. A range of chemical mediators including not only ACh but also bradykinin, serotonin, substance P and adenosine diphosphate trigger NO release from the endothelium by receptor mediated mechanisms [89]. Endothelial derived NO has the capacity to maintain vascular tone and to produce vasodilatation through the activation of guanylate cyclase [70]. Given the relative similarity between *L. ferrugineus* and ACh pharmacological properties, and the fact that ACh mediated activation of vascular muscarinic receptors triggers a cascade of events that ultimately generate NO, we predicted that inhibition of NOS, as with ACh, would perhaps attenuate the BP lowering effects of *L. ferrugineus*. Indeed, Nω-Nitro-L-arginine methyl ester hydrochloride (L-NAME), a NOS inhibitor, was shown to attenuate hypotensive responses to *L. ferrugineu*s *in vivo*, suggesting that BP lowering activity is not only evoked by activation of cardiovascular cholinergic receptors, but also through promoting NO release.

  It is common practice to subject crude plant extracts to activity guided fractionation to eliminate various types of complex or antagonistic molecules and derive a more purified and more potent form of the extract. Subsequent studies on *L. ferrugineus* have therefore aimed to obtain purer derivatives of the active LFME extract for further *in vivo* and *in vitro* testing. In one study [2], four chemical fractions including chloroform fraction (CF-LFME), ethyl acetate fraction (EAF-LFME), n-butanol fraction (NBF-LFME) and water fraction (WF-LFME) were derived as shown in Fig. 2 [60]. *In vivo* data from our laboratory showed that NBF-LFME significantly lowers BP in a dose dependent manner, and has a relatively longer duration of action compared to other fractions. Interestingly, it has been further observed that NBF-LFME has more BP lowering potency relative to its mother crude extract LFME, which indicates that fractionation is able to increase the number of bioactive molecules in the extract [60]. In keeping with our *in vivo* data, *in vitro* findings showed that, relative to other fractions, NBF-LFME produces a significant concentration dependent inhibition of aortic ring contraction against the α-agonist phenylephrine and depolarizing signals of potassium chloride [60]. These *in vivo* and *in vitro* results compare very closely to research findings on other plants used in hypertensive treatment, in which bioactivity guided fractionation contributes to a more potent pharmacological action. For example, Kane and colleagues [90] investigated an ethanolic extract and fractions of *Euphorbia thymifolia* for diuretic activity, and found that the dose dependent diuretic effect of this herb was more potent in the fractionated extract. In addition, Ouedraog *et al* [91] performed a bioassay guided fractionation of ethanolic extract of *Agelanthus dodoneifolius*, a plant traditionally used as treatment for hypertension, using rat aorta pre-contracted by norepinephrine to monitor the relaxant activity. They found that the vasorelaxant properties of *Agelanthus dodoneifolius* crude extract were markedly potentiated following column chromatographic separation of its active fractions.

  As we found previously, LFME exhibits reversible noncompetitive antagonism of norepinephrine induced aortic ring contraction [59, 70], and NBF-LFME fraction exhibits the greatest BP lowering effect *in vivo* relative to other LFME fractions [60]. Therefore, we further investigated the effect of the purified NBF-LFME fraction with the aim of finding a more molecular mechanistic explanation to its pharmacological action [92]. The active *L. ferrugineus* fraction was challenged against vascular endothelium dependent and independent mechanisms, using blockade with various pharmacological interventions including L-NAME, NO-cGMP pathway inhibitor methylene blue, cyclooxygenase inhibitor indomethacin, ATP dependent potassium channel blocker glibenclamide, β-blocker propanolol and α-receptor blocker prazosin. Those experiments revealed that NBF-LFME induced vascular relaxation is primarily driven by endothelium dependent mechanisms, stimulating muscarinic receptors, activating the endothelium derived NO-cGMP-relaxant pathway, promoting prostacyclin release and lengthening the released NO half life through its antioxidant effects [92]. This data therefore provide solid evidence that *L. ferrugineus* remarkable antihypertensive potentials extend beyond its muscarinic receptor activation, and further involve enhancement of key modulators of vascular function including NO and prostacyclin, and possible vascular protection through its antioxidant and free radical scavenging properties. The effectiveness of *L. ferrugineus* through its enhancement of NO or its antioxidant properties is of marked importance, as an imbalance between NO and reactive oxygen species production appears to be a common feature of experimental and human hypertension [93]. It is therefore possible that promoting NO release and antioxidant action of *L. ferrugineus* biologically active principles can potentially influence a range of vascular targets to offer diverse protective cardiovascular properties. For instance, ginsenosides and saponins from *Panax ginseng* have been shown to protect against myocardial ischaemia/reperfusion damage and decrease lipid peroxidation, and that those effects are mediated by release of NO from endothelial cells, especially from perivascular nitric oxidergic nerves [94]. *Crataegus pinnatifida*, a plant which has been used as a decoction for antihypertensive treatment for thousands of years in China, is believed to exert its effects through vasorelaxation resulting from NO stimulation and antioxidant activity [95]. Similarly, other plants such as *Theobroma cacao*, due to their enriched flavonoid constituents and resulting stimulation of NO formation [96], has been shown to improve endothelial function through mechanisms that enhance NO synthesis or those that decrease NO breakdown [97]. Accordingly, further investigation of *L. ferrugineus* cardiovascular effects and/or targets is warranted in order to fully understand its cardiovascular protective action.

4.2. Gastrointestinal effects

  Herbal medicines could benefit patients suffering from gastrointestinal disorders that cannot be treated using conventional drug therapy, including functional dyspepsia, constipation, and postoperative ileus. As reviewed by Langmead and Rampton, although most indications for the use of such remedies are traditionally derived, controlled research trials suggest some health benefits for ginger in nausea and vomiting, liquorice extracts in pep-

tic ulceration, Chinese herbal medicine in irritable bowel syndrome, opium derivatives in diarrhoea and senna, ispaghula and sterculia in constipation [98].

Following traditional uses of *L. ferrugineus* for gastrointestinal complaints [43], we aimed to investigate the effect of LFME and NB-LFME on the isolated guinea pig ileal preparation *in vitro* [69, 99], to test for gut contractility actions. Indeed, this work showed that crude LFME and its NB-LFME retain the ability to dose dependently increase intestinal smooth muscle contractility. Importantly, these effects were significantly reduced upon pre-incubation with atropine, enhanced in the presence of neostigmine, and unchanged in the presence of hexamethonium, suggesting a direct cholinomimetic action via muscarinic receptor activation, and that bioactive constituents within *L. ferrugineus* serve as a substrate for intestinal AChE. Having previously described a cholinomimetic hypotensive action for *L. ferrugineus*, our findings in the guinea pig ilium were not counterintuitive. Nonetheless, this data has undoubtedly highlighted a new target organ for *L. ferrugineus* health benefits, confirmed the ability of the extract's active constituents to induce a notable peristalsis, and most importantly provided an immense support for its folklore use. Accordingly, *L. ferrugineus* extracts, like other crude extracts obtained from *Carissa carandas* [100] and *Fumaria parviflora* which possess a cholinergic stimulatory action [101], can potentially be formulated to treat constipation disorders.

Muscarinic receptors are expressed abundantly throughout gastrointestinal tract. Muscarinic agonists elicit contraction through the M3 receptor present in smooth muscle ranging from the esophagus to ileum [102]. In the ileum, muscarinic agonists are known to have a dual effect on contraction: a direct M3 mediated contraction and an indirect M2 mediated inhibition of relaxation [102, 103]. Activation of gastric acid secretion by endogenous ACh, on the other hand, is triggered by M3 receptor subtype expressed on gastric parietal cells [104]. Despite a cholinomimetic action for *L. ferrugineus*, it remains undetermined if active constituents within its crude or purified extract would show a muscarinic receptor subtype specificity to elicit their contractile effect, or an ability to modulate acid, mucous or other gastrointestinal secretions.

5. Future therapeutic applications of *L. ferrugineus*

Alzheimer's disease is a common neurodegenerative condition that affects the elderly population, primarily resulting in memory loss. The memory dysfunction in Alzheimer's disease has been associated with cortical cholinergic deficiency and loss of cholinergic neurons within the brain [105]. Furthermore, chronic neuroinflammation and oxidative stress contribute to the neurodegeneration associated with Alzheimer's disease and represent targets for therapy [106]. *L. ferrugineus* antioxidant properties and effects on cholinergic pathways are of particular interest, because cholinergic abnormalities may affect memory or play a role in Alzheimer's disease [107]: perhaps *L. ferrugineus* action on cholinergic pathways may explain

its memory enhancement and gerontological effects that have been observed in elderly people. Though a variety of medicinal plants and their derivatives have been shown to have cholinomimetic activity in conjunction with improvement of memory and learning functions [108, 109], research linking mistletoe and Alzheimer's disease is limited [110, 111]. Hence, we propose this possible relationship between memory effects and *L. ferrugineus* cholinergic action tentatively, and emphasize that accurate scientific approach is required to test this hypothesis.

6. Conclusions

Natural products will continue to be important in three areas of drug discovery: as targets for production by biotechnology; as sources of new lead compounds of novel chemical structure; and as the active ingredients of useful treatments derived from traditional systems of medicine [112].

Though *L. ferrugineus* possesses a wide range of medicinal applicability, to date there is limited data available that evaluates this plants traditions, and few studies have been scientifically and experimentally translated toward evidence based phytomedicine. Despite the research reviewed, various aspects of *L. ferrugineus* remain to be addressed: for example, identification of chemical constituents and therapeutic mechanisms. Further studies on the isolation and structural elucidation of the pharmacologically active components of *L. ferrugineus* are likely to yield interesting results. Perhaps synthetic or chemical modification of functional groups attached to these constituents could be the base for a more desired pharmacologically active component. For example, the production of a compound that has longer NO enhancing properties that would benefit vascular health and serve as a better tool for hypertension control. In addition, to further characterize the BP lowering potential of *L. ferrugineus*, future investigations may focus on the effects of its extracts both *in vivo* and *in vitro* in hypertensive animal models, such as the spontaneously hypertensive rat. The effect of chronic treatment with *L. ferrugineus* extracts in animal models may also give rise to valuable research outcomes of the herb's effects in high BP treatment.

Finally, yet another research aspect of *L. ferrugineus* which remains to be addressed is its toxicology. Presently, no current toxicology studies have been performed to investigate its effect on living organisms, despite that toxicology in particular is an important aspect of herbal medicine, since all substances become toxic under certain conditions, whether it be through contaminated growth environments, contamination incurred during collection of the plant materials, or even unfavorable storage conditions [113].

The findings from *L. ferrugineus* mistletoe are likely to have important implications for understanding the beneficial actions of *L. ferrugineus* and for developing therapies for clinical problems involving the sites of action of this plant. More in depth research into *L. ferrugineus* will provide further justification for the employment of this herb in old traditional treatments, as well as provide greater in-

sight into the underlying mechanisms responsible for its therapeutic effectiveness, and thus enhance awareness toward evidence based phytotherapy.

Conflict of interest

The authors declare that there are no conflict of interest.

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