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Review

The pharmacological potential of *Phyllanthus niruri*

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Abstract

Introduction *Phyllanthus niruri* is a traditional shrub of the genus Phyllanthaceae with long-standing Ayurvedic, Chinese and Malay ethnomedical records. Preliminary studies from cell and animal model have provided valuable scientific evidence for its use.

Aim This review aims to summarize selected scientific evidence on the pharmacological properties of *P. niruri* over the past 35 years while identifying potential areas of further development of this herb as an economical adjunct.

Methods The review covers literature pertaining to the evidence base therapeutic potential of *P. niruri* spanning from 1980 to 2015 available on PubMed.

Results Evidence suggests that the extracts of *P. niruri* possess hepatoprotective, antiviral, antibacterial, hypolipidaemic, hypoglycaemic, analgesic, anti-inflammatory, cardioprotective, anti-urolithiatic and antihyperuricaemic properties due its novel bioactive compounds.

Conclusion Scientific evidence suggests that there is strong pharmacological potential in developing *P. niruri* as a drug to be used in liver disorders and in antiviral therapy. Despites this, large-scale heterogeneity in study protocol and unstandardized reporting standards limit the ability for valuable comparison and may mask the ability to replicate these studies. Thus interpretation of findings should be performed with caution and further studies should be performed in line with best practices. More cheminformatics, toxicological and mechanistic studies would aid the progress to clinical trial studies.

Introduction

Phyllanthus niruri is a perennial tropical shrub, which has been used for a wide range of diseases in South and south-east Asian traditional medicine, including but not limited to jaundice, diarrhoea, dyspepsia, genitourinary infections and renal stones. In Brazil, where the plant is known as '*Chanca Piedra*' or 'stone breaker', preparations of *P. niruri* are considered folk remedies for renal and vesicular calculi.[1] Traditional medicine systems, such as Ayurvedic and Unani medicine, have utilized the leaves and fruit, to treat gallstones and jaundice. In Malay traditional medicine, *P. niruri*, vernacularly known as '*dukong anak*', is used for kidney disorders and cough.[2] In South India, where the herb is called *Bhumyamalaki*, the herb is believed to treat constipation, gonorrhoea and syphilis.[3] In northern India, this herb locally known as '*pitirishi*' has gained a reputation as a household remedy for asthma, bronchitis and even tuberculosis.[4] The young shoots of this herb may at times be used as an infusion in cases of chronic dysentery.[5] Among traditional Chinese medicine circles, *P. niruri* or '*zhu zi cao*' has traditionally been used to alleviate liver injury secondary to various hepatotoxic agents. In fact, ever since the landmark animal study by Venkateswaran and colleagues which demonstrated for the first time *in vivo* the potential anti-hepatitis B activity of *P. niruri*,[6] this herb has received significant scientific interest leading to a range of studies looking at the various therapeutic potential of this plant species.

Phytochemical studies on this plant, from as early as 1861 when Ottow first isolated the lignan phyllanthin from this plant,[7] to as recent as the isolation of potential anti-HBV phytochemicals nirtetralin and niranthin,[8,9] have revealed that this plant is rich in tannins, flavonoids,

alkaloids, terpenes, coumarins, lignans and phenylpropanoids, which are responsible for the pharmacological activity of *P. niruri*. Table 1 summarizes the various compounds that have been isolated from this herb used in research. Despite its wide range of uses from an ethnomedicinal point of view, research regarding most of these potential therapeutic applications has not reached the level of clinical trials. As a matter of fact, there is a lack of consolidation regarding the current state of knowledge pertaining to *P. niruri* research. Heterogeneity of primary studies on *P. niruri* has also precluded an objective assessment of the plants potential and the mechanisms for most of the therapeutic activity of this herb have yet to be defined. *P. niruri* may potentially be an important drug lead as it should be reiterated that natural products from herbs are still crucial sources of novel therapeutic agents and new chemical entities. In addition, the previous over-reliance on combinatorial chemistry and the fact that it does not necessarily yield vast and pharmacologically feasible libraries has re-emphasized the importance of exploring natural products. The exploration of these natural products may lead to the development of innovative natural product-like libraries, which when coupled with the introduction of high-throughput screening assays, would be able to provide new drug leads for further development. Harnessing the therapeutic potential of common, multipurpose herbs like *P. niruri* provides more accessible and economical drugs, which not only target a wide range of chronic diseases, but have fewer side effects compared with synthetic agents. To enable more targeted future research on this plant, consolidation of scientific evidence and possible gaps in the knowledge need to be addressed. The present review aims to summarize and consolidate the current state of scientific evidence available on PubMed from 1980 until 2015 on the pharmacological properties of *P. niruri*. It will identify areas of further development of this herb as an economical adjunct or even as a novel alternative therapeutic agent and provide a direction for future research in the development of new *Phyllanthus*-based drugs.

Table 1 Phytoconstituents reported in *P. niruri* (Table adapted from Calixto *et al.* and Bagalkotkar *et al.*)

Class	Compound
Alkaloid	4-methoxy-nor-securinine
	Nirurine
	Ent-norsecurinine
Benzenoid	Gallic acid
Coumarins	Ellagic acid
	Ethyl brevifolin carboxylate
	Methyl brevifolin carboxylate
Flavonoid	Quercetin
	Rutin
	Astragalin
	Quercitrin
	Isoquercitrin
	Kaempferol-4′-rhamnopyranoside
	Eridictyol-7-rhamnopyranoside
	Fisetin-4-O-glucoside
	Nirurin (prenylated flavanone)
	Gallocatechin
	Niruriflavone
	Quercetol
Lignan	Phyllanthin
	Hypophyllanthin
	Niranthin
	Nirtetralin
	Phyltetralin
	Hinokinin
	Lintetralin
	Isolintetralin
	2,3-desmethoxy seco-isolintetralin
	Linnanthin
	Nirphyllin
	Phyllnirurin
	Demethylenedioxyniranthin
Tannin	Geraniin
	Repandusinic acid
	Corilagin
Triterpene	Limonene
	p-Cymene
	Lupeol acetate
	Lupeol
	Phyllanthenol
	Phyllanthenone
	Phyllantheol
	3,7,11,15,19,23-hexamethyl-2Z, 6Z,10Z,14E,18E, 22E-tetracoshenen-1-ol
Sterol	B-sitosterol
	Estradiol
	Isopropyl-24-cholesterol
Phytallate	Phyllester
Lipid	Ricinoleic acid
Saponins	Diosgenin
Miscellaneous	Beta-glucogallin
	1-O-galloyl-6-O-luteoyl-α-D-glucose
	Niruriside
	Triacontanal
	Tricontanol

Antioxidant and hepatoprotective activity

The antioxidant hepatoprotective activity of *P. niruri* may be due to its rich content of flavonoids, tannins, lignans and terpenes, which possess antioxidative traits. One of the earliest *in-vitro* studies on the antioxidative hepatoprotective role of *P. niruri* demonstrated that hexane extract of *P. niruri* contained lignans such as phyllanthin and hypophyllanthin, which protected rat hepatocytes against carbon tetrachloride and galactosamine-induced hepatotoxicity.[10] Several other studies have additionally proven the hepatoprotective effect of *P. niruri* in animal and cell

culture models exemplified by the reduction in liver enzyme levels.[11–27] It is, however, important to note that while such reduction in enzyme levels is observed, the exact mechanism for this reduction remains unknown. Additionally, the level and type of enzyme that have shown reduction appear to vary between studies suggesting that there may be multiple mechanism involved in the reduction of these liver enzyme levels. Methanolic extracts of *P. niruri* reduced the levels of TBARS (thiobarbituric reactive substances) in streptozotocin-induced diabetic rats besides improving the levels of reduced glutathione (GSH) and increasing the activity of endogenous antioxidants – superoxide dismutase (SOD) and catalase (CAT) in rat liver, kidneys, heart and brain tissues.[28] Similar results were also replicated in a study involving IDDM and NIDDM rats administered with ethanolic extracts of *P. niruri*.[29] When administered to diabetic Wistar rats, aqueous *P. niruri* extract not only normalized the activity of endogenous antioxidants and levels of plasma vitamin C and vitamin E, but also decreased malondialdehyde (MDA) lipid peroxidation rates.[30] It can be surmised that *P. niruri* contains bioagents, which inhibit lipid peroxidation and prevent excessive superoxide synthesis secondary to chronic hyperglycaemia. Hence, *P. niruri* may alleviate lipoprotein metabolism abnormalities, reduce cholesterol–phospholipid ratios, control biomembrane damage and decrease ROS-linked lipid peroxidation.[28–30]

P. niruri is also a potent NO-quenching agent. An Ayurvedic polyherbal formulation containing *P. niruri* had nitric oxide scavenging properties nearly 1.6 times greater than that of *Gingko biloba* preparations.[18] The role of *P. niruri* as an anti-NO agent is significant since abnormal elevation of NO is responsible for the production of intermediate compounds, which are associated with genotoxicity. While aqueous extracts were more efficacious than methanolic preparations in normalizing ALT levels, more importantly, it was the protein fraction, which had greater hepatoprotectivity against tetrachloride poisoning.[20] The fact that the hepatoprotective activity diminished after preadministration heating and upon addition of trypsin suggested that the hepatoprotective effect of *P. niruri* was due to its protein fractions. Subsequently, studies isolated a 35-kDa polypeptide chain, which displayed significant prophylactic and cytoprotective effects against tertiary butyl hydroperoxide (TBHP).[31] It is a repetition of what was mentioned prior and therefore can be excluded. However, more comprehensive studies to elucidate the exact antioxidative mechanism of *P. niruri* proteins have yet to be executed.

Other studies on the *in-vitro* antioxidant properties of methanolic and aqueous preparations of *P. niruri* have shown that leaf and fruit extracts of *P. niruri* displayed significant inhibition of iron-overload microsomal lipid peroxidation and significant DPPH radical-scavenging activity. Aqueous extracts possessed more potent ROS-quenching property than alcoholic extracts and even displayed DPPH and ABTS scavenging activities comparable with ascorbic acid.[32] Another study showed that crude aqueous extracts of *P. niruri* leaves exerted dose-dependent inhibition of Fe(II)-induced lipid peroxidation, which may suggest its potential use for the treatment of brain and liver iron toxicity.[33] In addition, the iron-chelating property of aqueous extracts may provide a novel neuroprotective therapy for Fe(II)-associated oxidative stress in the brain, which is involved in the pathobiology of Alzheimer's disease.[34]

P. niruri extracts also possess potential as prophylactic antioxidative agents. Rats pretreated with *P. niruri* before being administered carbon tetrachloride had lower hepatic malondialdehyde (lipid peroxidation product) levels.[22] Studies focusing on the *in-vivo* hepatoprotective activity of *P. niruri* against paracetamol-induced hepatotoxicity[15,16] observed the normalization of liver enzyme profiles and non-enzymatic antioxidant levels via the decrease in iron-induced peroxidation of hepatocyte biomembranes. This is significant since paracetamol poisoning has limited and often ineffective treatment options, which depend heavily on N-acetyl cysteine.[35] Studies on aspirin and iron hepatotoxicity have shown that ROS promote apoptosis by mediating ERK,[17] JNK[14] and p38[14,17] mitogen-activated protein kinase (MAPK) pathway activation. Therefore, the scavenging activity of *P. niruri* may interfere in ROS-induced apoptosis by inactivating these pathways.[13,14,17,19,23] However, no molecular studies focusing on this aspect have been executed.

P. niruri may also prevent the progression of thioacetamide (TAA)-induced liver cirrhosis in rats by regulating the expression of transforming growth factor (TGFbeta), collagen alpha 1 (Collalpha1), matrix metalloproteinase-2 (MMP2) and tissue inhibitor of metalloproteinase-1 (TIMP 1) genes through its two constituents: 4-O-caffeoylquinic acid and quercetin 3-O-rhamnoside.[11,12] This is the only study which has explicitly linked the antioxidant property of *P. niruri* with its epigenetic activity. Despite the preponderance of *in-vitro* and animal studies, there has only been one experiment on human subjects conducted concerning the antioxidant properties of *P. niruri*. This study investigated the plasmatic effect of *P. niruri* tea on healthy subjects[36] whereby it was observed that drinking *P. niruri* tea caused a transient rise in plasma ascorbic acid and a more sustained increase in plasmatic gallic acid within 24 hours. However, there was insignificant improvement in SOD and CAT activity, probably due to the short duration of tea intake. In general, this herb has been shown to significantly restore the reduced levels of GSH[13–15,17,23–25] and various antioxidant enzymes[15,19,20,23–25] and lower lipid peroxidation.[13,15,17,19,20,23–25] However, a study has shown that

P. niruri may have adverse effects on the kidneys and testes.[21]

Antidiabetic – hypoglycaemic action

Increased oxidative stress due to chronic hyperglycaemia is a widely accepted factor in the progression of diabetes and its complications. Animal studies using extracts of *P. niruri* have demonstrated dose-dependent improvements in fasting blood sugar, improved glucose tolerance and restoration of pancreatic tissue architecture, which may be due to inhibition of enzymatic pathways in intestinal carbohydrate digestion and glucose storage.[28,37,38,39] It is thought that the bioactive agents of the extract possess insulin-mimicking activity or potentially may stimulate the production of insulin as observed by the extracts ability to improve hepatic glycogen content and increase liver hexokinase activity.[29] Despite these findings, the antidiabetic activity of *Phyllanthus* remains uncertain with varying results from different members of the genus.[40,41] Review of existing studies suggest that the authors have used a variety of methods to induce diabetes, differing extraction method and dosages. This has invariably precluded the direct comparison of studies to ascertain the true functional properties of *P. niruri* as an antidiabetic agent although it has long been employed as a traditional treatment for alleviation of non-insulin-dependent diabetes.[42]

Anti-inflammatory, antinociceptive and analgesic activity

Studies on the anti-inflammatory, antinociceptive and analgesic activity of *P. niruri* have mainly revolved around animal models. Intraperitoneally administered methanol extract of dried callus tissue of *P. niruri* caused antinociceptive effects on five different models of pain, suggesting that *P. niruri* possessed analgesic properties. However, the mechanism of action is still debated on. Currently, there are still no molecular studies on the effect of *P. niruri* extracts on pain pathways. Obidike *et al.*[43] deduced that the anti-inflammatory and antinociceptive action of *P. niruri* was mediated via the peripheral nervous system. In his study on rats, whole plant chloroform extract was found to inhibit writhing response, reduce yeast-induced pyrexia, alleviate albumin-induced inflammation with an effect comparable to aspirin, increase pain threshold in the Randall–Selitto test but not the hot plate test for thermally induced nociception. Hence, Obidike deduced that *P. niruri* chloroform extract exerted antipyretic, anti-inflammatory and antinociceptive effects "..... the peripheral nervous system rather than the central nervous system."

However, other rat studies suggest that the hydroalcoholic[44–47] and spray-dried standardized[48,49] extracts may exert both significant peripheral and central analgesia. Hence, there remains a need for the further study of the effect of *P. niruri* extracts on major pain pathways to clarify the ambiguity that surrounds its analgesic mechanism. In an attempt to identify the specific analgesic and anti-inflammatory bioactive agents, two studies have shown that only spray-dried extract of leaves possessed analgesic anti-allodynic and anti-edematogenic and that these appeared to be a function of gallic acid concentration.[49,50] In addition, corilagin, which is found in abundance in *P. niruri* extracts, has also been identified as an antihyperalgesic tannin, which derives its activity from its involvement in the glutamatergic system.[50] Corilagin was found to reduce acetic acid writhing response in a dose-dependent manner and also displayed significant neurogenic analgesia, suggesting the possibility that corilagin either attenuates the release of inflammatory endogenous mediators in the peripheral circulation or induces analgesia via the direct interaction with peripheral nociceptors or bradykinins.[51,52] Moreover, the antihyperalgesic activity of corilagin may be due to its inhibition of the glutamatergic system via the possible prevention of NO synthesis by corilagin. This is also in accordance with the outcomes of a study by Martini *et al.*[53]

Hypolipidaemic activity

Studies pertaining to the lipid-lowering activity of *P. niruri* have all been conducted using rat models. It is interesting to note that no studies using rabbit models have been performed despite that fact that it is widely accepted that rabbits are more reliable hyperlipidaemic models. Additionally, no in-depth *in-vitro* or molecular studies have been conducted to date to elucidate the exact mechanism involves in the activity of lowering lipid levels. However, animal studies provide strong evidence that *P. niruri* possesses antioxidant-linked hypolipidaemic properties.[29,30,37,54–56] Of interest is the role of *P. niruri* in redox changes, disruption of lipoprotein export associated with alcoholic liver disease and lipid peroxidation secondary to alcohol-induced oxidative stress.[57,58] However, there is a need for comparative studies to examine the hypolipidaemic capacities of different types of *P. niruri* extracts, since there has yet to be uniformity between the studies concerning the type of extract used, with one using ethanolic extracts,[56] one methanolic extract,[37] three aqueous[29,30,55] and one unstated,[54] apart from the discrepancies in concentrations of decoctions used in these studies. Furthermore, animal studies on mammals other than rats are needed before one considers clinical trials, as the pathobiology associated with rodent Triton-induced

hyperlipidaemia may differ from human pathology. In addition, phytochemical screening plays a key role in narrowing down the range of phytochemicals likely to be responsible for *P. niruri* hypolipidaemic activity.

In a study on Triton- and cholesterol-induced hyperlipidaemic rats, *P. niruri* lowered the major serum lipid biomarkers significantly,[54] corroborating the results of a previous animal study[56] where administration of alcoholic extracts of *P. niruri* lowered the low-density lipoprotein levels. Hyperlipidaemic rats orally administered *P. niruri* experienced reduced serum levels of lipidaemic parameters such as total cholesterol, triglycerides, low-density lipoprotein, apo-LDL and VLDL-TG while partially reactivating plasma lecithin: cholesterol acyltransferase(LCAT) and postheparin lipolytic activity. It also restored hepatic lipoprotein lipase activity, normalized cholesterol biosynthesis and increased receptor-mediated LDL catabolism. Faecal excretion of cholic and deoxycholic acids was also normalized. The high-density lipoprotein and apo-HDL levels also recovered when rats were fed *P. niruri* extract, which could be linked to improved LCAT activity. It is thought that the LDL-lowering property of *P. niruri* may also be due to the increased binding of β-lipoproteins with hepatic LDL receptors.[59] The increased faecal excretion of bile acids may be due to the flavonoids present in *P. niruri*, as studies have proven that rats given phenolic flavonoids experience similar results due to increased lipid catabolism and reduced reabsorption of bile acids and cholesterol from the gut.[60,61]

In general, these studies demonstrated dose-dependent hypolipidaemic activity of *P. niruri* extracts, with a study showing that *P. niruri* may possess greater hypolipidaemic activity than glibenclamide.[37] There was, however, in one study, a degree of weight gain observed in the rats administered the *P. niruri* extract, which is similar to the side effects of thiazolidinediones.[37] As such, more studies are required to study the potential adverse effects of *P. niruri* as an antidiabetic agent especially when treating obese patients in need of urgent blood glucose and weight control. It is also evident that the hypolipidaemic action of *P. niruri* and its potential use among patients suffering from alcoholic liver disease is closely linked with its lipid peroxidation-quenching action, which may be a function of its high polyphenolic content.[29,55]

Cardioprotective activity

Only one major animal study has been conducted to investigate the attenuating action of *P. niruri* extracts in preventing doxorubicin-associated cardiotoxicity. Pretreatment of rats with *P. niruri* extract significantly protected rat myocardia from doxorubicin toxicity by normalizing cardiac biomarkers, restoring intracellular levels of enzymatic and non-enzymatic antioxidants and decreasing rat cardiac tissue peroxidation.[62]

Antiplatelet and vasorelaxant activity

Methyl brevifolin carboxylate isolated from *P. niruri* exerted vasorelaxant effect on rat aortic rings via inhibition of noradrenaline-induced vasocontraction mediated by a decrease in calcium ion influx through receptor-operated Ca^{2+} channels.[63] The same compound also acted as a platelet aggregation inhibitor.[64]

Wound healing and anti-ulcer properties

A rat study involving the oral administration of ethanolic extracts of the herb showed significant inhibition of the development of indomethacin-induced ulcers. The anti-ulcer activity has been attributed to gallic acid, beta-sitosterol, ellagic acid and alkaloids-4-methoxy-securinine.[65] Extracts of *P. niruri* also protect against ethanol-induced gastric mucosal ulceration in rats[66] and reverse dexamethasone-suppressed burn wound healing.[67] The exact mechanisms have not been elicited to date.

Antiviral activity

Perhaps the most prominent among the potential therapeutic effects of *P. niruri* is its antiviral activity. Studies conducted on sera obtained from chronic hepatitis B patients and woodchuck hepatitis (WHV)-infected woodchucks, which were treated with *P. niruri* extracts, showed decreased viral antigen levels.[68] Overall, aqueous extracts of *P. niruri* have been shown to possess significant antiviral potential and appear promising especially with regard to hepatitis B carriers.[6]

Clinical studies on hepatitis B patients showed that 50–60 per cent of patients who were administered *P. niruri* extract experienced HBsAg seroconversion. The reduction in HBsAg antigen may have been due to the inhibitory effect of *P. niruri* on hepatitis B viral genetic replication.[69,70] Of note, in a study where patients were treated with extracts of three different members of the genus *Phyllanthus*, it was observed that extracts of *P. niruri* were more likely to induce reductions in HBeAg titres.[71]

Although not all the bioagents responsible for the anti-hepatitis B activity of *P. niruri* have been identified, molecular studies have determined the molecular structure of a novel lignin found in *P. niruri*, nirtetralin B and its two stereoisomers, nirtetralin and nirtetralin A. Nirtetralin significantly inhibited HBsAg and HBeAg levels *in vitro*.[72] All three lignans had a dose-dependent inhibitory effect on

the *in-vitro* titres of HBV antigens. Moreover, inhibition ratios for nirtetralin and nirtetralin B were significant when compared with acyclovir, suggesting that these compounds were promising as novel anti-HBV antivirals. In general, lignans had low cytotoxicity on host cells, suggesting that these compounds could safely be given at non-toxic dosages without incurring undesirable adverse drug reactions.[73,74]

To date, no systematic review has been conducted on the anti-hepatitis B activity of *P. niruri per se*. However, there have been a number of reviews since the year 2000, on the utility of members of the *Phyllanthus* genus as potential antiviral agents for chronic hepatitis B infection.[75–77] One review concluded that *Phyllanthus* extracts were as effective as interferon in the terms of HBsAg seroconversion. Moreover, the extracts of members of the *Phyllanthus* genus were more effective than other herbal preparations in eliminating hepatitis B surface antigen from patient sera and in normalizing other hepatic parameters. *Phyllanthus* extracts also worked more effectively when used in combination with interferon. Although the above conclusions were not based on a single species of *Phyllanthus* in particular, the overall findings of this review were suggestive of the antiviral potential of the genus *Phyllanthus*, and by extrapolation, *P. niruri*. However, this review also reported that the evidence presented was still insufficient to confirm whether *Phyllanthus* extracts had clinically significant benefits given that most of the 22 studies analysed were rather heterogenous in terms of methodology, with a prevailing lack of consensus regarding the species of *Phyllanthus* tested and clinically relevant experimental parameters.[75]

Subsequent reviews have concluded that while there is insufficient evidence to suggest that the extracts of *Phyllanthus* are, by themselves, comparable in efficacy to current antiviral agents, and hence justify their use in chronic hepatitis B patients, such extracts may enhance the potency of an antiviral agent if used in combination.[76,77] Despite these evidences, to justify the use of *Phyllanthus* for chronic hepatitis B patients, there is a need for more high-powered studies to assess the effectiveness of *Phyllanthus* against current antiviral therapy. Additionally, it may be particularly important for investigator to assess the quality of existing preclinical research in terms of their scientific rigour and reproducibility.

The antiviral activity of *P. niruri* is not just limited to hepatitis B. Aqueous extracts of *P. niruri* containing repandusinic acid, a hydrolysable tannin, have been shown to exert a significant inhibitory effect on HIV-1 reverse transcriptase (HIV-1-RT).[78] Kinetic analysis suggests that repandusinic acid competitively inhibits the template primer during the process of reverse transcription. However, repandusinic acid does not bind to the same site as dTTP analogues such as AZT (azidothymidine). Despite the promising results with respect to the inhibition of HIV-1-RT activity, repandusinic acid seemed to exert less significant inhibition of DNA pol alpha.[78] With regard to the resultant degree of cytopathogenicity, repandusinic acid reduced the amount of pathogenic changes in HIV-infected MT4 cells, and the results even suggested that repandusinic acid may be more potent than AZT in inhibiting HIV cytopathogenicity. Moreover, azidothymidine and repandusinic acid may work in synergy when administered as a combination. However, the action of repandusinic acid has only been studied at the cellular level, and no animal or human studies on the anti-HIV therapeutic effects of repandusinic acid have been carried out. The study by Ogata *et al.* was a milestone in itself as it highlighted that hydrolysable tannins were important potential antiviral agents in HIV therapeutics. While it was previously known that phenols, flavonoids and alkaloids were major chemical constituents of *P. niruri* extracts and had great potential as antiviral agents, this study highlighted hydrolysable tannins such as repandusinic acid, as another novel HIV-1-RT inhibitor. In addition, although it was previously believed that tannins generally fared poorly on the therapeutic index and, due to their lack of specificity, could interfere in macromolecular interactions, repandusinic acid clearly demonstrated high specificity for HIV-1-RT, hence implying that it may have less cross-interactions with other macromolecules than previously believed.[78] This significant toxic selectivity for virus-infected cells was replicated in a subsequent study on alkaloidal extracts of *P. niruri*,[79] with a greater preference for HIV-2-infected cell lines. Like repandusinic acid, the alkaloid extract of *P. niruri* was also found to have an inhibitory effect on HIV-1 replication and dose-dependent cytoprotectivity against HIV infection. A separate study on a cohort of ten HIV patients who were administered the extracts of five different traditional herbs (with *P. niruri* being one of them) for at least half a year resulted in 7 of the 10 patients experiencing a rise in CD4 count. Despite this significant finding, no follow-up studies have been conducted on the mechanisms of action underlying these clinical findings.

Apart from repandusinic acid, there are other *P. niruri* phytochemicals, which may form new classes of anti-HIV agents. Qian-Cutrone and colleagues isolated a glucopyranoside, niruriside, which was found to inhibit REV/RRE binding during the movement of viral RNA from the cell nucleus to the cytoplasm. However, despite being found to be a specific REV/RRE inhibitor, niruriside did not display satisfactory levels of cellular protection in cases of acute HIV-1 infection.[80]

A study exploring the antidengue activity of members of the genus *Phyllanthus* showed that *Phyllanthus* extracts

worked best when administered simultaneously with DENV-2 inoculum implying that the *Phyllanthus* extract most probably affected the early phases of viral infection such as the viral attachment and entry.[81] Proteome analysis showed that the expression of 13 host and viral proteins involved in viral entry and replication, molecular chaperoning, cytoskeletal assembly and cellular metabolisms was altered, including calreticulin, Trim 1, heat-shock 70-kDA protein, beta-actin, DNA topoisomerase I, NS3, G3PD (glyceraldehyde-3-phosphate dehydrogenase), RBM1 (RNA-binding motif 1), DNA mismatch repair protein Msh2, dengue virus NS2bNS3 and polysialyltransferase.[81] Apart from that, *P. niruri*-synthesized silver nanoparticles demonstrated significant larvicidal, pupicidal and adulticidal activity against *Aedes aegypti* both in laboratory and field settings.[82]

Antibacterial activity

With the increase in antibiotic resistance rates and the need for novel antibiotics, which have optimal antimicrobial activity with minimal toxicity, there is renewed interest in exploring phytochemicals from everyday plants. A possible reason for the increased interest in extracting phytochemicals for the development of novel antibiotics is the threat of plant species extinction, hence inciting a need to explore the medicinal potential of these resources before they are lost.[83]

P. niruri contains various phytochemicals, which exert antimicrobial and antiprotozoal properties.[84] These include rutin,[4,85] gallocatechin,[86] prenylated flavanone glycosides,[87] quercetin,[88] quercitrin,[89] p-Cymene,[90] corilagin,[91] diosgenin,[92] securinine[93] and β-glucogallin.[94] Overall, studies on the antimicrobial activity of *P. niruri* extracts are still limited to *in-vitro* models and have not advanced to animal studies as of the time of writing.[32,95–101] These studies utilize different types of *P. niruri* extracts, with one study a comparison of methanolic, ethanolic and aqueous extracts, recognizing that different preparations yielded different compositions of pharmacophores.[101] To date, there are no studies known to specifically elicit the exact mechanism of action for the antimicrobial activity of the extracts. Of the 8 studies conducted to date concerning the antimicrobial potential of *P. niruri*, only four have carried out phytochemical screening of the extracts used.[97,98,100,101]

An agar well diffusion study conducted on the antimicrobial activity of aqueous and ethanolic extracts of the leaves and roots of 4 Indian herbs, including *P. niruri*, showed that the ethanolic extract was more effective against *Escherichia coli* and *Staphylococcus aureus*, whereas the aqueous preparation had greater activity against *Proteus vulgaris* and *Bacillus subtilis* but poor anticoliform activity.[95] It was observed that methanol extracts of *P. niruri* were twice as strong as that of aqueous preparations, with an MIC of approximately one-third that of aqueous extracts. In addition, both aqueous and methanolic extracts of *P. niruri* demonstrated significant activity against *Listeria monocytogenes*, the bacteria responsible for listeriosis, suggesting the potential of *P. niruri* as a food preservative.[96] A subsequent disc diffusion study found that both ethanolic and aqueous extract of *P. niruri* failed to inhibit the growth of the Gram-negative bacilli but demonstrated statistically more significant inhibitory activity against Gram-positive bacteria.[32] The apparent difference in results between this study and that of Cheah and colleagues in 2011,[96] could be due to the use of varying solvents. This could suggest that the aqueous extracts contained a higher content of phenolic compounds compared with the ethanolic extract.[102]

Agar diffusion assays in a study on *Helicobacter pylori* and three species of probiotic *Lactobacilli* revealed that *P. niruri* inhibited *H. pylori* in a dose-dependent manner while it did not affect the growth of lactic acid bacteria.[97] The addition of proline to *H. pylori* agar did not reverse the inhibiting activity of *P. niruri* aqueous extract, suggesting that the anti-*H. pylori* property of *P. niruri* did not involve the inhibition of proline dehydrogenase, a membrane-associated protein linked with prokaryotic energy production.[103] The anti-*H. pylori* activity recorded in this study could be due to ellagitannins such as geraniin and corilagin contained in the aqueous extract, which have also been previously shown to act in a concentration-dependent manner against various antibiotic-resistant *H. pylori* strains[104] by rapidly precipitating agglutination of *H. pylori* cells.[105] The lack of activity against lactobacilli implies that the ellagitannins-rich aqueous extract was selective for the targeted pathogen (*E. coli*) instead of probiotic organisms. In terms of its anticoliform activity, methanolic extracts of *P. niruri* followed by its seeds, displayed greatest inhibition of *E. coli* activity. Overall, root extracts displayed the weakest antibacterial activity.[98] The optimal bacteriostatic action against *E. coli, S. viridans, S. aureus* and *P. aeruginosa* observed with methanolic leaf extract could be due to the action of flavonoids.

Methanolic extracts of *P. niruri* leaves acted directly on the cell wall in a concentration-dependent trend. There is a possibility that certain morphological or molecular features of Gram-positive cell walls are targets of the active agents of *P. niruri* methanolic extracts.[102] These binding sites have yet to be identified.

In short, different parts of the herb and the usage of different solvents could yield extracts with differing compositions of pharmacophores, hence affecting the antimicrobial spectrum of activity of this plant.

There is a need for more molecular studies to elicit exact mechanism for bioactive agents before we can proceed to animal studies. In general, methanolic extracts are more potent against Gram-positive microbes, followed by aqueous extracts and ethanolic extracts. The antibacterial activity of *P. niruri* is also dose dependent. However, the compatibility of methanolic extracts to the mammalian subject may need to be investigated using animal models, as methanol, a polar organic solvent, may be disruptive of cellular phospholipid membranes. Although there is noticeably poor Gram-negative activity, there is still a need for large-scale molecular studies to investigate the relationship between the morphology of Gram-negative bacteria and the lack of Gram-negative activity in *P. niruri* extracts.

Antiplasmodial and nematicidal properties

In-vivo[106–108] and *in-vitro*[109–112] studies show that *P. niruri* extracts display antiplasmodial properties. This may be due to the terpene-rich content of *P. niruri* extracts.[111] Of interest, methanolic extracts displayed chemosuppressive action comparable with chloroquine and demonstrated better prophylactic activity than pyrimethamine.[108] Of note, a study on the nematicidal activity of *P. niruri* against *Meloidogyne incognita* and *Rotylenchulus reniformis* identified two prenylated flavanones as being responsible for the nematicidal activity of *P. niruri*.[113]

Anti-urolithiatic activity

A study on the effect of *P. niruri* extract on calcium oxalate (CaOx) crystallization in vitro showed that *P. niruri* restricted CaOx crystal growth and aggregation, showing its potential to disrupt the early stages of stone formation.[114] *P. niruri* also changed the shape of calculus in rats into a smoother and possibly more fragile form which could ease removal or dissolution of calculi.[115] A clinical study showed that *P. niruri* lowers urinary calcium in hypercalciuric patients subset among 69 calcium stone-forming patients.[116] Another clinical study demonstrated that in postextracorporeal shock wave lithotripsy patients who underwent therapy with Uriston, a *P. niruri* extract had higher stone-free rates than the control group.[117]

Antihyperuricaemic activity

Lignans from *P. niruri* were found to be antihyperuricaemic in animals; the effects were comparable with drugs for treating hyperuricaemia and gout-like allopurinol and probenecid.[118] A later study showed that the antihyperuricaemic property of the lignans was due to their uricosuric action. This study also showed that *P. niruri* methanol extract had antihyperuricaemic effect primarily attributed to its uricosuric action and partly via xanthine oxidase inhibition.[119]

Antineoplastic activity

Spray-dried extract of *P. niruri* (SDEPN) was found to be selectively toxic against various cancer cell lines – colorectal carcinoma (HT29)[120] cells, human hepatocellular carcinoma (HepG2),[120,121] Huh-7 cells[121] and ovarian cancer cell lines SKOv3ip and Hey[122] – in addition to reduction in tumour incidence, tumour yield and tumour burden in mice.[123]

Spasmolytic activity

Alkaloid extracts of *P. niruri* demonstrated smooth muscle relaxation in urinary and biliary tracts.[124] Extracts of *P. niruri* leaves, stems and roots showed antispasmodic properties on several smooth muscles *in vivo* such as guinea pig ileum, rat uterus and canine vascular smooth muscles. Ether extracts were found to be most effective as antispasmodics.[125,126]

Immunomodulatory activity

Extracts of *P. niruri* have proven to be potent murine lymphocytes mitogens and are able to induce surface activation maker (CD69), B and T lymphocyte proliferation. The production of interferon-gamma (IFN-gamma) and interleukin-4 (IL-4) by *P. niruri* extract-stimulated naive splenocyte cultures was also increased in a concentration-dependent manner. Various indices of activation and functions of murine bone marrow-derived macrophages, such as phagocytosis, lysosomal enzymes activity and TNF-alpha release, were significantly enhanced by pretreatment with *P. niruri* extract, which also modulated macrophage nitric oxide release.[127] *P. niruri* also increased the expression of major histocompatibility complex-II and markers for dendritic cell maturation (CD40), activation (CD83) and costimulation (CD86) in a concentration-dependent manner. In a transgenic T-cell activation model, *P. niruri*-treated dendritic cells also presented Ova antigen to Ova-specific CD8(+) T cells more efficiently.[128]

Anti-amnesic property

Isocorilagin from *P. niruri* has been shown to be two to three times more potent than galanthamine, the clinically used AChE inhibitor. Kinetic analyses suggested that isocorilagin is a non-competitive inhibitor for AChE. *In-silico*

molecular docking revealed that isocorilagin effectively blocks substrate entry by forming hydrogen bonding with residues at the entrance of the AChE active site.[129]

Discussion and Conclusion

The present review provides a comprehensive overview of selected scientific studies using the plant *P. niruri*. The plants' extensive use in traditional and complementary medicine for variety of diseased condition has led to a significant amount of scientific studies particularly using animal models. The present review demonstrates that *P. niruri* extracts have pharmacological potential in a large range of conditions as summarized in Appendix 1. Of the conditions investigated, it appears that *P. niruri* has commonly been investigated for its antiviral, hepatoprotective, hypolipidaemic and antibacterial activity. The positive findings seen in the articles reviewed here is likely due to the fact that there may be multiple bioactive compounds within crude extracts used in these studies. It is widely accepted that crude extracts are made up of a range of bioactive compounds and each compound may exert differing activity on body tissues. Nonetheless, only a handful of studies assessed the pharmacological potential of these bioactive compounds as most studies described the activity of methanolic and aqueous crude extracts. Therapeutic findings using crude extracts have limited translational value as investigators are unable to determine whether the findings are related to the action of a single bioactive compound or that of synergy between multiple bioactive compounds. This additionally relates to the extraction process used when preparing crude extracts. It is widely known that different extraction methods and use of solvents with different polarity yield different bioactive compounds and as such limits our ability to compare findings between studies. In performing our review, we observed that there was significant heterogeneity in study protocols and in some instances conflicting results, which suggest that some studies may not provide reproducible data. Additionally, the lack of information in some studies prevents our ability to replicate these studies to make an independent assessment of the therapeutic potential of the plant. When assessing pharmacological potential of novel therapeutic agents, it is essential that authors provide a comprehensive account of the experimental design and protocol and ensure proper standardization of material and techniques, an aspect that appears to be lacking in some studies in this review. Thus while the overall findings suggest an abundance of therapeutic potential of *P. niruri*, such findings must be interpreted with caution. There are still many aspects of research on this herb that need to be considered such as larger sample sizes, toxicological studies, mechanism studies and molecular analyses. Current evidence is largely limited to correlation between identified phytochemicals and their biological activities. There is a lack of mechanisms of action studies to understand the interaction of bioactive phytochemicals from *P. niruri* and their respective molecular targets. Essentially, more robust scientific methodologies are necessary before confirmatory decisions can be made on the potential of *P. niruri*. A key step before clinical trials may be considered.

Conflict of interest

The authors declared they have no conflict of interest to disclose.

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Appendix 1 General overview of the therapeutic potential of *P. niruri*

Key information	Major findings	References
Hepatoprotectivity	*In-vivo* and *in-vitro* experiments suggesting the protective role of *P. niruri* against various hepatotoxic agents	Syamasundar *et al.*[10], Amin *et al.*[11], Amin *et al.*[12], Bhattacharyya *et al.*[14], Sarkar *et al.*[13], Bhattacharjee *et al.*[15], Bhattacharyya *et al.*[17], Bhattacharjee *et al.*[19], Bhattacharjee *et al.*[20], Manjrekar *et al.*[21], Harish *et al.*[22], Chatterjee *et al.*[23], Chatterjee *et al.*[24], Chatterjee *et al.*[25] Sarkar *et al.*[27]
Antiviral properties	Antiherpes simplex virus (HSV)-1 effect In-vitro experiments suggest that ethanolic extract of *P. niruri* extract inhibits of HSV-1 replication	Faral-Tello *et al.*[130]
	Anti-hepatitis B virus (HBV) effect *In-vitro* experiments suggest that nirtetralin B (isolated from *P. niruri* extract) effectively suppressed the secretion of the HBV antigens in Human HBV-transfected liver cell line *In-vivo* experiments suggest that nirtetralin reduced the serum m duck hepatitis B virus DNA (DHBV DNA), HBsAg and HBeAg in ducklings	Liu *et al.*[73]
	In-vitro experiments suggest that nirtetralin A and B effectively suppressed the secretion of the HBV antigens	Wei *et al.*[8]
	Anti-human immunodeficiency virus (HIV) effect *In-vitro* experiments suggest that alkaloidal extract of *P. niruri* showed suppressing activity on strains of HIV-1 cells cultured on MT-4 cell lines	Naik *et al.*[79]
	In-vitro experiments suggest that repandusinic acid A monosodium salt (RA) isolated from *P. niruri* inhibited HIV-1-induced cytopathic effects in MT-4 cells, HIV-1-induced giant cell formation of SUP-T1 and HIV-1-specific p24 antigen production	Ogata *et al.*[78]
	In-vitro experiments suggest that niruriside isolated from *P. niruri* showed specific inhibits the REV/RRE activity in the HIV thus making the infection non-productive	Qian-Cutrone *et al.*[80]
Antibacterial activities	*In-vitro* experiments suggest that aqueous extracts of *P. niruri* is effective against Gram-positive bacteria only – Staphylococcus aureus and Streptococcus agalactiae	Amin *et al.*[32]
	In-vitro experiments suggest that methanol extract of *P. niruri* is effective against Gram-positive bacteria (*B. cereus*, *B. subtilis* and *S. aureus*) compared with Gram-negative bacteria (*E. coli, P. rettgeri* and *P. aeruginosa*)	Ibrahim *et al.*[23]
Anticholinesterase activity (AChE)	*In-vitro* experiments suggest that methanol extract of *P. niruri* showed potential inhibitory activity against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) due to the presence of isocorilagin	Koay *et al.*[129]
Hyperalgesic activity	*In-vivo* experiments suggests that corilagin, isolated from *P. niruri* presented hyperalgesic activity in chemically and thermally based nociception in mice model	Moreira *et al.*[50]
	In-vivo experiments suggest that *P. niruri* given intraperitoneally or orally caused marked inhibition of capsaicin-induced pain in mice models	Santos *et al.*[45]
Antidengue activity	*In-vitro* experiments suggest that *P. niruri* extract and *P. niruri*-synthesized nanoparticles were highly effective against *A. aegypti* larval instars and pupae of *A. aegypti*	Suresh *et al.*[82]

Appendix 1 *Continued*

Key information	Major findings	References
Antidiabetic activities	*In-vivo* experiments suggest that *P. niruri* extract lowered blood glucose, suppressed postprandial rise in blood glucose following a glucose meal, reduced haemoglobin glycation and increased absolute and relative weights as well as glycogen content of liver in diabetic rats	Okoli *et al.*[38]
Antihyperuricaemic effects	*In-vivo* experiments suggest that intra peritoneal treatment with methanol extract of *P. niruri* and treatment with hypophyllanthin and phyltetralin mainly increase urinary excretion of uric acid and partly inhibit xanthine oxidase in rats	Murugaiyah *et al.*[119]
Anti-inflammatory and antipyretic activities	*In-vivo* experiments suggest that chloroform soluble fraction of *P. niruri* methanol extract demonstrated antipyretic and anti-inflammatory effects in rats	Obidike *et al.*[43]
Antineoplastic activities	*In-vitro* experiments suggest that spray-dried extract of *P. niruri* has cytotoxic effects on HT29 and HepG2 cells, and this effect is enhanced when combined with cisplatin	Araujo *et al.*[120]
	In-vitro experiments suggest that spray-dried extract of *P. niruri* has a cytotoxic effect on human hepatocellular carcinoma cells (HepG2, Huh-7) and colorectal carcinoma cells (Ht29) but has protective effect on keratinocytes (HaCaT) normal cells	Araujo *et al.*[121]
	In-vitro experiments suggest that corilagin extracted from *P. niruri* has a cytotoxic effect against the growth of ovarian cancer cells (SKOv3ip, Hey and HO-8910PM)	Jia *et al.*[122]
	In-vivo experiments suggest that oral administration of *P. niruri* extract reduce tumour incidence, tumour yield, tumour burden and cumulative number of papillomas in mice model with skin carcinogenesis by enhancing the antioxidant defence mechanism	Sharma *et al.*[123]
Antioxidative properties	*In-vitro* experiments suggest that ethanol extract of *P. niruri* possesses a high level of flavonoid content, while the aqueous extract possesses the high free radical-scavenging activities with high phenol content and elevated levels of ferric reducing antioxidant power (FRAP)	Amin *et al.*[32]
	In-vivo experiments suggest that *P. niruri* tea ingestion is associated with a modest increase in antioxidant markers (ascorbic and gallic acid) in human plasma	Colpo *et al.*[36]
	In-vivo experiments suggest that *P. niruri* leaf extract protects the kidney from oxidative stress induced by diabetes in rats	Giribabu *et al.*[131]
	In-vivo experiment suggest that aqueous and alcoholic extract of *P. niruri* has the ability to scavenge radiation induced free radicals in irradiated mice	Thakur *et al.*[132]
Antiplasmodial activities	*In-vivo* experiment suggest that *P. niruri* methanol extract (chloroform fraction) demonstrated chemosuppression of *Plasmodium berghei* in mice	Ifeoma *et al.*[108]
	In-vitro experiments suggest that ethyl acetate and acetone extract of *P. niruri* demonstrated moderate antiplasmodial activity against *Plasmodium falciparum*	Venkatesalu *et al.*[112]
	In-vitro experiment suggest that ethanolic extract of *P. niruri* demonstrates anti plasmodial effect against *Plasmodium falciparum*	Tona *et al.*[110]
	In-vivo experiment suggest that ethanolic and dichloromethane extracts of *P. niruri* produced significant chemosuppressions of *Plasmodium berghei* in mice	Tona *et al.*[106]
	In-vitro experiment suggest that ethanolic and dichloromethane extracts of *P. niruri* demonstrated antiplasmodial activity against *Plasmodium falciparum*	Tona *et al.*[109]

Appendix 1 *Continued*

Key information	Major findings	References
	In-vitro experiment suggest that ethanolic extract of the whole *P. niruri* plant demonstrated better antiplasmodial activity against *Plasmodium falciparum* compared with callus cultured extracts	Cimanga *et al.*[111]
	In-vitro experiment suggest that the aqueous and methanolic extract of *P. niruri* has better antiplasmodial activity against *Plasmodium falciparum* compared with aqueous and chloroformic extracts *In-vivo* experiments suggest that methanolic extract demonstrated the best antiplasmodial activity against *Plasmodium berghei* in mice	Mustofa *et al.*[107]
Platelet aggregator inhibitor	*In-vitro* experiments suggest that ethanol extracts *P. niruri* leaves demonstrated greater antiplatelet aggregatory effects as compared with adenosine	Iizuka *et al.*[64]
Cardioprotective activity	*In-vivo* experiments suggest that pretreatment of mice with aqueous extract of *P. niruri* protected the myocardium from the toxic effects of doxorubicin	Thippeswamy *et al.*[62]
Gastroprotective activity	*In-vitro* experiments suggest that aqueous extracts of *P. niruri* are effective against *H. pylori* and do not affect beneficial lactic acid bacteria	Ranilla *et al.*[97]
Hypolipidaemic activities	*In-vitro* experiments suggest that *P. niruri* leaf extract protects against alcohol and polyunsaturated fatty acid-induced hyperlipidaemia in rats	Latha *et al.*[55]
	In-vivo experiments suggest that treatment with *P. niruri* reduced the level of low-density lipoprotein (LDL), phospholipids (PL), triglycerides (TG), apo-LDL and very low-density lipoprotein (VLDL)/TG ratio in hyperlipidaemic rats	Khanna *et al.*[54]
Immunomodulation activity	*In-vitro* experiments suggest that extract of *P. niruri* induce several immuno-activities including increase in the expression of surface activation maker (CD69), proliferation of B and T lymphocytes increased production of interferon-g (IFN-g) and interleukin-4 (IL-4)	Nworu *et al.*[128]
	In-vitro experiments suggest that aqueous extract of *P. niruri* enhances the structural and functional maturation of bone marrow dendritic cells (BM-DCs) and their antigen-presenting function	Nworu *et al.*[129]
Antinematicidal activity	*In-vitro* experiments suggest that 2 prenylated flavanones isolated from the hexane extract of *P. niruri* has antinematicidal activity against Meloidogyne incognita and Rotylenchulus reniformis	Shakil *et al.*[113]
Uro and nephrolithiasis activity	*In-vitro* experiments suggest that the concentrated stock solutions of *P. niruri* decreased the growth rate of calcium oxalate crystal in the synthetic urine	Rodgers *et al.*[133]
	In-vivo experiments suggest that aqueous extracts of *P. niruri* induce changes in calculi that might aid in elimination or dissolution of calculi in rats	Barros *et al.*[115]
	In-vitro experiments suggest that aqueous extract of *P. niruri* has an inhibitory effect on the calcium oxalate crystal growth and aggregation in human urine	Barros *et al.*[114]
	In-vitro experiments suggest that aqueous extract of *P. niruri* exhibited an inhibitory effect on the calcium oxalate crystal internalization	Campos *et al.*[134]
	In-vivo experiments suggest that treatment with aqueous extract of *P. niruri* inhibited the growth rate of the matrix calculus and reduced the number of stone satellites in rats	Freitas *et al.*[135]

Appendix 1 *Continued*

Key information	Major findings	References
	A randomized prospective study involving patients with renal stones suggested that regular self-administration of *P. niruri* after shock wave lithotripsy for renal stones results in an increased stone-free rate	Micali *et al.*[117]
	A randomized prospective study involving calcium stone-forming patients suggested that consumption of capsule of lyophilized aqueous extract of *P. niruri* induced a reduction in the mean urinary calcium in hypercalciuric patients	Nishiura *et al.*[116]
Vasodilatory effects	*In-vitro* experiments suggest that methyl brevifolin carboxylate isolated from the leaves of *P. niruri* inhibited norepinephrine-induced vasocontractions in isolated rat aortic strips	Iizuka *et al.*[63]
Wound healing	*In-vivo* experiments suggest that *P. niruri* reversed dexamethasone-suppressed wound contraction in rats	Devi *et al.*[136]