Indian Journal of Traditional Knowledge
Vol 20(2), April 2021, pp 329-334

Study of phytochemical content, antioxidant and larvicidal property of different solvent extracts of *Clerodendrum infortunatum* and *Citrus grandis*

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Received 16 October 2019; revised 10 October 2020

The present study was carried out to investigate the phytochemical, antioxidant and larvicidal property of different solvent extracts of leaves of *Clerodendrum infortunatum* and fruit peel of *Citrus grandis*. The antioxidant property was studied by ferric reducing antioxidant power (FRAP), total antioxidant capacity (TAC), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) and thiobarbituric acid reactive species (TBARS) assays. The total phenolic and flavonoid contents of the extracts were estimated following standard protocols. Larvicidal activity of the plant extracts were evaluated following standard WHO protocol. In a series of test doses (100 to 2000 µg/mL), 20 numbers of *Aedes aegypti* larvae were exposed and the mortality was recorded after 24 h and LC_{50} were calculated. The study showed that the *C. grandis* extracts have better phytochemical, antioxidant and larvicidal activity compared to *C. infortunatum*. Among the four solvent fractions, diethyl ether extract showed higher activity in both the plants. The present study thus showed potential larvicidal property of the plants against *A. aegypti*. However, further characterization and identification of active compound (s) need to be carried out to study the exact mode of action.

Keywords: Antioxidant, *Aedes aegypti*, *Clerodendrum infortunatum*, *Citrus grandis*, Larvicidal activity
IPC Code: Int Cl.[21]: A61K 9/00, A61K 8/97, A61K 36/752, A01H 5/10

Mosquitoes are one of the most important insects that are involved in the transmission of many diseases. Today, there are about 3500 known species of mosquitoes globally with highest density in tropical and sub-tropical countries[1]. Major vector-borne diseases (VBD) such as malaria, dengue, chikungunya, yellow fever, etc. are transmitted from one infected person to the other by mosquitoes[2,3]. Along with malaria and others vector-borne diseases, dengue is one of the major VBDs causing huge economy losses. According to WHO, dengue causes an estimate of about 390 million infections every year worldwide out of which 2.5% of the people die[4]. *Aedes aegypti* (L.) belonging to the Family *Culicidae* is a vector for the transmission of dengue fever which is endemic to many countries including Asia, Africa and America[2]. Over the last few years, there is an increasing trend of dengue cases in India spreading the length and breadth of the country because of drastic climatic changes, urbanization, inadequate vector control measures, mass migration and most importantly the insecticide resistance capacity developed by *Aedes* mosquitoes. The use of commercial insecticides such as organochlorines, organophosphates, pyrethroids, or carbamates has been the most common mosquito control strategy since long time. However, repeated exposure to same insecticides has developed insecticide resistance capacity in many mosquito populations[5]. Moreover, the use of commercial insecticides has several side-effects and imposes serious threat not only to the human health but also to the ecosystem. Like many other mosquito vectors, the development of insecticide resistance has also been reported by many researchers in *A. aegypti* mosquitoes[1,3].

Plants have been used as medicines since ancient times in many parts of the world. Because of its rich bioactive compounds and lesser side-effects there is a growing attention for plant-derived medicines throughout the world. Many researchers have showed potential larvicidal activities of several plant extracts and isolated complounds[6,7]. North East India is one of the biodiversity hotspots of the world with rich in flora and fauna. The use of medicinal plants for curing common health problems has been the tradition of many ethnic groups of NE India. The

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pharmacological property of several medicinal plants has been investigated by many researchers in this part of India including their insecticidal activity and insect repellent property[8-10]. *Clerodendrum infortunatum* and *Citrus grandis* are two of the most commonly known mosquito repellent plants used by the tribal communities of Kokrajhar district of Assam. As a traditional practice, the leaves of *C. infortunatum* and fruit peel of *C. grandis* are used to repel mosquitoes and flies from edible items.

C. infortunatum (L.) (Family Lamiaceae) is a wild flowering shrub distributed mostly in the tropical countries. Various parts of this plant are used to cure health complications such as colic, scorpion sting and snake bite, tumours, skin-disorders, smallpox, etc. In Indian traditional medicine, *C. infortunatum* is used for the treatment of bronchitis, asthma, fever, helminthiasis, blood diseases, inflammation, burning sensation, epilepsy, and many others[11,12]. Several bioactive compounds such as 6-Oxa-bicyclo3,1,0-hexan-3-one, 2-Methoxy-4-vinylphenol, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, etc. have been reported from different parts of *C. infortunatum*[13]. *C. grandis* (L.) Osbeck (Family Rutaceae) is a profusely branched small tree with spines on the branchlets, old limbs and trunk. Flowers are fragrant, borne singly or in cluster; fruit shape ranges from nearly round to pear-shaped. The peels are clinging, more or less easily removable, greenish-yellow, or pale yellow colour[14]. *C. grandis* is known to possess several medicinal properties against many diseases such as nervous problem, hemorrhagic, cough, diabetic, cancer, liver problem, epilepsy etc[15]. Several bioactive compounds, phenolics, flavonoids and essential oils have been reported and isolated from *C. grandis*[15].

Materials and Methods

Collection, identification and preparation of crude extracts of plants

Clerodendrum infortunatum (L.) and *Citrus grandis* (L.) Osbeck were collected from Kokrajhar area and sample plants were submitted to Department of Botany, Bodoland University for identification. The identification numbers were BUBH2018047 and BUBH2018064 for *C. infortunatum* and *C. grandis*, respectively. Fresh samples of *C. infortunatum* (leaves) and *C. grandis* (fruit peel) were collected and washed properly with distilled H_2O and dried completely in a hot-air oven at 45°C - 50°C. Crude plant extracts were prepared in four different solvents – n-hexane, diethyl ether, ethyl acetate and methanol as described in our earlier publication and the crude extract obtained was kept at -20°C in an air-tight container for further use[12].

Phytochemical analysis

Total Phenolic Content (TPC)

The total phenolic content was estimated following Folin-Ciocalteu reagent method[17]. The amount of phenolic content was calculated from a calibration curve of gallic acid and results expressed as µg (microgram) gallic acid equivalent (GAE)/mg plant extracts.

Total Flavonoid Content (TFC)

The method of Ordonez *et al.*[18] was used to estimate the total flavonoid content of the plant extracts. TFC was calculated from the standard curve of quercetin and the values were expressed as µg quercetin equivalent (QE)/mg of plant extracts.

Antioxidant Study

Total Antioxidant Capacity (TAC)

Phosphomolybdate method was used to estimate the total antioxidant capacity of the plant extracts using ammonium molybdate reagent[19]. The reaction mixture was incubated at 95°C for 30 min and the colour developed was read at 695 nm against a blank solution. TAC was expressed as µg ascorbic acid equivalent (AAE)/mg plant extracts.

Ferric Reducing Antioxidant Power assay (FRAP)

FRAP activity of the plant extracts was estimated following the method by Iloki-Assanga *et al.*[20]. The FRAP activity of plant extracts was compared with the standard ascorbic acid. The values were expressed as µg Fe^{2+}equivalent (FE)/mg plant extracts.

1,1-Diphenyl-2-Picrylhydrazyl Radical Scavenging (DPPH) Assay

DPPH radical scavenging activity of the plant extracts were estimated following the method as described by Mamta *et al.*[21]. DPPH inhibition was calculated as per the following formula:

$$\text{DPPH (\% inhibition)} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100 \quad \ldots (1)$$

Where,

Abs control means absorbance without plant extract or reference chemical.

Abs sample means absorbance with sample or reference chemical.

Lipid peroxidation scavenging activity (Thiobarbituric acid reactive species; TBARS assay)

The lipid peroxidation inhibitory activity of plant extracts was estimated following the modified thiobarbituric acid reactive species (TBARS) assay using egg yolk homogenates as lipid-rich media[22]. The coloration of the assay mixture was measured at 532 nm. The percentage inhibition was calculated following the formula (1).

2,2'-Azinobis-(3-Ethylbenzothiazoline-6-Sulfonate) (ABTS) Assay

The ABTS activity was measured following the protocol described by Re *et al.* using gallic acid as standard reference[23]. The percentage inhibition was calculated following the formula (1).

Larvicidal bioassay

Standard protocol of WHO was followed to study the larvicidal property of plant extracts[23]. In a series of test doses of plant extract (100 to 2000 µg/mL), 20 numbers of 3rd and 4th instar larvae of *A. aegypti* were exposed for 24 h under standard experimental conditions. Larval mortality was noted down after the treatment and lethal concentrations (LC_{50}) were calculated. All the experiments were conducted for three replicates (n=3).

Statistical analysis

Statistical calculations were carried out in Microsoft Excel, Origin Pro and SPSS software. The data were represented as mean ± standard deviation (SD) for at least 3 replicates (n = 3) for each set of experiments. The results are considered significant at $p \leq 0.05$ level.

Results

Phytochemical and antioxidant study

The TPC and TFC content of all the four solvents extracts - hexane, diethyl ether, ethyl acetate and methanol of *C. infortunatum* and *C. grandis* are presented in Figure 1. The present study observed that all the solvent contains high phenolic and flavonoid content. Both the TPC and TFC were found to be higher in *C. infortunatum* compared to *C. grandis*. TPC was found highest in methanolic extracts of both the plants, 154.54±3.89 µg/mg and 24.136±1.81 µg/mg extract for *C. infortunatum* and *C. grandis*, respectively. While lowest TPC was seen in hexane extracts of both the plants (Fig. 1a). Similarly, highest TFC was found in diethyl ether extract of *C. grandis* (80.58±5.13 µg/mg extract) and both the diethyl ether

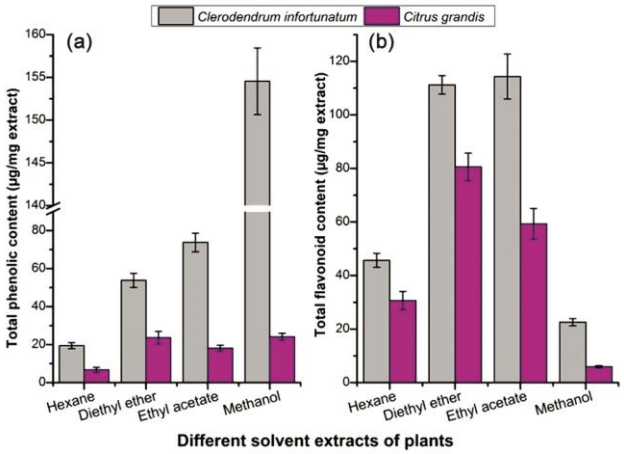

Fig. 1 — Total phenolic and flavonoid content of different solvent extracts of *Clerodendrum infortunatum* and *Citrus grandis*. Values expressed as mean ± SD, n = 3.

and ethyl acetate extract of *C. infortunatum* (111.22±3.44 µg/mg and 114.34±8.40 µg/mg extract, respectively) (Fig. 1b). Unlike TPC, lowest TFC was seen in the methanolic extract of both the plants. The TPC and TFC of *C. infortunatum* were found to be significantly different from *C. grandis* (at $p \leq 0.05$ level).

Five different antioxidant assays such as FRAP, TAC, DPPH, ABTS and TBARS were conducted to assess the antioxidant properties of the plant extracts. The antioxidant values and 50% inhibition concentrations (IC_{50}, µg/mL) of plant extracts for different antioxidant assays were presented in Table 1. In *C. infortunatum*, the ferric reducing power of plant was found to be ranged from 8.53 to 25.18 µg/mg extract. Highest FRAP activity was observed in ethyl acetate extract and lowest in hexane extract. The *C. grandis* extracts also showed almost similar result and FRAP value ranged from 4.98 to 17.59 µg/mg extract, highest in ethyl acetate extract, and lowest in methanolic extract (Table 2). The total antioxidant capacity was found to be highest in the methanolic extract of *C. infortunatum* and lowest in hexane extract. However, the methanolic extract showed lowest TAC activity in *C. grandis* and highest in diethyl ether extract. For DPPH and ABTS antioxidant assays, diethyl ether extract showed strongest activity while the hexane extract showed weakest activity in *C. infortunatum*. Similar kind of result was observed in different solvent extracts of *C. grandis*. Unlike DPPH and ABTS assays, the hexane extract of *C. infortunatum* showed strongest antioxidant property in TBARS assay (IC_{50},

Table 1 — Antioxidant profile of different solvent extracts of *Clerodendrum infortunatum* and *Citrus grandis.*

Antioxidant parameters	*Clerodendrum infortunatum*				*Citrus grandis*				Standard reference
	Hexane	*Diethyl ether*	*Ethyl acetate*	*Methanol*	*Hexane*	*Diethyl ether*	*Ethyl acetate*	*Methanol*	
FRAP (µg/mg extract)	8.53±0.61	14.59±1.06	25.18±1.08	17.43±2.05	11.59±0.57	17.59±0.20	15.88±0.91	4.98±0.10	-
TAC (µg/mg extract)	46.07±1.57	121.97±5.35	96.04±1.59	147.19±2.42	107.16±5.44	180.97±9.72	133.95±3.74	77.61±4.14	-
DPPH (IC_{50}, µg/mL)	6062.88±42.93	589.79±5.95	1897.05±13.33	1166.25±28.76	2493.81±46.81	655.97±17.38	296.17±22.86	29.74±3.63	0.39±0.11*
ABTS (IC_{50}, µg/mL)	325.71±19.49	22.26±1.02	36.19±1.58	118.43±9.31	191.32±4.53	35.41±2.26	42.61±1.02	72.09±4.28	1.27±0.11**
TBARS (IC_{50}, µg/mL)	60.81±5.93	98.34±10.87	238.72±6.81	148.77±18.38	123.03±9.78	74.88±3.27	166.61±13.06	188.32±4.53	24.33±1.15**

*gallic acid, **ascorbic acid, values are expressed as mean ± SD for three replicates (n = 3).

Table 2 — LC_{50} values of larvicidal activity of different solvent extracts of *Clerodendrum infortunatum* and *Citrus grandis.*

Solvent extracts of plants	*Clerodendrum infortunatum*	*Citrus grandis*
	LC_{50} (µg/mL) (95% Confidence limit)	LC_{50} (µg/mL) (95% Confidence limits)
Hexane	659.69 (523.71 – 952.13)	236.70 (160.29 – 321.16)
Diethyl ether	423.57 (332.04 – 544.67)	218.97 (163.57 – 280.44)
Ethyl acetate	608.29 (443.58 – 863.64)	645.56 (90.195 – 972.679)
Methanol	945.29 (739.08 – 1263.16)	356.36 (271.31 – 458.49)

60.81±5.93 µg/mL) followed by diethyl ether, methanol and ethyl acetate extracts. On the other hand, diethyl ether extract of *C. grandis* showed the strongest activity in TBARS assay (IC_{50}, 74.88±3.27 µg/mL) followed by hexane, ethyl ether and methanolic extracts. The result did not show any kind of correlation between the plants, solvent extracts and the antioxidant properties. However, both *C. infortunatum* and *C. grandis* extracts showed similar trend of ABTS radical scavenging activity with highest in diethyl ether followed by ethyl acetate, methanol and hexane extracts. The antioxidant activities, though, did not show any kind of positive correlation among the solvent extracts and their antioxidant properties, diethyl ether extract was found to have slightly better antioxidant activity in both the plants while the hexane extract showed slightly weaker antioxidant property compared to other solvent extracts of both the plants.

Larvicidal activity

The present study also tested the larvicidal efficacy of the solvent extracts of both the plants on *A. aegypti*. The concentration-dependent mortality of

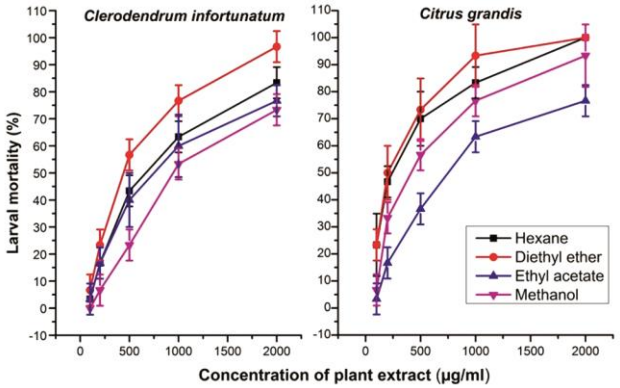

Fig. 2 — Larvicidal activity of different solvent extracts of *Clerodendrum infortunatum* and *Citrus grandis* against *Aedes aegypti* larvae. Values expressed as mean ± SD, n = 3.

A. aegypti in different solvent extracts of *C. infortunatum* and *C. grandis* is shown in Fig. 2 and the LC_{50} values in Table 2. On exposure to different concentrations of plant extracts, *A. aegypti* showed concentration-dependent mortality after 24 h of treatment. In *C. infortunatum*, diethyl ether extract showed strongest larvicidal activity with LC_{50}= 423.57 µg/mL followed by ethyl acetate (LC_{50}= 608.29 µg/mL), hexane (LC_{50}= 659.69 µg/mL) and methanol (LC_{50}= 945.29 µg/mL). Similarly, the extracts of *C. grandis* also showed almost the same result with diethyl ether extract possessing the strongest larvicidal activity with LC_{50}= 218.97 µg/mL followed by hexane (LC_{50}= 236.70 µg/mL), methanol (LC_{50}= 356.56 µg/mL) and ethyl acetate (LC_{50}= 645.56 µg/mL). However, in terms of overall larvicidal efficacy, *C. grandis* showed better larvicidal activity compared to *C. infortunatum*. The extracts of both the plants showed significant difference in term of the mortality of *A. aegypti* larvae at $p \leq 0.05$ level of significance. It is also seen that the extracts of less polar solvents possess higher

larvicidal activity against *A. aegypti*. Both the plants showed strongest larvicidal activity in diethyl ether extract of the plants.

Discussion

The search for plant-derived medicines as an alternative to the synthetic drugs is gaining attention due to the safety and desirable therapeutic potential. The rich source of phytochemicals present in the plants can be ascribed to their medicinal property. Due to its ethnomedicinal values, the phytochemical content, antioxidant and larvicidal property of *C. infortunatum* and *C. grandis* was studied. Phenolics and flavonoids are important bioactive compounds having tremendous medicinal values. Green plants, fruit, vegetables and cereals are among the richest sources of phenolics and flavonoids. Fruit peel and citrus plants are rich sources for flavonoids[25]. The present study revealed high phenolic and flavonoid content in both the plants. TFC was found to be higher than TPC in both the plants except the methanolic extract of *C. infortunatum*. Diethyl ether and ethyl acetate fraction of both the plants showed the highest flavonoid content. In accordance with our study, Abifarin *et al.* also showed higher values of TFC compared to TPC in different solvent extracts of *Cucumis africanus*[26]. Similarly, antioxidant studies showed higher activity in *C. grandis* compared to *C. infortunatum*. In both the plants, diethyl ether and ethyl acetate showed comparatively better activity. Similar to our study, Saeed *et al.* showed that the antioxidant properties of different solvent extracts (LC_{50}) of *Torilis leptophylla* ranged from 10.0±0.09 μg/mL to 395±5 μg/mL and the n-butanol extract was found to be having the strongest antioxidant activity while n-hexane extract showed lowest activity[27]. Similar kind of result has been revealed by Ohikhena *et al.* in the solvent fractions of *Phragmanthera capitata*[28]. Dhawan and Gupta also showed that out of the six solvent extracts of *Datura metel*, chloroform extract showed better antioxidant activity[29]. Similarly, other studies also showed that the plant extract from less polar solvents possessed higher antioxidant activity compared to other solvent extracts[30]. The solvent extracts of both *C. infortunatum* and *C. grandis* showed considerable larvicidal activity against *A. aegypti* larvae. Fruit peel extract of *C. grandis* showed better activity compared to *C. infortunatum*. Like antioxidant activity, the larvicidal activity was found to be the highest in diethyl ether extract of both the plants. Similarly, Johnson *et al.* evaluated the larvicidal activity of different solvent extracts of four plants and found that chloroform extracts all the plants possessed stronger larvicidal activity[31]. Hari and Mathew also reported similar kind of result showing better larvicidal activity of petroleum ether extracts of several plants against *Aedes* and *Culex* mosquito larvae[32].

Conclusion

Plants have been used in traditional medicine practices since prehistoric times. In the present study, two most commonly used mosquito repellent plants namely *C. infortunatum* and *C. grandis* collected from Kokrajhar area were investigated for their phytochemical, antioxidant and larvicidal properties. From the present study, it can be suggested that the plant extracts having stronger antioxidant properties also possess stronger larvicidal activity. It can also be said that phytochemical that are extracted in less polar solvents have better larvicidal activity against *Aedes aegypti*. However, further characterization and isolation of principle compounds of the plants need to be carried out to ascertain their larvicidal efficacy.

Acknowledgement

Authors would like to acknowledge the informants for providing the ethnomedicinal information. Our thanks also go to the village elderly people and head of the village (Gaonbura) for granting the permission for collecting the ethnobotanical data. We also acknowledge Department of Zoology, Bodoland University, for providing the necessary facilities.

Financial support

There is no specific financial grant received from any funding agencies for this work.

Conflict of interest

Authors declare no conflict of interest

Authors Contribution

AS - research design, manuscript writing, KB – experimental work, TB – experimental work, MD – literature survey and manuscript drafting, MKR – statistical calculation, manuscript drafting

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