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Anti-inflammatory Activity of the Phenolic Compounds from *Vanda roxburghii* R. Br.

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Abstract

Inflammation is implicated in the pathogenesis of several neurodegenerative diseases including Alzheimer's disease (AD). *Vanda roxburghii* R. Br. is used as folk medicine in the treatment of various ailments and diseases including inflammation, rheumatoid arthritis and neurological disorders. We reported earlier that the plant possesses anti-inflammatory activity and the activity is high in the chloroform fraction among other fractions tested. Therefore, the present work was designed to isolate the compounds from the chloroform fraction responsible for anti-inflammatory activity. Four compounds namely syringaldehyde (**1**), vanillin (**2**), dihydroconiferyl dihydro-*p*-coumarate and (**3**) and gigantol (**4**) were isolated and purified using chromatographic methods. The isolated compounds were identified by analysis of their NMR spectral data. The compounds were evaluated for anti-inflammatory activity using carrageenan-induced hind paw edema model in mice. All the compounds showed significant activity at a dose of 100 mg/kg. Among the compounds, gigantol displayed the highest activity which was comparable to the activity of the standard drug indomethacin. At the same dose the other three compounds syringaldehyde, vanillin and dihydroconiferyl dihydro-*p*-coumarate exhibited similar activity. These results suggest that the isolated compounds from the chloroform fraction have potential anti-inflammatory activity that might be useful in the prevention and treatment of AD.

Key words: *Vanda roxburghii*, chloroform fraction, phenolic compounds, anti-inflammatory activity acetycholinesterase, antioxidant.

Introduction

Neurodegenerative diseases are heterogeneous diseases of the nervous system characterized by degeneration of neurons. Alzheimer's disease (AD) ranks first among the neurodegenerative diseases in terms of severity and prevalence. About 50 million people are now suffering from AD worldwide and the number has been projected to be tripled by 2050. The prevalence of AD is high in the developing countries than the developed countries (Scheltens *et al.*, 2021). Until now, only five drugs have got approval of FDA for treatment of AD. These drugs only make symptomatic relief, but can not stop the progression of the disease (Atri, 2019). Therefore, development of new drug or alternative medicine is urgently required for the management of this disease.

Beta-amyloid composed of aggregated Abeta protein and neurofibrillary tangles composed of misfolded tau protein are the hallmarks of AD pathology. Extensive studies on the mechanism of AD pathogenesis revealed several hypotheses including inflammatory hypothesis (Guo *et al.*, 2020). According to inflammatory hypothesis, the amyloid Abeta in the brain of AD induces activation of microglia and astrocytes which initiate a pro-inflammatory cascade and release the potentially neurotoxic substances, cytokines and other related compounds that result the degeneration of neurons

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(Boyd *et al.*, 2022; Kinney *et al*., 2018; Park *et al*., 2020). Therefore, prevention of inflammation is suggested to be an important approach in the treatment of AD. Numerous compounds from medicinal plants with anti-inflammatory activity have been found to improve and prevent neurodegeneration in AD (Shin *et al*., 2020).

Traditional medicine is still practiced in many countries to treat different diseases and ailments. It has been reported that about 40% people of Bangladesh still depends on traditional medicine as the first line therapy (Kadir *et al*., 2014; Uddin and Zidorn, 2020). Importantly, traditional medicine is preferred to treat the chronic diseases including AD, where modern drugs are limited. Bangladesh has a rich heritage of traditional medical systems which utilize plants to treat neurological disorders. Investigation of those plants by modern scientific methods may aid to discover new drugs or to establish them as an alternative medicine for AD. *V. roxburghii*, is an ornamental plant belonging to the Orchidaceae family. The plant has reputation for its folkloric use in the treatment of nervous system disorders. It is also used in the management of inflammation, pain, bronchitis and rheumatoid arthritis (Kirtikar and Basu, 1999; Ghani, 2003; Prasad and Achari, 1966). The therapeutic uses of this plant have been documented in Unani and Ayurvedic systems of medicines. We reported earlier that the plant has high phenolic content and antioxidant activity, and possesses cholinesterase inhibitory potential (Uddin *et al.*, 2015; Ahammed *et al.*, 2021). In a preliminary study, we observed the anti-inflammatory activity in the crude extract of the plant and the activity was high in the chloroform fraction among other fractions examined (Uddin *et al.*, 2015). Therefore, this work was planned to isolate the compounds from the chloroform fraction of *V. roxburghii* having anti-inflammatory activity.

Materials and Methods

Experimental animals: Swiss albino mice of either male or female sex (weighing 25-27 gm) were collected from the Department of Biochemistry and Molecular Biology, Rajshahi University and maintained in the animal house under standard environmental condition. They were given standard diet and water. Ethical clearance for the experimental protocol were obtained from the Institutional Animal, Medical Ethics, Biosafety and Biosecurity Committee (IAMEBBC), Institute of Biological Sciences, University of Rajshahi, Bangladesh (Ethical approval no. 104).

Plant collection: The roots of *V. roxburghii* were collected in the month of June 2017 from Rajshahi district, Rajshahi, Bangladesh. The plant was authenticated and a voucher specimen (No. PH12) has been preserved at the herbarium of the Department of Botany, University of Rajshahi, Bangladesh.

Extraction, fractionation and isolation of the constituent: The crude methanol extract and its chloroform fraction was prepared by the method as described earlier (Ahammed *et al.*, 2021). In summary, the dried coarse powder of the plant root (1 kg) was soaked in methanol (5 litres) and kept at room temperature with gentle shaking for 5 days. It was then filtered with Whatman filter paper number 1 and evaporated under reduced pressure by a rotary evaporator to yield the crude methanol extract (28.37 gm of dry extract). The crude extract was defatted with petroleum ether and then partitioned with chloroform to obtain the chloroform fraction (6.78 gm).

For isolation of compounds, chloroform fraction was subjcted to column chromatography with with silica gel as the stationary phase eluting with n-hexane, dichloromethane and methanol in an increasing ratio. All the column fractions were examined on TLC and then the fractions with similar pattern were combined together that yielded five fractions F1-F5. They were futher chromatographed by PTLC on silica gel with solvent system n-hexane: dichloromethane (1;1) to afford the compounds **1**-**4**.

Identification of the compounds: The isolated compounds were identified as syringaldehyde (**1**), vanillin (**2**), dihydroxyconiferyl dihydro-*p*-coumarate (**3**) and gigantol (**4**) by comparing their R_f values

with the authentic samples (Uddin *et al.*, 2015a; Ahammed *et al.*, 2021).

Anti-inflammatory activity: The animals were randomly selected and divided into seven groups: Group I to Group VII. Each group consisted of four mice. The mouse was weighed accurately and the doses of the test samples were calculated. All the drugs and test samples were dissolved in 1% Tween 80 in normal saline. Group I, control group received only Tween 80 (1 % in normal saline); Group II, standard group, received indomethacin (IND) (10 mg/kg); Group III, test group treated with chloroform fraction (200 mg/kg); Group IV, test group treated with compound **1** (100 mg/kg); Group V, test group treated with compound **2** (100 mg/kg); Group VI, test group treated with compound **3** (100 mg/kg) and Group VII, test group treated with compound **4** (100 mg/kg).

Sub plantar injection of 0.1 mg / 25 µl suspension of carrageenan with normal saline was given to the right hind paw of the mice to generate acute inflammation, one hour after oral administration of the test materials. Indomethacin at 10 mg/kg body weight was used as standard anti-inflammatory agent. The paw edema thickness was measured carefully at 0, 1, 2, and 3 hours after the carrageenan injection. The mean increase of thickness in paw edema was recorded at the different time intervals and thus edema thickness in control and in groups treated with test materials were calculated. The anti-inflammatory activity was expressed as percentage of inhibition (%) of paw edema which was calculated by using the following formula:

% of edema inhibition = [1- (C_t-C_0) treated group / (C_t-C_0) control group] x 100%

where,

C_0=Paw thickness at zero time

C_t= Paw thickness at t time

(C_t-C_0) = paw edema

Statistical analysis: Data were presented as mean ± SD. All statistical data were analyzed using Microsoft Excel program. The statistical significance (p value <0.05) between the means was determined using one way analysis of variance (ANOVA).

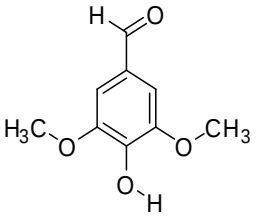
Results and Discussion

Medicinal plants continue to offer a good number of modern drugs having diverse pharmacological activities. In Bangladesh, many plants have been used by the rural practitioners to treat different diseases. The inherited knowledge of folk medicine may contribute to the development of new drugs. *V. roxburghii* is a potential herb that have been indicated for the management of different ailments and diseases (Kirtikar and Basu, 1999; Ghani, 2003; Prasad and Achari, 1966). We previously reported that the chloroform fraction of this plant has substantial anti-inflammatory activity (Uddin *et al.*, 2015). In this work, we report the bioactivity of the isolated compounds from the chloroform fraction responsible for anti-inflammatory activity.

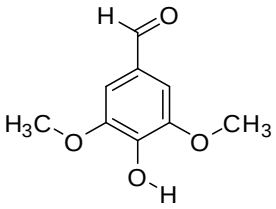
Purification of compounds from the chloroform fraction: Chromatographic separation and purification processes of the chloroform soluble fraction of a methanolic extract roots of *V. roxburghii* led to the isolation of four compounds. The compounds were found to be pure when analyzed on TLC. The R_f values of the compounds were 0.5, 0.6, 0.9 and 0.76. The compounds were identified as syringaldehyde (**1**), vanillin (**2**), dihydroconiferyl dihydrocoumarate (**3**) and gigantol (**4**), by comparison of the R_f values with that of the authentic compounds reported earlier from this plant (Uddin *et al.*, 2015a; Uddin *et al.*, 2015b). The structure of the compounds has been given in the Figure 1. All the purified compounds were evaluated for anti-inflammatory activity.

Anti-inflammatory activity of the chloroform fraction: The model of carrageenan-induced hind paw edema is considered as a standard experimental model for evaluation of anti-inflammatory potential of the test agents (Boominathan *et al.*, 2004; Panthong *et al.*, 2007). Carrageenan is a phlogistic agent of choice that has no antigenicity and systemic

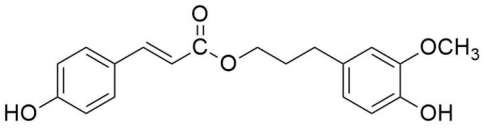
effects and the anti-inflammatory model with carrageenan shows a high degree of reproducibility (Sarkhel, 2016). Therefore, this model was employed to test the anti-inflammatory activity of the chloroform fraction of the plant extract. The result has been presented in the Table 1. Chloroform fraction exhibited a significant anti-inflammatory activity which was in accordance with the result of Uddin *et al*. (2015a).



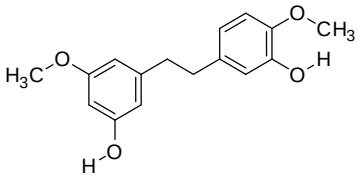
Syringaldehyde (**1**)



Vanillin (**2**)



Dihydroconiferyl dihydrocoumarate (**3**)



Gigantol (**4**)

Figure 1. Structures of the isolated compounds **1**-**4** from the chloroform fraction of *V. roxburghii*.

Table 1. Anti-inflammatory effects of the chloroform fraction and the compounds from the chloroform fraction of *V. roxburghii* in carrageenan-induced paw edema in mice.

Group	Test materials	Dose	Swelling thickness ($\times 10^{-2}$ mm +SD (% Inhibition)			
		(mg/kg)	1 hr	2 hr	3 hr	4 hr
I	Control		42.01±3.26	45.34±4.31	48.98±4.68	52.45±4.76
II	Indomethacin	10	29.75±3.46 (29.2%)*	31.9±3.34 (29.6%)*	30.16±3.48 (38.4%)*	29.63±3.35 (43.5%)*
III	Chloroform Fr.	200	33.18±3.69 (21%)	35.06±4.16 (22.7%)*	33.72±3.35 (31.2%)*	34.22±3.24 (34.8%)*
IV	Compound **1**	100	36.11±3.36 (14.1%)	39.2±4.37 (18.8%)	36.83±3.61 (24.8%)*	36.68±3.85 (30.1%)*
V	Compound **2**	100	36.24±3.80 (13.7%)	41.42±4.4 (19.8%)	36.39±3.93 (25.7%)*	36.1±3.61 (31.2%)*
VI	Compound **3**	100	36.97±4.32 (12%)	41.68±4.52 (19.7%)	38.63±4.28 (21.1%)*	34.99±3.25 (33.3%)*
VII	Compound **4**	100	30.84±3.65 (26.6%)*	33.4730.46 (26.1%)*	32.26±3.78 (34.1%)*	30.87±3.52 (41.2%)*

Data represent the mean±SD. *$p<0.05$, significantly different compared with carrageenan control.

Anti-inflammatory activity of the isolated compounds: Inhibition of inflammation has been shown to reduce the progression of the pathogenesis of AD (Kinney *et al.*, 2018). Since the chloroform fraction have shown anti-inflammatory activity in carrageenan-induced hind paw edema model in mice, further test was done to evaluate the activity of the isolated compounds. The results of the activity of the tested compounds is shown in the Table 1. Among the compounds, gigantol showed the highest activity with edema inhibition ranging between 26.2% to 41.2% ($p<0.05$) at a dose of 100 mg/kg, which was comparable to the activity of reference drug indomethacin. At the same dose, the inhibition of edema was 14.1% to 30.1% for syringaldehyde, 13.7% to 31.2% for vanillin and 12% to 33.2% for dihydroconiferyl dihydro-*p*-coumarate. These results suggest that all the compounds isolated from the

chloroform fraction are attributable to the anti-inflammatory activity of the plant.

In this work we isolated four phenolic compounds from the chloroform fraction and evaluated their anti-inflammatory activity in carrageenan induced hind paw edema model. Gigantol is a stilbene that occurs in Orchidaceae and other family. Earlier reports revealed that this compound has strong antioxidant activity and possesses anti-inflammatory activity in RAW 3 cell models. The activity of gigantol obtained in this study is consistent with the results of the earlier reports (Won *et al.*, 2006; Simmler *et al.*, 2010). We show here that gigantol is the most potent anti-inflammatory compound among all the compounds isolated from the chloroform fraction. Vanillin and syringaldehyde are two common phenolic compounds found in nature. The compounds have shown earlier the antioxidant and anti-inflammatory activity in different in vitro model (Ahammed *et al.*, 2021; Salau *et al.*, 2020; Shahzad *et al.*, 2018). Using the carrageenan-induced hind paw edema model we report here that these two compounds have similar anti-inflammatory activity. Dihydroconiferyl dihydro-*p*-coumarate is a phenolic compound of the orchid plants. This compound has potential antioxidant activity as well as moderate AChE inhibitory properties (Ahammed *et al.*, 2021). In this report, we show for the first time that dihydroconiferyl dihydro-*p*-coumarate has also anti-inflammatory activity. It has been reported that carrageenan on intraplantar injection generates prostaglandins, TNFalpha, TNFgamma, IL-1 and IL-2 and these mediators are able to stimulate nociceptors inducing inflammation in mice. Therefore, it is reasonable to assume that the anti-inflammatory action of these phenolic compounds might be due to an inhibition of the mediators that provoke inflammation.

Conclusion

In conclusion, four phenolic compounds have been isolated from the chloroform fraction of *V. roxburghii* extract that possess the anti-inflammatory activity. The potential of the compounds suggests that they may be useful in the treatment of AD. Additional studies in animal model are warranted to establish the therapeutic activity of the compounds.

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Conflicts of interest

The authors declare that there is no conflict of interests.

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