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MINI REVIEW

Bioprospecting of *Vernonia cinerea* for nutraceutical and homemade first aid remedies: a review update

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Abstract

Vernonia cinerea (VC) is a ubiquitous weed in tropical and subtropical regions. It was traditionally used to treat health issues by various cultures, but today little is known to the new generation about its high medicinal values, how to use it as a quick first aid, and its commercialization potential. A literature search found that VC has been extracted using various kinds of solvents for different polarities of phytochemical extraction. Interestingly, the food-grade solvents such as ethanol and water were found to be suitable to extract the active ingredients from VC for major therapeutic applications such as antioxidants, anti-diabetic, anti-inflammatory, anti-cancer, and anti-microbial as well as being a wound healer, hepato-protective, and ease the smoking withdrawals symptoms. The spray-dried water extracts are currently innovated into different forms of nutraceutical products. The high toxicity threshold allows the utilisation of VC as home remedies in the form of fresh, dried, and water extracts for oral intake, while the extracts using common alcohol/spirit and oil can be made for topical applications. This review aimed to give an insight into the commercialization potential of VC as a nutraceutical product and the reestablishment of homemade first-aid remedies for various health conditions especially among the people in rural areas.

1. Introduction

World Health Organisation reported that 70% of the world population is using herbs in their health management (Mohamad *et al*., 2019). The developing countries especially in the rural areas use them regularly as a part of their medication system. The drive to use herbs for health management is mainly due to costless, accessibility and supported by folkloric practices (Islahudin *et al*., 2017). Many scientific investigations have been conducted to validate the therapeutic potential of medicinal herbs (Mohamad *et al*., 2022; Zainol *et al*., 2021; Abdul Majid *et al*., 2022). *Vernonia cinerea* (VC) is one of the common tropical weeds which is highly therapeutic. Nonetheless, there is limited information on the bioprospecting of VC.

Traditionally, VC has been used in the form of raw coarse powder and extract (powder or liquid) for the treatment of many illnesses. The decoction of the whole plant is traditionally used to treat cough, insomnia, fever, dysentery and hepatitis (Quattrocchi, 2012). Meanwhile, the leaves of VC and its paste are used to reduce headaches. The leaf juice can be combined with honey to treat malaria, while its ground form is used for conjunctivitis and skin disease treatments (Quattrocchi, 2012; Hussain *et al*., 2015). The leaf juice also can be either used alone or in combination with the leaves of *Lynonia ovalifolia* (Wall.) Drude for treating wounds (Manandhar, 1991; Joshi and Joshi, 2000; Kumar *et al*., 2013). The root of VC is traditionally used as a remedy for filariasis, elephantiasis, helminthic infestation and viral fevers. Meanwhile, its seeds are used to treat arthritis, dysuria, colic, cough, worm infestation, and skin diseases (Quattrocchi, 2012; Hussain *et al*., 2015).

VC has been intensively studied in Asian countries,

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especially India, Thailand, Malaysia, China, Bangladesh, Philippines, Cambodia, and Nepal. Most of the studies were done for the phytochemical screening and pharmacological activities of VC (Hussain *et al*., 2015). In this review, we aim to highlight the most effective processing techniques in extracting the active ingredients of VC and its commercialization potential. It is hoped that this review may enlighten the wisdom of wild herbs utilization towards any interested parties or researchers in producing nutraceutical products specifically from VC.

2. Botanical background of *Vernonia cinerea*

VC can be found in tropical and subtropical areas (Johnson, 2012). The species are weed-like plants, fast-growing, and can easily grow unintentionally in gardens, in a vase, on the roadside, or any ground alongside normal weed. The plant is an Angiosperms or flowering seed plant due to the presence of flowers. The plant is a type of annual plant where that can be spotted in a single season and die the next season (Harborne and Baxter, 1996). The plant is herbaceous as the stem tissue is green, flexible, and has no woody structure. The plant grows as a shrub or in dense patches which can be seen clearly by its structure of multi-stemmed. The mode of nutrition is autotrophic; produces energy and food from inorganic compounds and light for growth (Weathers *et al*., 2021).

The nature of the stem is solid, covered with a hair-like structure and the growth form is erect as the stem grows vertically and upwards. VC has a white or brown taproot system which has a bulky mother root that grows vertically downwards and the remaining root is sprouting from the mother root. The colour of the leaf is green, attached to the stem via petiole which is a short or winged petiole, and only one leaf per petiole. The arrangement pattern is in an alternate manner in which a single leaf attaches at one node of the stem in one direction, while the next leaf attaches further than the nodes in the opposite direction. The leaf shape is ovate, an egg-like shape, or can be obovate which has a broader tip and the leaf venation or veins are observed as a pinnate structure where multiple secondary veins are attached to the main veins and then run parallel to each other from the centre towards leaf margin. The side of the leaf or leaf margin shows a blunt serrated or toothed and comes with no fixed shape or distance from another. The apex or tip of the leaf is seen as an acute shape as the tip ends with a short sharp point, while the base of the leaf is seen as obtuse or broader (Zakir *et al*., 2020).

The flower is seen in white, light purple, or pinkish colour. The flower location is terminal where the flower grows at the top of the stem, while the symmetry of the flower is radial. The flower of VC is in a composite family where the flowers form an inflorescence; the arrangement of small flowers into a big flower at the floral axis (Allred, 1982). One inflorescence can have up to five flowers from different branches, while one plant can have multiple inflorescences. The individual flower is shaped as urceolate, a look of swelling at the bottom and curving slightly inwards at the top. VC's fruits are oval achenes covered with fine whitish hairs and slightly tapered at the bottom (Zakir *et al*., 2020). The overview of VC is illustrated in Figure 1.

Figure 1. Overview of *Vernonia cinerea*'s whole plant, flowers and leaves.

3. Bioactivities of *Vernonia cinerea*

The therapeutic potential of VC had been reported immensely in the previous reports of its bioactivities such as anti-inflammatory, antimicrobial, anticancer, antidiabetic, anti-smoking aid, antioxidant and ameliorative activity of various plant parts (aerial, leaves, root, stem, or as a whole plant). The bioactivities of VC are summarized in Table 1.

3.1 Anti-inflammatory activity

In a Wistar rats model of carrageenan-induced paw oedema, different kinds of solvent extracts (aqueous, hydroalcoholic, ethanol, and chloroform) from VC stem showed inhibition of the inflammation in the range of 11.11 – 16.66% at a dose of 500 mg/kg while the positive control (diclofenac sodium) showed comparable inhibition of 11.11% at a dose of 50 mg/kg (Singh *et al*., 2014). There were also similar observations reported in an anti-inflammatory study of VC's methanol extract (whole plant) tested on a similar animal model. In addition, the study reported a decrease in pro-inflammatory cytokines (IL-1β, TNF-α, and IL-6) levels detected in the rat serum (Pratheesh and Kuttan, 2009).

3.2 Antimicrobial activity

The aerial part of VC was reported to have antimicrobial activity against *Pseudomonas aeruginosa, Citrobacter freundii*, *Escherichia coli*, *Acinetobacter calcoaceticus*, *Acinetobacter anitratus*, *Bacillus*

Table 1. Summary of reported *V. cinerea's* bioactivities

Plant's part	Extracts	Bioactivities	References
Whole plant	Methanol	Anti-inflammatory and antioxidant Chemo-protective Neuroprotective Anticancer	Khay *et al*. (2012); Pratheesh Kumar and Kuttan (2009); Pratheesh Kumar and Kuttan (2010); Reddy *et al*. (2012)
	Ethanol	Antidiabetic	Choudhary *et al*. (2013)
	Hexane, chloroform, and ethyl acetate fractions	Antimicrobial and antioxidant	Sonibare *et al*. (2016)
	Alkaloid and dichloromethane	Antimalarial	Soma *et al*. (2017)
	Water	Antidiabetic Anticancer Hepato-protective	Gokilaveni *et al*. (2006); Naowaboot *et al*. (2018); Supabphol *et al*. (2013)
	Spray and freeze-dried water extract	Antioxidant and anti-smoking aid	Kanthain *et al*. (2020)
Aerial	Methanol	Antimicrobial	Latha *et al*. (2009)
	Water	Anticancer	Supabphol *et al*. (2013)
Stem	Methanol	Antioxidant	Goggi and Malpathak (2017)
	Water, hydro-alcoholic, ethanol, and chloroform	Anti-inflammatory	Singh *et al*. (2014)
	Chloroform and ethanol	Antibacterial	Singh *et al*. (2014)
Leave	Methanol	Antioxidant Antidiuretic	Goggi and Malpathak (2017); Adeboye *et al*. (1997)
	Water	Antidiuretic	Adeboye *et al*. (1997)
	Chloroform	Diuretic Antibacterial	Adeboye *et al*. (1997); Singh *et al*. (2014)
	Ethanol	Antibacterial Antioxidant Hepato-protective	Leelaprakash *et al.* (2011); Nishadh *et al*. (2013); Singh *et al*. (2014)
Root	Methanol	Antibacterial Antioxidant	Goggi and Malpathak (2017); Tantengco *et al*. (2016)
	Water	Antioxidant	Goggi and Malpathak (2017)
	Pill (6 g)	Antidiabetic	Sayeed *et al*. (2013)

licheniformis, Micrococcus spp., *Staphylococcus epidermidis*, *S. aureus*, *Erwinia* sp., *Bacillus cereus*, *Serratia marcescens*, *S. saprophyticus*, *Yersinia enterocolitica*, *Shigella sonnei*, *Morganella morgana*, *Enterobacter aerogenes*, *Candida albicans*, and *Cryptococcus neoformans*, with inhibition zones of 8-20 mm and MIC value of 1.56-50 µg/mL. The methanol extract of VC showed the most potent antimicrobial activity compared to its fractions (diethyl ether, chloroform, ethyl acetate and butanol), especially against *C. albicans* (Latha *et al*., 2009). A similar observation was observed for hexane, ethyl acetate, and chloroform fractions of VC (whole plant) whereby all extracts were mostly active against *C. albicans* compared to the tested bacteria such as *S. aureus, P. aeruginosa, P. vulgaris, E. coli* and *K. pneumonia* (Sonibare *et al*., 2016). Antibacterial activity against *S. aureus* was also reported on the chloroform and alcoholic extracts of VC's leaf and stem. The inhibition zones were observed in the range of 19.33 to 21.00 mm. Ciprofloxacin was used as a positive control and showed 26.66±1.20 mm of inhibition zone (Singh *et al*., 2014). In another study, methanol extract of the VC's root showed inhibition activity against *S. aureus* with the highest inhibition zone (16.93 mm) at the lowest concentration tested, 25 mg/mL (Tantengco *et al*., 2016).

3.3 Antidiabetic activity

Antihyperglycemic activity of ethanol extract of VC as well as its isolated compound, sesquiterpene lactones, had been reported whereby a reduction of the blood glucose level was observed in the alloxan-induced diabetic mice (Choudhary *et al*., 2013). A clinical trial of VC had also been carried out on 48 patients diagnosed with type 2 diabetes mellitus. The patients received the treatment for six months with the consumption of 2 g pills of VC, three times a day. The first group received the treatment of VC for three months, followed by a placebo for another three months, while the second

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group received the treatments in the opposite sequence. This study showed that the blood glucose and glycated haemoglobin levels were reduced among the patients for both groups receiving the treatment and well-tolerated with no effect on the vital organs such as kidneys and liver. The blood biochemical parameters were observed to be in the normal range as well (Sayeed *et al*., 2013). Furthermore, it had been reported that the water extract of VC potentially improved insulin sensitivity in the obese mice induced with a high-fat diet by the increase of AMPK, PI3K, and Akt phosphorylation in the liver, skeletal muscle, and epididymal white adipose tissue as well as by the reduction in metabolic parameters such as hyperinsulinemia, hyperlipidemia, and hyperleptinemia at 250 and 500 mg/kg of treatment (Naowaboot *et al*., 2018).

3.4 Ameliorative and antioxidant activity

A study was done to investigate the protective role of VC's methanol extract (whole plant) by intraperitoneal treatment on Balb/c mice induced with cyclophosphamide (CTX), an anti-neoplastic drug. The administration of the extract and CTX synergistically reduced the tumour in mice as well as reduced the intestinal damage caused by the CTX. The reduction of pro-inflammatory cytokines was observed with an improved haematological and biochemical profiles of the treated mice (Pratheeshkumar and Kuttan, 2010). The ameliorative activity of VC also had been studied on catalepsy-induced rats (haloperidol-induced) in the evaluation of its neuroprotective effect. Ethanol extract of VC may contribute to enhanced locomotion by causing a reduction in the glutamate level of treated rats. The neuroprotective effect was observed to be comparable to the positive control, L-DOPA, in rats treated with 400 mg/kg of ethanol extract (Reddy *et al*., 2012).

The hepatoprotective effect of aqueous extract of VC (whole plant) had been studied on albino Wistar rats which were induced with carbon tetrachloride (CCl4). There was a total of five groups observed in this study whereby it was found that the activity of liver enzymes such as alkaline phosphatase (ALP), aspartate aminotransferase (AST), acid phosphatase (ACP), and alanine transaminase (ALT) was inhibited in the treatment groups (Gokilaveni *et al*., 2006). Thus, the study suggested that the oral treatment of VC's aqueous extract may potentially possess an ameliorative role by its hepatoprotective activity with no adverse effect. In another study done on the CCl4-induced albino Wistar rats, ethanol extract from VC's leaves was investigated for its hepatoprotective effect in 14 days of treatment. An increased level of oxidative liver enzymes and inhibition of lipid peroxidation by VC extract was observed. The study findings showed the association of the antioxidant activity of VC extract with its hepatoprotective role in the liver damage of rats (Nishadh *et al*., 2013). A similar observation was also reported in a seven-day treatment of ethanol extract of VC's leaves on the albino rats. This study also tested the radical scavenging activity of the ethanol extract by DPPH and ABTS scavenging assay whereby the antioxidant activity was observed to be in a dose-dependent manner (Leelaprakash *et al*., 2011). Ethyl acetate fraction of VC (whole plant) had been reported to show the highest antioxidant activity in a DPPH scavenging assay, followed by hexane and chloroform fractions (Sonibare *et al*., 2016). Besides, a total of 21 extracts of different solvent extraction (petroleum ether, water, acetone, methanol, ethanol, chloroform, and ethyl acetate) were tested for antioxidant activity from the leaf, stem, and root of VC. The methanol extract of all plant parts showed the highest antioxidant activity in the DPPH and ABTS scavenging assay (Goggi and Malpathak, 2017).

3.5 Anti-smoking aid

Previous studies have also been reported on the potential of VC as an anti-smoking aid. In 2009 and 2010, VC was formulated in the form of condensed juice and tea (3 g per tea bag) to evaluate the smoking cessation rate among smokers whereby reductions in smoking rates were observed among smokers as compared to the control group (Wongwiwatthananukit *et al*., 2009; Leelarungrayub *et al*., 2010). Meanwhile, another study reported on the inhibitory activity of VC's isolated compounds against monoamine oxidases (MAO-A and MAO-B) and human cytochrome P450 2A6 (CYP2A6) which are responsible for dopamine and nicotine metabolism (Prasopthum *et al*., 2015). The content of nicotine in VC (water extract) was also previously studied and the findings showed that leaves contained higher nicotine content than flowers while none was identified in the stem of VC (Ketsuwan *et al*., 2017). A systematic review and meta-analysis of five randomized controlled trials were conducted in a previous study to summarize the smoking cessation effect of VC. The study findings showed an increase in the smoking cessation rate in the treatment group compared to the control group (Puttarak *et al*., 2018). Recently, a study also was conducted on VC (whole plant) to evaluate its antioxidant activity and anti-smoking potential. The antioxidant activity was reported on the spray-dried and freeze-dried extract in the DPPH and ABTS scavenging assays. Pilot-scale production of hard candy was done using the spray-dried extract, with a nicotine content of 2.35 mg for each candy, and further suggested as an anti-smoking aid (Kanthain *et al*., 2020).

3.6 Anticancer activity

Cytotoxicity study of VC (whole plant) had been carried out on Human Cervix Epithelioid Carcinoma (HELA) and Human Embryonic Kidney (HEK293) cells induced with cisplatin. The aqueous fraction of VC showed the highest cytoprotective effect by enhancing the cell viability of the induced cells as compared to its crude aqueous extract and butanol fraction (Amuthan *et al*., 2019). The cytoprotective effect of VC's water extract (aerial part) was also evident in a study conducted on human umbilical vein endothelial cells that were exposed to nicotine toxicity. The study reported a decrease in vacuole-like appearance in the cells treated with 100 µg/ml of VC in time (24 to 72 hrs) and dose-dependent manner (Supabphol *et al*., 2013). In another previous study done on VC (whole plant), the cytotoxicity of extracts and its major compound (sesquiterpene lactone) was evaluated whereby methanol extract was the most potent extract compared to water and dichloromethane extracts against HepG2 hepatoma and HT29 colon adenocarcinoma cell lines (Khay *et al*., 2012). Besides, the major compound showed the highest cytotoxicity against the two cell lines. A similar observation was also reported in a study done on vernolides A, a sesquiterpene lactone isolated from VC which was tested against NCI-661, DLD-1, KB, and Hela tumour cell lines (Kuo *et al*., 2003).

3.7 Miscellaneous activities

Stovetop oil infusion of VC (whole plant) or Sirangak oil was reported for the wound healing treatment in mice whereby the infused oil was applied near the wound area at a 15 cm radius. A higher percentage of wound recovery was observed in mice treated with Sirangak oil 85.6% as compared to 71.6% in control mice (Fadillah and Santoso, 2019) A previous study was reported on the antidiuretic activity of water and methanol extracts of VC's leaves. Though, its chloroform extract was observed to have diuretic activity in albino rats (Adeboye *et al*., 1997). Meanwhile, a combination of hexane extract of *Rhinacanthus nasutus* and ethyl acetate extract of VC (aerial) at a 2:1 ratio was reported to produce synergistic toxicity against *Aedes aegypti* mosquito larvae (Duangkaew *et al*., 2018). Antimalarial activity of VC (whole plant) was also reported on its crude extracts (Soma et al. 2017) as well as its isolated compound (sesquiterpene lactones) (Chea *et al*., 2006) against chloroquine-resistant *Plasmodium falciparum* strain.

4. Extraction and phytochemicals analysis of *Vernonia cinerea*

Extraction is an important process in the recovery of phytochemicals from plant matrix. It can be done either through conventional or unconventional methods. The most common extraction methods are Soxhlet extraction, supercritical fluid extraction (SFE), microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) (Alara *et al*., 2019). Based on previous reports, phytochemical screening of VC extracts has been done through several methods of extraction. A different method of extractions may give a different type of phytochemicals with varying amounts of yield. The extraction yield and biological activity of the resulting extract are not only affected by the extraction technique but also by the extraction solvents. Many solvents, including methanol, ethanol, acetone, and water, have been used for extracting phytochemicals from plant materials. Due to the wide range of phytochemicals content and differences in their solubility properties in different polarities of solvents, the optimal solvent for extraction depends on the particular plant materials as well as the targeted compounds (Truong *et al*., 2019).

One herb may contain within it, hundreds if not thousands of phytochemicals of different polarities. The phytochemicals could be the primary or secondary metabolites serving the overall metabolic mechanisms of the plant. The processing of herbs plays a vital role in elucidating the suitable phytochemicals for specific usage. The common practices of scientific communities use a variety of solvents to extract phytochemicals from herbs. Nonetheless, the greenest solvent and processing methods in the phytochemical extractions were prioritized especially for oral consumption (Awang *et al.*, 2016). In this section, various processing methods and solvents utilised in the previous studies of VC are reviewed and presented in Table 2. Based on the previous findings on the phytochemicals screening of VC, the presence of alkaloids, terpenoids, fatty acids, sesquiterpenes, terpenoids, flavonoids, esters, diterpenes, fatty acid ester, phenolics, steroids, glycosides, saponins, and linoleic acid were reported. The identification of phytochemicals in the VC was previously done using Gas Chromatography-Mass Spectrometry (GC-MS), High-Performance Liquid Chromatography (HPLC), High-Performance Thin Layer Chromatography (HPTLC), Liquid Chromatography Mass Spectrometer (LC-MS), Thin Layer Chromatography (TLC), Nuclear Magnetic Resonance (NMR) and Fourier Transform Infrared (FTIR) spectroscopy.

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Table 2. Phytochemicals analysis of *V. cinerea.*

Part plant	Solvent extraction	Phytochemicals	Compound	Method of detection	References
Whole plants	Methanol	Alkaloid	-	Mayer's test, Dragendroff's test and Wagner's test	Vijayakumar and Gangaprasad (2019)
		Terpenoid	-	Salkowski test	
		Flavanoid	-	Dilute with ammonia solution and conc. H_2SO_4	
		Alkoloid, Anthraquinone, courmarin, tannin, glycoside, xanthoprotein, sugar	-	-	Prabha (2015)
	Ethanol	Triterpenoid	β-amyrin, taraxasterol, lupeol, betulin	HPTLC	Thongkhao *et al*. (2020)
	Aqueous Ethanol	Flavonoids	Dihydrokaempferol-5-O-β-D-glucopyranoside, Quercetin-3-O-β-D-xylopyranosyl (1-2)-β-D-glucopyranoside, kaempferol-3,7-diglucoside, Quercetin-7-O-rutinoside, kaempferol, Flavone,5,7-dihydroxy-4'-O-α-D -glucoside	LC-Q-TOF.MS	Alara *et al*. (2018)
		Saponins	Phytolaccoside, curcligo saponin I, Mubenoside A, Soyasaponin βg, 3-O-α-L-Rhamnopyranosyl-(1-2)-α-L-arabinopyranosylgypsogenin, (25R)-Ruscogenin-1-O-β-D-xylopyranosyl (1-3)-β-D-fucopyranoside, clinopodiside F		
	Methanol and Dichloromethane	Sesquiterpenes lactone	8α-tigloyloxy-hirsutinolide-13-O-acetate	HPLC	Khay *et al*. (2012)
Aerial	Methanol	Steroids, Glycosides, triterpinoids, ester	-	-	Prabha (2015)
Leaves	Methanol (Soxhlet method)	Sesquiterpenes	caryophyllene, α-guaiene, α-humulene, valencene, α-bulnesene	GC-MS	Rajamurugan *et al*. (2011)
		Diterpene	Phytol		
		Triterpene	Squalene		
		Fatty acid			
		Fatty Acid Ester	Hexadecanoic acid,ethyl ester		
			9,12,15-Octadecatrienoic acid, methyl ester,(Z,Z,Z)-		
			8,11,14-Eicosatrienoic acid,(Z,Z,Z)-		
			11-Eicosenoic acid, methyl ester		
			13-Docosenoic acid, methyl ester		
			15-Tetracosenoic acid, methyl ester		

Table 2 (Cont.). Phytochemicals analysis of *V. cinerea*.

Part plant	Solvent extraction	Phytochemicals	Compound	Method of detection	References
Leaves	Methanol (Soxhlet method)	Phenolics	Gallic acid	HPTLC	Rajamurugan *et al*. (2011)
			Rutin		
			Quercetin		
			Caffeic acid		
			Ferulic acid		
	Distilled water (Microwave-assisted hydrodistillation and Soxhlet method)	Fatty acid ester	Hexadecanoic acid,ethyl ester	GC-MS	Alara and Abdurahman (2019)
			8,11,14-Eicosatrienoic acid,(Z,Z,Z)-		
			11-Eicosenoic acid, methyl ester		
			13-Docosenoic acid, methyl ester		
			15-Tetracosenoic acid, methyl ester		
		Linolenic acid	9,12,15-Octadecatrienoic acid,(Z,Z,Z)-		
			9,12-Octadecadienoic acid methyl ester		
		Palmitic acid	n-Hexadecanoic acid		
		Plasticizer	1,2-Benzenedicarboxylic acid diisooctyl ester		
		Phenolics	Phenol-2,4-bis(1-phenylethyl)		
		Triterpene	Squalene		
		Terpene Alcohol	3,7,11,15-Tetramethyl-2-hexadecen-1-ol		
		Sesquiterpene	caryophyllene, α-guaiene, , α-bulnesene		
		Aromatic	Benzene,1,4-dichloro-		
		Hydrocarbon	Azulene, 1,4-dimethyl-7-(1-methylethyl)-		
	Ethanol	Phenolic acids/phenolic aldehyde/flavonoids	Caffeoylquinic acid derivatives	TPC/TFC/LC-MS-QTOF/ FTIR	Alara, Abdurahman, Ukaegbu and Azhari (2018)
			2'-Hydroxy-3',4'-dimethoxy-isoflavan-7-O-β-D-glucoside		
			β-Glucogallin		
			Apocynin		
			Dihydroresveratrol		
			Kukoamine A		
		Phenolic acids	1-galloyl-β-D-glucose	TPC/TFC/LC-MS-QTOF/ FTIR	Alara, Abdurahman and Ukaegbu (2018)
			3,5-O-dicaffeoylquinic acid		
			4-hydroxyacetophenone		
			Kukoamine A		
			6-gingerol		
			caesalpins P		
			kakuol		

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Table 2 (Cont.). Phytochemicals analysis of *V. cinerea.*

Part plant	Solvent extraction	Phytochemicals	Compound	Method of detection	References
Leaves	Ethanol	Flavonoids	Quercetin-7-O-rutinoside	TPC/TFC/LC-MS-QTOF/ FTIR	Alara, Abdurahman and Ukaegbu (2018)
			Kaempferol		
	Chloroform, ethyl acetate and methanol	Alkaloids, Carbohydrates and glycosides, glycosided, saponins, proteins and amino acids, phenolic compounds	-	TLC	Dhanalakshmi *et al.* (2013)
	Aqueous, Ethanol, Acetone and Ethyl Acetate	Favonoid, Saponin, Steroid, Phenolic, Terpenoids Tannis	-	TLC	Ramaswamy and Mani (2016)
Stem bark and leaves	Methanol	Triterpenoids, glycosides, steroids, ester	Lupeol, 12-oleanen-3-ol-3β-acetate, Stigmasterol, β-sitosterol	NMR	Haque *et al.* (2012)
Roots	Hydroalcoholic	Flavonoids	Quercetin	HPLC	Acharya *et al.* (2019)
	Essential oil	-	α-Muurolene, β-Caryophyllene, α-Seline,Cyperene, α-Gurjunene, β-Elemene, 4,5-di-epi-Aristolochene, Caryophyllene oxide, γ-Himachalene, α-Bulnesene, β-Selinene, β-Humulene, α-Copaene, 7-epi-α-Selinene, Selin-11-en-4-α-ol, Cyclosativene, Valerianol,5-Cedranone, Globulol, Seline-3,7-11-diene, Seychellene,β-Chamigrene, Sesquirtepenes hydrocarbons, Oxygenated sesquiterpenes	GC-MS	Joshi (2015)
	Essential oil	-	trans-β-bergamotene, cyperene, germacrene A, β-pinene and β-elemene,	NMR	Boué *et al.* (2019)
Flower	Essential oil	-	trans-β-bergamotene, γ-humulene, β-pinene, (E)-β-farnesene and (E)-β-caryophyllene	NMR	Boué *et al.* (2019)

5. *Vernonia cinerea* processing for therapeutic application

In this section, the processing is outlined to suit VC's final therapeutic application both orally and topically. The processing of herbs for therapeutic applications revolves around the usage of fresh or dried herbs, water, and/or ethanolic extracts as well as newer technology such as supercritical extraction using CO_2 with or without the addition of food-grade ethanol (Fadhlina *et al*., 2020). The potential active ingredients for products may come in the form of crude extracts, active fractions, or pure compounds. Based on the data reviewed in this paper, most of the active ingredients of VC were obtained from the water or methanol/ethanol extracts. In view of processing specific therapeutic compounds for specific applications, one could utilise a suitable processing method following the protocols presented in this paper. However, intensive downstream scale-up studies are needed for the optimisation process to obtain optimum yield. It is also important to apply green technology in phytochemical processing. The following processing methods could be referred to produce active ingredients in the form of crude extract from VC.

5.1 Water Extraction

The water extraction of VC was previously done using a microwave oven, whereby the VC's powder was immersed in the boiled distilled water (150 mL). The water extract was then filtered prior to the lyophilisation process (Khay *et al*., 2012).

5.2 Ethanol Extraction

The ethanol extraction of VC (whole plants) was done by soaking the plant powder (20 g) in ethanol for 48 hours at room temperature. The mixture was centrifuged (10,000 rpm, 25°C) for 10 min to collect the supernatant for further analysis (Thongkhao *et al*., 2020).

5.3 Spray-drying and freeze-drying

The spray-drying and freeze-drying methods had been used recently for the processing of VC, however, the method was not discussed in detail. The processing was done at Chiang Mai University, Thailand (Kanthain *et al*., 2020).

5.4 Oil infusion

The whole plant of VC was used for the oil infusion method. VC was cleaned prior to the process and then fried in pre-heated oil until it turned a dark colour. The infused oil was then ready to use and can be applied around wound areas (Fadillah and Santoso, 2019).

5.5 Supercritical fluid extraction

In a previous study, a range of temperature (25°C-60°C at 5°C intervals) and pressure (100, 200, 300 and 350 bar) was tested for the extraction of VC. The flow rate of CO_2 and ethanol (10%) was kept constant at 3 L/min and 2 L/min, respectively. The best extraction parameters were reported at 60°C and 350 bar (Muhammad Niza and Rafaie, 2017).

6. Toxicity studies

An acute oral toxicity study on mice (10 males and 10 females) and a brine shrimp test were conducted to evaluate the toxicity of VC's (whole plant) methanol extract. The findings of both studies revealed no toxicity effect observed at the maximum recommended dose for LD50 (Latha *et al*., 2010). A similar observation was reported in an acute oral toxicity study of VC's (leave) methanol extract on Wistar albino rats (6 males and 6 females) treated at 2000 mg/kg. The study reported that the biochemical and histopathological parameters of the treated group were comparable to the control group (Rajamurugan *et al*., 2011). Besides, another acute toxicity study also showed no toxicity observed in rats after 48 hours treated with VC's methanol extract even at the highest dose, 3500 mg/kg (Haque *et al*., 2013).

7. Formulation of *Vernonia cinerea*-based products

Natural remedies that are easily accessible to people, especially in rural areas are needed. VC is one of these natural remedies and its efficacy has been traditionally and scientifically established. The raw materials of VC are easily accessible as it is wild and ubiquitous with minimal maintenance. *Vernonia cinerea*-based products are currently introduced in the form of tablets, candies, and polyherbal formulations (Table 3). These products are mostly produced in India as well as Thailand. They are formulated for multiple health-related problems with some of them proven scientifically for their therapeutic claims such as anti-smoking aid, anti-malaria, and anti-microbial activities. Thus, the commercialisation of VC-based products should be expanded in the future considering their clinically proven therapeutic effects, especially as an anti-smoking aid.

8. Conclusion

VC is a fast-growing weed that resides well on the garden soil including the house compound, along the roadside, and in the farming area of tropical countries. This allows easy access to the plant for homemade first aid as well as nutraceutical uses. The common folk remedy preparation is mainly using fresh, dried, water extracts, alcohol and oil infusion for oral/topical

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Table 3. Current VC-based products.

Product Name	Formulation	Therapeutic uses/ Scientific data	References
KSG tablet Origin: India	Aqueous extract	Characterization of bioactive compounds (anthocyanin, phenolic acids, flavonoids, and phenolic glycosides).	Sulaiman *et al*. (2021)
Anti-smoking aid	Jelly candies and cookies	In a quasi-experimental, patients taking VC jelly candies and cookies showed a better smoking cessation rate than those in the control group.	Chaikoolvatana, Ayuthaya, Suthipinittharm *et al*. (2017); Chaikoolvatana, Thanawirun, Chaikoolvatana *et al*. (2017)
Hard candy/ lozenge	A mixture of water extract of VC, glucose syrup, and refined glucose.	The spray-dried extract showed higher scavenging activity than the freeze-dried extract and was chosen for pilot hard candy production. Nicotine and other compounds were detected in the extracts.	Kanthain *et al*. (2020)
Dashapushpam	Ten flowering herbs including VC	Anti-microbial, anti-malaria activity and a polyherbal oil formulated for healthy skin and hair.	Vijayan *et al*. (2010); Varghese *et al*. (2020)
Herbal Mix Tea for weight loss	A raw course of herbs: VC, rose, stevia, yerba mate, navy bean.	Weight loss and energy booster.	Amazon.com (2020)
Sahadevi Taila - New Bhuvanendra Ayurvedic	VC, false daisy, licorice	An Ayurvedic hair tonic that is formulated with herbs for healthy hair.	AyurCentralonline.com (2019)
Sahadevi Taila	VC, Coconut oil, milk, water hyssop, false daisy, liquorice, red sandalwood, white sandalwood, spikenard, Vetiver grass, spiked ginger lily, moonseed, Hribera, and camphor.	A polyherbal oil formulated for healthy hair.	Indiamart.com (2021)

applications. Combining traditional practices and scientific methods, several processing steps are suggested using either fresh or dried herbs as follows: The soft aerial part of fresh CV can be blanched in the boiling water (5 mins), tossed and eaten as 'ulam' (salad). It also can be fried with sautéed onion and garlic or cooked as one of the soup's ingredients. Meanwhile, the fresh herb (aerial/whole part) can be processed as dried herbs by drying and then cutting into smaller pieces (4-10 mm: tea size) for preparation of tea or in the form of fine powder (>80 mesh) for capsuling. The tea-size dried VC can be steeped in boiling water at a ratio of 1:100 and is best to drink with the addition of one teaspoon of tea. Besides, tincture and oil infusion can be prepared using fresh or tea-size dried VC. It can be soaked in alcohol at a ratio of 1:10 for a few weeks and used accordingly. As for the oil infusion preparation, fresh or dried herbs can be cooked in cooking oil using low heat for 1 hr and ready to be used upon cooling.

Based on the reviewed studies, VC exhibited various bioactivities with varying types of solvent extracts from different plant parts. The polar extract of VC was reported to be the best extract for its bioactivities such as anti-smoking aid, antidiuretic, antidiabetic, anti-inflammatory, antimicrobial, ameliorative, and antioxidant activities, whereby the whole plant of VC was the most studied plant part as compared to the individual plant part. Thus, water extraction could be the best approach for the development of VC-based products. Water extract has been scientifically proven to exhibit numerous bioactivities which can be due to the presence of its bioactive compounds. In addition, the use of a green solvent such as water for the extraction is practical, cost-effective, environmentally friendly, and safe for humans. In terms of drug safety, VC was reported to be safe in acute toxicity studies on rats and mice. Hence, *V. cinerea* or VC could be a great candidate for developing nutraceutical products without adverse effects on consumers.

Conflict of interests

The authors have no relevant conflict of interest to disclose.

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