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ORIGINAL ARTICLE

Evaluation of Antioxidant and Analgesic Activities of Three Medicinal Plants

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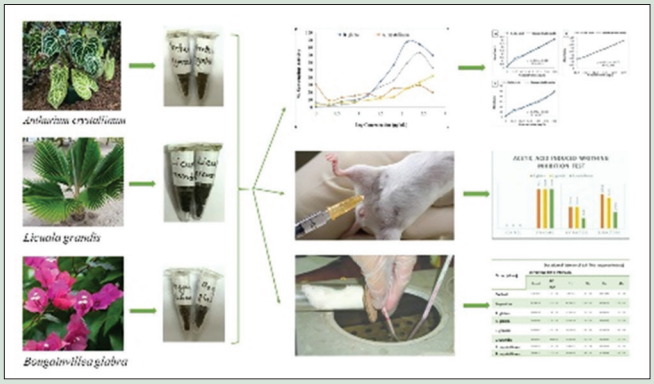
ABSTRACT

Background: Medicinal plant species with antioxidant and pro-oxidant properties contemplate in high number of flavonoids, carotenoids, and phenolic acids. This present study has been designed to evaluate antioxidant and analgesic activities of three Bangladeshi medicinal plants. **Materials and Methods:** In this study, after the phytochemical screening, antioxidant activity of the crude methanol extracts of three medicinal species naming *Bougainvillea glabra* (*Nyctaginaceae*), *Licuala grandis* (*Arecaceae*), and *Anthurium crystallinum* (*Araceae*) were evaluated using the spectrophotometric 1,1-diphenyl-2-picrylhydrazyl-free radical scavenging assay. In addition, total phenolic and total tannin contents have also been determined using standard gallic acid. Finally, analgesic activities of these plants have also been evaluated by acetic acid writhing inhibition and tail flick test in mice. **Results:** The inhibitory concentration 50% values of methanol crude extract of *B. glabra*, *L. grandis*, and *A. crystallinum* were 1.72, 2.18, and 13.21 µg/mL, whereas the standard (ascorbic acid) showed the value 3.90 µg/mL. The extracts of *B. glabra*, *L. grandis*, and *A. crystallinum* also showed the total phenolic contents as 40.97 ± 0.004, 247.272 ± 0.016, and 302.22 ± 0.011 mg gallic acid equivalent (GAE)/100 g, and the total tannin contents as 2172 ± 0.002, 435.7 ± 0.01, and 2414.98 ± 0.028 mg GAE/100 g of dried plant extract, respectively. The three-plant extracts have reduced the pain by both these methods in mice in a dose-dependent manner. **Conclusion:** In sum, it can be said that the three-plant species showed potent antioxidant and analgesic activities.

Key words: Analgesic, *Anthurium crystallinum*, antioxidant, *Bougainvillea glabra*, *Licuala grandis*

SUMMARY

- The *Licuala grandis* is a small palm tree, prospered in shaded location growing over 3 feet tall. On the other hand, *Anthurium crystallinum* is a flowering plant, native to rainforest margins and *Bougainvillea glabra* is an evergreen, climbing shrub with thorny stems that grows 9 m tall. These three plant species have some traditional uses, for instance, wound healing, analgesic etc. In the present study, we confirmed some common phytochemicals such as flavonoids, tannins and alkaloids which indicate the presence of antioxidant and analgesic activities that resembles with our findings.



Abbreviations Used: DPPH: 1,1-diphenyl-2-picrylhydrazyl, ROS: Reactive oxygen species, RNS: Reactive nitrogen species, SEM: Standard Error of Mean, IC_{50}: Inhibitory concentration 50%, GAE: Gallic acid equivalent, 5-HT3: 5-hydroxytryptamine 3, 5-HT2A: 5-hydroxytryptamine 2A.

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INTRODUCTION

In living organisms, the both reactive oxygen species and reactive nitrogen species are known to cause damage to lipids, proteins, enzymes, and nucleic acids, leading to some disorders such as neurodegenerative disease, cancer, aging, malaria, atherosclerosis, diabetes, liver injury, Alzheimer, Parkinson, and some others pathological events.[1,2] Finding antioxidant effects of different compounds derived from a number of plants drawing much more focus and interest that could be relevant in relation to their nutritional incidence and their role in health and disease.[3-7] Several studies have shown the presence of different natural antioxidative compounds in various plant species. Largely, these compounds include phenolic- or nitrogen-containing compounds and carotenoids.[8-10] Compounds with antioxidant activity act as pro-oxidants under certain conditions or in high concentrations. Prooxidant activity can accelerate oxidative damage to different cells.[11-16] Potential antioxidant should therefore be tested for pro-oxidant activity as well.

In a study, *Bougainvillea glabra* has been shown to accumulate large amounts of flavanols.[17] The antidiabetic and antilipidemic effects have also been evaluated for this plant species.[18] It should be noted that no report on antioxidant and analgesic activities were found. According to Hostettmann and Terreaux,[19] the predictable number of higher plant species in the world is of about 400,000, by the same token, secondary metabolites from different plants are categorized by a vast chemical variety, and currently one-fourth of all prescribed pharmaceuticals complexes in developed countries are directly or indirectly (semi-synthetic) derived from these plants. As plants produce a huge number of antioxidants, they can represent a source of new compounds with antioxidant activities.[20,21] Among the huge number of plant secondary metabolites, some compounds such as flavonoids indicate the presence of both antioxidant as well as analgesic activity.[22] From this point of view, the

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main goal of this research was to study the antioxidant activities through 1,1-diphenyl-2-picrylhydrazyl (DPPH)-free radical scavenging method, and determination of total phenolic and tannin contents as well as analgesic activities of crude methanol extracts from three-plant species, namely *B. glabra, Licuala grandis*, and *Anthurium crystallinum*, belonging to the family *Nyctaginaceae*, *Arecaceae*, and *Araceae*, respectively.

MATERIALS AND METHODS

Collection of plant material

The extraction and other investigation processes were carried out on the bark of *B. glabra* and leaves of *L. grandis* and *A. crystallinum*. These plant samples were collected from botanical garden, Dhaka, Bangladesh, in September 2017. The collected plant parts were then carefully handled and any sort of undesirable materials were detached from the collected parts.

Drying and grinding

The collected plant samples were washed with water and allowed to dry for few weeks. When the plant parts were suitable for grinding, they were grinded into coarse powder. Finally, the powder material was stored in a sealed container and kept in a dark, cool, and dry place until farther processing.

Cold extraction

The finely powered three-plant parts were taken in separate clean glass containers and they were soaked with methanol. Then, the container was sealed and kept for 15 days. During this time, it was allowed to intermittent stirring and shaking. The mixture was then filtered by cotton. Finally, it was filtered by Whatman filter paper.

1,1-diphenyl-2-picrylhydrazyl-free radical scavenging assay

In vitro antioxidant activity of the three plant extracts was assessed by DPPH-free radical scavenging assay.[23-26] To begin with, 0.004% DPPH solution (w/v) was prepared in ethanol and 3 mL of this solution was mixed with different concentrations of 1 mL ethanolic crude extract. Subsequently, the mixture was vigorously shaken and kept in a dark place for 30 min at room temperature. After that, absorbance was measured spectrophotometrically at 517 nm, and percentage inhibition was evaluated using following equation; % DPPH-free radical scavenging activity = $([A_0–A_1]/A_0) \times 100$. Here, A_0 and A_1 indicate the absorbance of control or blank and absorbance of plant extract/positive control. The % scavenging activity was then plotted against log concentration and a graph for inhibitory concentration 50% (IC_{50}) was calculated.

Estimation of total phenolics

Total phenolic contents of all the extracts were assessed by Folin–Ciocalteu (FC) technique.[27,28] At first, 2 mL FC (1/10) reagent was mixed with 2 mL sodium carbonate (75 g/L) and an aliquot of the extracts or positive control. The tubes were shaken for 15 s for the development of the color and then, the mixtures were allowed to stand 20 min at room temperature. Ultraviolet (UV) absorbance was then taken at 750 nm in a UV spectrophotometer. Then, a gallic acid standard calibration curve was developed having a positive control curve equation of y = 0.062x − 0.0154, $R_2 = 0.9818$ (*B. glabra*); y = 0.0559x + 0.0728, $R_2 = 0.9948$ (*L. grandis*); and y = 0.072x − 0.0411, $R_2 = 0.9734$ (*A. crystallinum*).

Determination of total tannins

Total tannin contents of the extracts were also determined FC reagent.[29,30] To begin the process, 0.1 mL extracts and/or positive control was mixed with 7.5 mL distilled water and 0.5 mL FC reagent. This preparation was then shaken vigorously and kept for 5 min. Then 35% sodium carbonate solution (1 mL) was added following the dilution with 10 mL distilled water. This preparation was then kept at room temperature for 30 min. UV absorbance was taken at 725 nm using UV spectrophotometer. Then, gallic acid standard calibration curve was developed and positive control curve equation was y = 0.0219x − 0.0037, $R_2 = 0.8626$ (*B. glabra*); y = 0.0214x − 0.0034, $R_2 = 0.8099$ (*L. grandis*); and y = 0.0307x − 0.0159, $R_2 = 0.9377$ (*A. crystallinum*).

Analgesic activity test

Acetic acid-induced writhing inhibition test

Analgesic activity test was evaluated using Swiss albino mice of both sexes (weighing about 25–40 g). All the animals were unfed for about 2 h before the start of the test. Mice were divided into four groups taking five mice in each group, where Group I has been received 1% Tween-80 solution in water (at dose of 10 mL/kg) and Group II has been received Diclofenac-Sodium (Na) as a positive control at a 25 mg/kg body weight dose. The other two groups, groups III and IV were treated with those three extracts at a dose of 250 and 500 mg/kg body weight, respectively. After 30 min, 0.7% acetic acid was injected intraperitoneally. Afterward, 5 min later writhing number was counted for 15 min.[31]

Tail flick test

The effect of plant extracts on the latency of tail-flick response representing the pain threshold was investigated in mice with minor modification of the method of Yerima *et al.*[32] The tail withdrawal from the heat (flicking response) was taken as the end point. A cutoff period of 10 s was kept to avoid damage to the tail. The drug was administered once daily for 5 consecutive days. On the 5th day, 1 h after drug administration, tail-flick response was recorded at 30, 60, 120, 180, and 240 min.

Statistical analysis

All analyses were double checked and passed in two replications. Mean ± standard error of the mean was used to the present data. All experimental parameters were evaluated for their significance level by correlation and regression analysis; the *t*-tests ($P < 0.05$) were used. Microsoft excel 2016 was used for both statistical analysis and graphical presentation.

RESULTS

Phytochemical screening

In phytochemical screening test, *B. glabra* showed the presence of reducing sugar, combined reducing sugar, tannins, flavonoids, and

Table 1: Result of phytochemical screening of methanol leaves extract of *Bougainvillea glabra*, *Licuala grandis*, and *Anthurium crystallinum*

Phytochemical group	*Bougainvillea glabra*	*Licuala grandis*	*Anthurium crystallinum*
Reducing sugar	+	+	+
Combined reducing sugar	+	+	-
Tannins	+	+	+
Flavonoids	+	-	+
Saponin	-	-	-
Gums	-	-	-
Steroids	-	+	+
Alkaloids	+	+	+
Glycoside	-	+	-
Proteins	-	+	+
Acidic compounds	-	+	+

+: Presence; -: Absence

alkaloids. On the other hand, *L. grandis* confirmed reducing sugar, combined reducing sugar, tannins, steroids, alkaloids, glycoside, proteins, and acidic compounds; and *A. crystallinum* revealed the presence of reducing sugar, tannins, flavonoids, steroids, alkaloids, proteins, and acidic compounds [Table 1].

1,1-diphenyl-2-picrylhydrazyl-free radical scavenging activity

Quantitative antioxidant activity was performed by DPPH-free radical scavenging assay where the IC_{50} values of methanol crude extract of *B. glabra*, *L. grandis*, and *A. crystallinum* were 1.72, 2.18, and 13.21 µg/mL, whereas the standard (ascorbic acid) showed the value as 3.90 µg/mL [Figure 1].

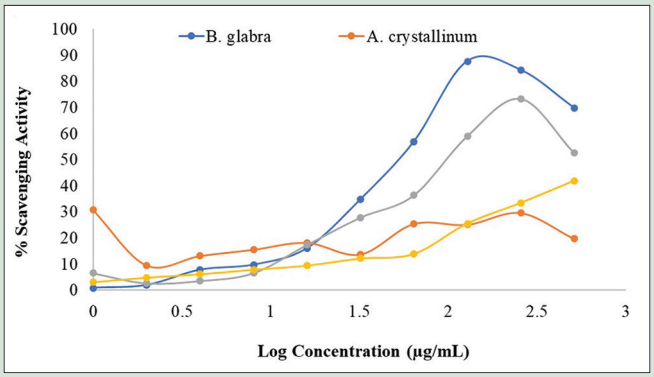

Figure 1: Comparison of 1,1-diphenyl-2-picrylhydrazyl scavenging activity of *Bougainvillea glabra*, *Licuala grandis*, and *Anthurium crystallinum* with positive control (Ascorbic acid)

Total phenol content

Gallic acid equivalent (GAE) was used to determine and express total phenolic contents of the three extracts [Figure 2]. The total phenolic content of *B. glabra*, *L. grandis*, and *A. crystallinum* was 40.97 ± 0.004, 247.272 ± 0.016, and 302.22 ± 0.011 mg GAE/100 g of dried plant extract, respectively, as represented in Table 2.

Total tannin content

GAE was also used to determine the total tannin content of the three extracts [Figure 3]. Total phenolic contents of *B. glabra*, *L. grandis*, and *A. crystallinum* were 2172 ± 0.002, 435.7 ± 0.01, and 2414.98 ± 0.028 mg GAE/100 g of dried plant extract, respectively [Table 2].

Analgesic activity

Acetic acid-induced writhing inhibition test

The three extracts *B. glabra*, *L. grandis*, and *A. crystallinum* showed 60.68%, 54.71%, and 29.06% writhing inhibition at doses 500 mg/kg, and 38.46%, 38.46%, and 18.81% writhing inhibition at doses 250 mg/kg body weight, respectively. On the other hand, the standard Diclofenac-Na exhibited 70.01%, 70.09%, and 70.09% writhing reduction in mice [Table 3].

Tail flick test

In tail-flick response test, ibuprofen showed prolongation in the response time 1 h after drug administration. The three-plant extracts displayed analgesic activities in mice in a dose-dependent manner [Table 4]. However, it is important to say that they did not show any statistically significant differences.

DISCUSSION

Medicinal plants, certainly, possess a great potential to treat a number of mild-to-severe disorders. This is why, medicinal plants from all around

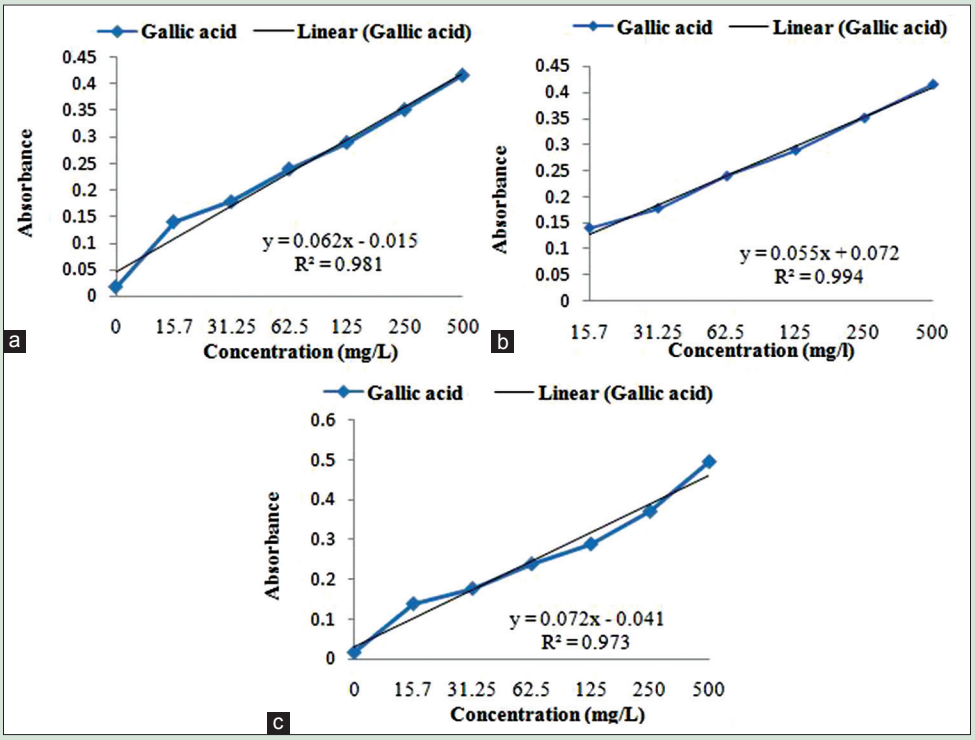

Figure 2: Determination of total phenolic contents of (a) *Bougainvillea glabra* (b) *Licuala grandis* and (c) *Anthurium crystallinum* with the help of gallic acid standard calibration curve

the world demonstrate a lot of pharmacological activities counting antioxidant, analgesic, anthelmintic, and antidiarrheal activities. All the three-plant extracts exhibited the presence of reducing sugars, tannins, and alkaloids. They also ensured the presence of some others secondary metabolites [Table 1]. On studying several literatures, it has been understood that the presence of flavonoids and tannins secondary metabolite in plants confirms the existence of antioxidants or free radical scavenging activity.[33] Some other studies showed that flavonoids are responsible for analgesic[34] and tannins for antidiarrheal and anthelmintic activities,[34] whereas the presence of alkaloids in plants possess antibacterial activity.[35]

Table 2: Polyphenolic content of methanol leaves extract of *Bougainvillea glabra, Licuala grandis,* and *Anthurium crystallinum*

Polyphenolic compounds	Methanol crude extract	Content (mg GAE/100 g)
Total phenol	*B. glabra*	40.97±0.004
	L. grandis	247.272±0.016
	A. crystallinum	302.22±0.011
Total tannin	*B. glabra*	2172±0.002
	L. grandis	435.7±0.01
	A. crystallinum	2414.98±0.028

Each value represents the total content of the analysis±SEM. Expressed in terms of mg GAE/100 g dried plant extract. SEM: Standard error of mean; GAE: Gallic acid equivalent; *B. glabra: Bougainvillea glabra; L. grandis: Licuala grandis; A. crystallinum: Anthurium crystallinum*

The free radical scavenging activity of three-plant species growing in Bangladesh, *B. glabra*, *L. grandis*, and *A. crystallinum* belonging to the family *Nyctaginaceae*, *Arecaceae*, and *Araceae*, respectively, was determined consuming DPPH-free radical. The DPPH scavenger capacity of the extracts was compared with known antioxidative substances (ascorbic acid). The antioxidant activity is one of the most important pharmacological properties of plants. To test the free radical scavenging activity or the antioxidant activity of food and plant extracts, DPPH is widely used.[36-38] which is readily scavenged by antioxidants.[39] The scavenging ability of the extract was concentration dependent and expressed as IC_{50} (Sample concentration required to decrease the initial concentration of DPPH by 50%) values. A higher antioxidant activity is indicated by a lower IC_{50} value. In the present study, among the three-plant extracts, *B. glabra* and *L. grandis* showed a potent IC_{50} values as 1.72 and 2.18 µg/mL compared to another plant extract, *A. crystallinum* showed 13.21 µg/mL, whereas the standard ascorbic acid showed the value of 3.90 µg/mL [Figure 1]. Comparing to the standard, the three crude extracts possess potent antioxidant activities. The result also suggests that the plant contains phytoconstituents which are capable of donating hydrogen to protect the cell from potential damage.

Table 3: Percentage analgesic activity of leaves extract of *Bougainvillea glabra, Licuala grandis,* and *Anthurium crystallinum* in acetic acid-induced pain model

Animal group	*Bougainvillea glabra*			*Licuala grandis*			*Anthurium crystallinum*		
	SE	Percentage writhing inhibition	*t*-test (*P*)	SE	Percentage writhing inhibition	*t*-test (*P*)	SE	Percentage writhing inhibition	*t*-test (*P*)
Negative control	3.14	0	-	3.141	0		3.141	0	
Positive control (25 mg/kg)	1.04	70.1	4.9538 (0.0011)	1.049	70.09	4.9538 (0.0011)	1.048	70.09	4.95 (0.0011)
Extract (250 mg/kg)	1.86	38.46	2.466 (0.0390)	1.327	38.46	2.6402 (0.0297)	1.6	18.81	2.55 (0.034)
Extract (500 mg/kg)	0.58	60.68	4.4462 (0.0022)	0.509	54.71	4.0236 (0.0038)	1.536	29.06	1.94 (0.087)

Positive control used Diclofenac-Na. SE: Standard error

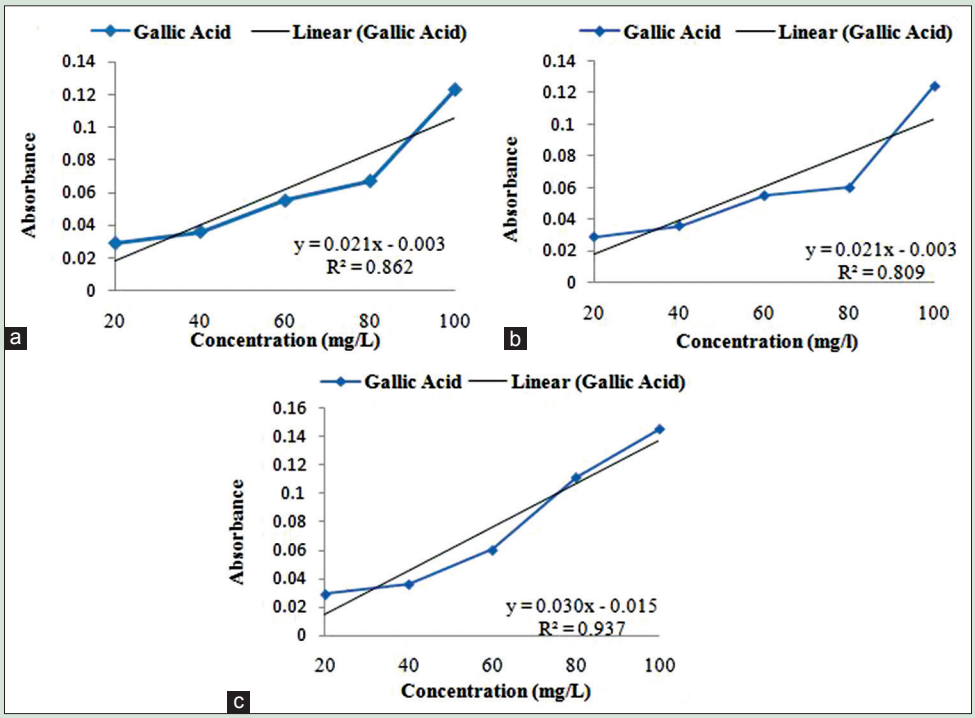

Figure 3: Determination of total tannin contents of (a) *Bougainvillea glabra* (b) *Licuala grandis* and (c) *Anthurium crystallinum* with the help of gallic acid standard calibration curve

Table 4: Analgesic activity of leaves extract of *Bougainvillea glabra, Licuala grandis*, and *Anthurium crystallinum* in tail flick test

Group (dose)	Duration of latency of tail-flick response (s) at various time intervals					
	Basal	30 min	1 h	2 h	3 h	4 h
Control (10 ml/kg)	4.19±0.31	4.01±0.30	4.27±0.34	4.45±0.52	4.35±0.74	4.28±0.37
Ibuprofen (100 mg/kg)	5.32±0.39	4.07±0.38	5.42±0.43	4.89±0.48	5.61±0.34	5.20±0.52
B. glabra (500 mg/kg)	4.08±0.39	4.45±0.28	4.22±0.38	3.83±0.31	4.39±0.30	4.47±0.28
B. glabra (250 mg/kg)	3.88±0.50	4.18±0.25	4.43±0.35	4.49±0.48	4.67±0.65	4.37±0.30
L. grandis (500 mg/kg)	4.36±0.51	4.24±0.39	4.51±0.44	4.76±0.33	5.19±0.23	3.56±0.60
L. grandis (250 mg/kg)	4.61±0.51	3.8±0.35	4.83±0.65	4.49±0.42	4.57±0.50	4.24±0.47
A. crystallinum (500 mg/kg)	4.34±0.42	3.74±0.50	4.75±0.32	4.23±0.79	4.34±0.36	4.83±0.44
A. crystallinum (250 mg/kg)	3.63±0.32	4.12±0.38	4.67±0.58	4.96±0.55	4.11±0.57	4.26±0.40

B. glabra: Bougainvillea glabra; L. grandis: Licuala grandis; A. crystallinum: Anthurium crystallinum

Plant phenolics such as flavonoids, phenolic acids, and tannins present in the fruits and vegetables have potential biological activities including anti-atherosclerotic, anti-cancer, and anti-inflammatory activities and such activities might be associated with their antioxidant activity.[40] Plant phenolics containing hydroxyl groups have a good scavenging capacity.[41,42] The extracts of *B. glabra*, *L. grandis*, and *A. crystallinum* also showed the total phenolic contents as 40.97 ± 0.004, 247.272 ± 0.016, and 302.22 ± 0.011 mg GAE/100 g of dried plant extract, respectively. The result proved that the extract possesses phenolic compounds which indicate the presence of hydroxyl groups [Table 2].

Bitter plant polyphenols such as tannins have astringent properties which mean that they can bind to protein molecules and precipitate or shrink them. Tannins contain sufficient hydroxyl groups and other free radicals for instance carboxyl and they bind proteins and other macromolecules. These free radicals protect cell damage.[43] Some studies specify that tannins are effective against ulcerated or inflamed tissues and they also possess a good anticancer activity.[44,45] The extract of the three plants, *B. glabra*, *L. grandis*, and *A. crystallinum* also showed the total tannin contents as 2172 ± 0.002, 435.7 ± 0.01, and 2414.98 ± 0.028 mg GAE/100 g of dried plant extract, respectively, which conclude that the plant extract contains a considerable amount of tannins which may be a source of free radicals that protect the cell from death and satisfy the possible mechanism how the plant extracts exerts its scavenging activities [Table 2].

In analgesic activity test, the intraperitoneal administration of acetic acid makes pain sensation[46] and creates writhing reflex in animals by activating the chemosensitive nociceptors.[47] Two different test methods have been employed in evaluating the analgesic activity of methanol extracts of three medicinal species. It is necessary to apply tests which differ with respect to stimulus quality, intensity, and duration, to obtain as complete a picture as possible of the analgesic properties of a substance using behavioral nociceptive tests.[48] The results obtained indicate that the extract possesses a moderate dose-dependent analgesic effect on the various pain models used. Centrally acting analgesic drugs elevate the pain threshold of animals toward heat and pressure. The effect of the extract on these pain models indicates that it might be centrally acting.[49] Acetic acid in this assay causes algesia by the release of some endogenous substances, which subsequently stimulated the pain nerve endings.[50] It has also been noticed that in acetic acid-induced models, the analgesia level is reduced by a number of abdominal constrictions.[51] It has been reported that flavonoids show analgesic action by increasing endogenous level of serotonin or interacting with 5-hydroxytryptamine 3 and 5-hydroxytryptamine 2A receptors.[52] The three extracts showed significant writhing inhibition comparable to the standard drug Diclofenac-Na [Table 3]. The presence of flavonoid compounds in the plant extracts may be responsible for analgesic activity. The results showed a higher writhing inhibition at dose 500 mg/kg than dose 250 mg/kg body weight that was comparable to that of standard Diclofenac-Na. Among the three extracts, *B. glabra* showed the highest writhing inhibition and all the results were statistically significant [Table 3].

CONCLUSION

The present study reveals that the methanol extracts of *B. glabra*, *L. grandis*, and *A. crystallinum* plants possess both antioxidant and analgesic activities. These preliminary studies do not describe the actual mechanism due to which the pharmacological activities were shown. However, a more extensive study (including the analysis and identification of the responsible compounds) is required to determine the exact mechanism of action of the extracts and their active compound(s) to authenticate them as a potent analgesic and antioxidant agent.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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