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Intra-specific variation of bioactive principles in select members of the genus *Clerodendrum* L.

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ABSTRACT

To explore the phytoconstituents present in the leaves of various species of the genus Clerodendrum. Aqueous, petroleum ether, chloroform, ethanol and acetone extracts were prepared by adding 50 g of dried leaf powder with 200 ml of these solvents, the constituents were shaken at room temperature for 24 hours. After incubation, the extracts were filtered using Whatman No.1 filter paper, collected and stored at 4^0C until further use. Preliminary phytochemcial screening was performed using the standard methods given by Harborne. The various leaf extract of the genus Clerodendrum showed the presence of phytochemicals such as alkaloids, carbohydrates, coumarins, glycosides, flavonoids, phytosterols, proteins, quinones, saponins, steroids, tannins and terpenoids. The solvents used for extraction may be responsible for the presence or absence of the phytoconstituents. The findings of the present study recommended that the leaves of the genus Clerodendrum have potential bioactive agents that may be of use for developing plant based drugs.

Keywords: *Clerodendrum*, Leaf extract, Phytochemistry, Verbenaceae.

INTRODUCTION

The genus *Clerodendrum* L. (Family – Verbenaceae) with about 500 species, is widely distributed in tropical and subtropical areas of the world and being used in various indigenous system of medicine for the treatment of life threatening diseases such as syphilis, typhoid, cancer, jaundice and hypertension [1]. Apart from the medicinal uses, some species of the genus are ornamental and cultivated for aesthetic purposes. To validate the ethnomedicinal claims associated with the genus, it is essential to elucidate the active principles adhered with them, since plants possesses a wide array of chemical compounds that produce a definite physiologic action on human body.

A straggling shrub, *Clerodendrum inerme* Gaertn. locally known as 'Chanku Kuppi' in Tamil, one of the major ingredient for the preparation of medicinal oil "Kayathirumeni" is widely used as pain reliever and to heal external wounds among the inhabitants of Kanyakumari district. The various parts of the plant are used in the treatment of skin diseases, venereal infections, elephantiasis, asthma, tropical burns and for rheumatism [2-4].

Clerodendrum infortunatum Linn. (= *C. viscosum* Vent.) locally known as 'Peruvilai', is distributed throughout the Western Ghats. The leaves of this species are bitter, tonic, vermifuge, laxative and cholagogue. It is also used in Indian folk medicine for the treatment of bronchitis, asthma, fever, disease of blood, certain skin diseases, inflammation, burning sensation and epilepsy [1]. The plant is used in folk medicine and Homeopathy in case of ailments like diarrhea, skin disorders, venereal and scrofulous complaints, wounds, post-natal complications, as a vermifuge, laxative and cholagogue, for the removal of ascarids in anus, and as external applications on tumours, etc. [5]. In addition to that, the leaves of this species along with the leaves of *Adhathoda vasica*, *Anacardium occidentale*, *Calycopteris floribunda*, *Careya arborea*, *Ficus benghalensis*, *F. gibbosa*, *F. glomerata*, *F. religiosa*,

Musa paradisica, *Piper nigrum*, *Psidium guajava*, *Quassia indica*, *Tamarindus indica* and *Vitex negundo* are boiled in water and used for bathing after delivery to rejuvenate the body [6].

Clerodendrum paniculatum Linn. is commonly known as Pagoda flower. The plant is used therapeutically in Indian, Chinese, Thai, Korean and Japanese systems of medicine for the treatment of various life threatening diseases such as HIV, syphilis, typhoid, cancer, jaundice and hypertension [7].

Clerodendrum philippinum Schauer (= *C. fragrans* (Vent.) Willd.) is an ornamental plant, commonly known as Chinese Glory Bower and locally called 'Scent Malli'. Traditionally the roots and leaf extracts of the species have been used for the treatment of rheumatism, asthma and other inflammatory diseases [7-13].

Clerodendrum phlomidis Linn.f. (= *C. multiflorum* (Burm.f.) locally known as 'Vathamudakki' a constituent of more than 50 indigenous medicinal formulations is used in the treatment of inflammation, diabetes, nervous disorders, asthma, rheumatism, digestive disorders and urinary disorders and also a bitter tonic. Non-clinical investigations have revealed anti-inflammatory, hypoglycemic, immune modulatory, antidiarrhoeal and antiplasmodial properties [14]. A decoction of *C. phlomidis* leaves is used along with other plants for inflammation and is effective in treating bronchitis, headache, weakness, drowsiness and digestive problems [15,16]. In Haryana, the rural population uses the leaf paste on breast to increase lactation among nursing mothers [17].

Clerodendrum thomsoniae Balf. commonly called as 'bleeding heart-vine'is grown as an ornamental plant for its decorative two coloured flowers. *Khamti* tribe living in Lohit district of eastern Arunachal Pradesh make a paste from the powdered leaves of this species with the powdered root bark of *Sterculia villosa* to relieve pain and inflammation caused by sudden prickling of thorn or metallic objects that occur between the finger tip and nail [18]. In *Akha's* traditional medicine of China and Thailand, the plant is used to treat urethral stones [19]. It is also prevalently used as herbal home medicine among Jamaicans [20].

Perusal of the literature proved that the various species of the genus *Clerodendrum* have been extensively used in indigenous medicinal system to treat various ailments. To prove the ethnobotanical claim of the traditional usage, the present study was conducted to evaluate the phytochemicals present in the select species of the genus *Clerodendrum*.

EXPERIMENTAL SECTION

The fresh healthy and disease free leaves of *Clerodendrum inerme*, *C. infortunatum*, *C. paniculatum*, *C. philippinum*, and *C. phlomidis*, were collected from Nagercoil and its neighbourhood of Kanyakumari district, Tamilnadu, India and authenticated by a plant taxonomist of the Department of Botany, Scott Christian College, Nagercoil by using the *Flora of Scott Christian College Campus* [21]. The collected specimens were shade dried and ground into fine powder with the help of mortor and pestle. For extraction of phytochemicals, 50 gm of leaf powder was taken and mixed with 200 ml of water, petroleum ether, chloroform, ethanol and acetone in a conical flask and shaken at room temperature for 24 hours. After incubation the extracts were filtered using Whatman No.1 filter paper and subjected to phytochemical analysis using the standard methods given by Harborne [22].

RESULTS

Phytochemical screening was carried out in various solvent extracts of the leaves of *Clerodendrum inerme*, *C. infortunatum*, *C. paniculatum*, *C. philippinum*, *C. phlomidis* and *C. thomsoniae* and the results are presented in Table 1. Alkaloids and quinones were noticed in all the solvent extracts of *Clerodendrum inerme*, whereas alkaloids, carbohydrates, flavonoids, glycosides, phytosterols, quinones, saponins, steroids and terpenoids are present in chloroform extract. Tannins are present in aqueous, petroleum ether and ethanol extracts. Proteins are present in aqueous and acetone extracts. Coumarins are present in aqueous and petroleum ether extracts. Terpenoids are present in aqueous and chloroform extracts. Flavonoids and steroids were absent in ethanol and acetone extracts.

Crude leaf extracts of *C. infortunatum* showed the presence of alkaloids in all the extracts. Glycosides and saponins are present in petroleum ether and chloroform extracts; terpenoids in aqueous, chloroform, ethanol and acetone extracts; steroids in petroleum ether, chloroform and acetone extracts; proteins in aqueous and chloroform extracts. Flavonoids were present only in aqueous extract; flavonoids and tannins are absent in chloroform extracts whereas, coumarins were found to be absent in all the extracts.

Flavonoids were found to be present in all the extracts of *C. paniculatum*. Glycosides are present in aqueous, petroleum ether and chloroform extracts; tannins in chloroform, ethanol and acetone extracts; steroids in petroleum ether, chloroform and ethanol extracts and phytosterols in aqueous, petroleum ether and chloroform extracts. Coumarins and quinones are present only in petroleum ether, chloroform and ethanol extracts whereas, carbohydrates were found only in the chloroform extract. Terpenoids and proteins are absent in aqueous and acetone extracts.

Alkaloids were noticed in the leaf extracts of *C. philippinum* and phytosterols were found in all the extracts. Tannins and proteins are absent in petroleum ether and aqueous extracts. Carbohydrates, phytosterols and saponins are present in chloroform and aqueous extracts. Flavonoids are present in aqueous, petroleum ether and ethanol extracts. Quinones are present in chloroform, ethanol and acetone extracts. Terpenoids and steroids are present only in chloroform extract and saponins are present only in aqueous extract.

Clerodendrum phlomidis showed the presence of alkaloids, tannins, steroids in acetone, petroleum ether and aqueous extracts. *Clerodendrum phlomidis* showed the presence of alkaloids, tannins and steroids in acetone, petroleum ether and aqueous extracts. Glycosides are present in petroleum ether, chloroform and ethanol extracts. Flavonoids are present in aqueous, petroleum ether and ethanol extracts. Proteins are present in petroleum ether and chloroform extracts. Carbohydrates and phytosterols are present only in chloroform extract; saponins are present only in aqueous extract, whereas coumarins and quinones are totally absent in all the extracts.

Leaf extracts of *C. thomsoniae* showed the presence of saponins, steroids and terpenoids in all the extracts; phenols are present in aqueous, chloroform, ethanol and acetone extracts; quinones are present in aqueous, petroleum ether and acetone extracts; carbohydrates are present in chloroform and ethanol extracts; glycosides, flavonoids and alkaloids were present only in aqueous extracts; proteins, coumarins and phytosterols are absent in all the extracts.

DISCUSSION

Plants are the repositories of phytochemical constituents and components of phytomedicine [23-27]. The secondary metabolites can be derived from any part of plants such as leaves, flowers, roots, bark, fruits, seeds etc [28-32]. In the present study phytochemical screening was performed using various organic solvent extracts of the leaves of *Clerodendrum* species.

The leaf extracts of *Clerodendrum inerme* showed the presence of carbohydrates, proteins, tannins and phenolic compounds, glycosides, alkaloids, steroids and flavonoids. Similar studies conducted by Garima *et al*. [33] on *C. inerme* leaves showed the presence of phytoconstitutents such as alkaloids, steroids, carbohydrates, glycosides saponins and flavonoids. A sterol, 4α-Methyl-24β-ethyl-5α-cholesta-14,25-dien-3 β-ol [34], megastigmane glycosides (Sammongaosides A and B), aliphatic glycoside pentadecanoic acid-β-D-glucoside [35] and three neo-clerodane diterpenoids, inermes A, B and 14,15-dihydro-15β-methoxy-3-epicaryoptin, have been isolated from the hexane extract of the leaves in addition to an epimeric mixture of 14,15-dihydro-15-hydroxy-3-epicaryoptin were isolated from this plant [36]. The plant *C. inerme* have been documented pharmacologically and clinically which are endowed in phytochemicals with marked activity on human pathogenic bacteria [37]. Gurudeeban *et al*. [38] proved that antioxidant activity and radical scavenging ability may be due to the presence of flavonoids and phenolic compounds which is present in the leaf extracts of *C. inerme*. The ethanolic extract of the leaves showed significant hepatoprotective active against CCl_4 induced liver damage in Swiss albino rats [39].

The phytochemical constituents such as alkaloids, flavonoids, steroids and terpenoids are believed to be responsible for the therapeutic properties [40-52]. *Clerodendrum infortunatum*, alkaloids are present in all the extracts. Shareef *et al*. [53] studied the aerial part of the related species *C. serratum*, and found that alkaloids were present in all extracts except ethyl acetate. It has been supported by previous studies that the leaf extract of *C. infortunatum* showed activity against various life threatening microorganisms [54,55]. Roy *et al*. [56] reported antifungal activities of the plant *C. infortunatum* extract. Jirovet *et al*. [57] study the active principles in essential oil content of the leaves and bark of the plant. A diterpenoid Clerodin (CI3H1803) extracted from the plant by Barton *et al*. [58] and it is supposed to be the main active compound that may interact with some target molecules of the human system.

Table 1. Qualitative phytochemical screening of leaf extracts of *Clerodendrum* sps.

	Phytoconstituents											
	Alkaloids	Carbohydrates	Coumarins	Flavonoids	Glycosides	Phenols	Phytosterols	Proteins	Quinones	Saponins	Steroids	Terpenoids
C. inerme												
Aqueous	+	-	+	+	-	+	-	+	+	-	+	+
Pet. Ether	+	-	+	+	-	+	-	-	+	-	+	-
Chloroform	+	+	-	+	+	-	+	-	+	+	+	+
Ethanol	+	-	-	-	-	+	+	-	+	-	+	-
Acetone	+	-	-	+	-	-	-	+	+	-	-	-
C. infortunatum												
Aqueous	+	-	-	+	-	+	-	+	+	-	-	+
Pet. Ether	+	-	-	-	+	+	-	-	-	+	+	-
Chloroform	+	+	-	-	+	-	+	+	+	+	+	+
Ethanol	+	-	-	-	-	+	-	-	+	-	-	+
Acetone	+	-	-	-	-	+	-	-	+	-	+	-
C. paniculatum												
Aqueous	-	-	-	+	+	-	+	+	-	-	-	-
Pet. Ether	+	-	+	+	+	-	+	+	-	+	+	+
Chloroform	-	+	+	+	+	+	+	+	+	+	+	+
Ethanol	+	-	-	+	-	+	-	+	+	-	+	+
Acetone	+	-	-	+	-	+	-	-	-	-	-	+
C. philippinum												
Aqueous	+	+	-	+	+	+	+	-	-	+	-	-
Pet. Ether	+	-	+	-	+	-	+	+	-	-	-	-
Chloroform	+	+	+	-	+	+	+	+	+	-	+	+
Ethanol	+	-	-	+	+	+	+	+	+	-	-	-
Acetone	+	+	-	+	-	+	+	+	+	-	-	-
C. phlomidis												
Aqueous	+	-	-	+	-	+	-	-	-	+	-	-
Pet. Ether	+	-	-	+	+	-	-	+	-	-	+	+
Chloroform	+	+	-	-	+	+	+	+	-	-	+	+
Ethanol	+	-	-	+	+	+	-	+	-	-	+	-
Acetone	+	-	-	-	-	+	-	-	-	-	+	-
C. thomsonie												
Aqueous	+	-	-	+	+	+	-	-	+	+	+	+
Pet. Ether	-	-	-	-	-	-	-	-	+	+	+	+
Chloroform	-	+	-	-	-	+	-	-	-	+	+	+
Ethanol	-	-	-	-	-	+	-	-	-	+	+	+
Acetone	-	+	-	-	-	+	-	-	+	+	+	+

Abbreviations: (-) present; (+) absent.

The leaf extracts of *C. paniculatum* showed the presence of carbohydrates, glycosides, flavonoids, phenolic compounds, saponins, terpenoids, steroids, proteins, alkaloids and phytosterols. The presence of phenolic compounds, carbohydrates, glycosides, sterols, tannins, sugars etc., were noticed and the presence of these secondary metabolites confirms that the plant can be used for pharmaceutical manufacturing and drug discovery [59]. It is also evident that the leaf extract of this plant extract possesses antioxidant [60] and antibacterial activity against *Vibrio parahaemolyticus* [61].

In *Clerodendrum philippinum*, steroids were present in all the tested extracts except aqueous extract. Previous research report denotes that steroid is the major chemical constituent present in *C. philippinum* [62,63]. The phytochemicals present in *C. philippinum* are responsible for the antioxidant property either by scavenging free radicals or by preventing their formation due to the presence of flavonoids [64]. Since the leaves possess potent phytochemcial constituents it can be used to cure various diseases and also used as therapeutic agents [13]. Kang *et al*. [9], Pathong *et al*. [10] and Choi *et al*. [11] proved that root and leaves extracts of *C. philippinum* have been used for the treatment of rheumatism, asthma and inflammatory diseases. It is also used for the treatment of fever, jaundice, typhoid and syphilis [65].

In *Clerodendrum phlomidis*, steroids and alkaloids were detected in all extracts except aqueous and acetone. Similar studies were conducted by Agilandeswari [66] on the leaves of *C. phlomidis*. Phenolics, glycosides, proteins, flavonoids and terpenoids were detected in one or two extracts. A sterol glucoside 24-ethyl-cholesta-5(6), 22(23), 25(26)-triene-3-O-β-D-glucopyranoside is a rare glucoside of 22-dehydroclerosterol isolated from the species showed the antihepatotoxic activity [67]. A new triterpene ester, together with tetratriacontanol and 24β-ethylcholesta-5,22*E*,25-triene-3β-ol, has been isolated from this plant [68]. The phytochemical compound pectolinaringenin isolated from *C. phlomidis* showed the larvicidal activity against the early fourth-instar larvae of the filarial vector *Culex quinquefasciatus* and dengue vector *Aedes aegypti* [69]. Ethnopharmacological studies of *C. phlomidis* displays considerable potency in anti-inflammatory action and prominent antiarthritic effect on adjuvant induced arthritis [70]. Apart from tremendous medicinal uses to treat human pathogenic infections, it is also used to treat plant fungal pathogens [37].

CONCLUSION

The species of the genus *Clerodendrum* are commonly found in Kanyakumari district is widely used in traditional system of medicine to cure various ailments. The presence of wide range of phytochemical constituents indicates that the species could serve as pilot for the development of novel antimicrobial agents. Further studies regarding isolation and purification of bioactive principles of the genus *Clerodendrum* to be done.

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