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Evaluation of Anti-oxidant and Anti-pyretic Activity of Leaf of *Dendrobium chrysanthum*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This investigation is based on a very remarkable and interesting orchid of the genus *Dendrobium* widely available in Meghalaya (India) and it is the world second largest orchid genus. The purpose of the study was to evaluate the anti-oxidant and anti-pyretic activity of leaf of *Dendrobium chrysanthum*. As the plants believed traditionally to have some anti-inflammatory activity used by the rural people of Meghalaya, use of medicinal plants and plants extract for the health procurement has always remain a common choice for the North- eastern people of India since ancient time. The leaf of *Dendrobium c.* was collected from Shillong, Meghalaya and extraction is done by using methanol as a solvent. The methanol extract found to have the constituents such as carbohydrate, glycoside, alkaloids, phenol and flavonoids. Acute anti-pyretic activity for the extract was investigated in pyrexia rat. Temperature level was determined after 1hours, 2 hours, 4 hours and 6 hours after giving the extract dose of 100mg/kg and 200mg/kg body weight, and it was found to show potent anti-pyretic activity by reducing the temperature in rat. *In-vitro* anti-oxidant activity was studied by DPPH radical scavenging method of methanolic extract which shows 36.20 as IC_{50} (µg/ml) whereas the standard Ascorbic acid in the same concentration shows 32.81 IC_{50} (µg/ml). The obtained result justified the traditional use of *Dendrobium c.* as anti-pyretic and antioxidant purpose.

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1. INTRODUCTION

Plants as a source of traditionally used medicaments have been stood up to the test of time and also contribute with several novel compounds for the healthcare purpose in modern science. Among the ancient civilizations traditional system was well established, India is considered to be as rich repository for the medicinal plants, as per WHO (world health organization) almost 80% of world population are dependants on plants and herbs for the procurement of healthcare [1,2].

Orchids are the largest groups of angiosperm with diverse Genus and around 25,000 species have been established [3]. The genus *Dendrobium* has been reported to be used for the pharmacological activities such as hepatoprotective, anticancer, antimicrobial, immunomodulatory, neuroprotective, anti-diabetic and antioxidant activities. Diverse activity of *Dendrobium* is due to the presence of different chemical constituents as per the data reported alkaloids, flavonoids, phenolic compounds, phenanthrenes, glycosides and terpenoids [4]. There are near to 1,200 species of *Dendrobium* orchids mostly grow in to the high altitudes and mountainous region, with humid environment at mild temperature. Geographically distributed in a huge characteristics, possessing various morphological features and mostly explored in Asian country, India; Sri Lanka; mainly, in China; Australia, Japan; Europe, Korea; Caledonia and Guinea [5].

Increase in body temperature more than the normal ranges 97.7–99.5°F (36.5–37.5°C) is considered as fever, characterized by elevated set point in thermoregulatory region, elevated body temperature mostly results from various interaction in between immune system and central nervous system [6]. Fever is a natural body defense mechanism mediated by different infectious agent leading to the damages in tissue. The triggering of inflammation is generally initiated by the formation of certain inflammatory mediators such as Prostaglandin PGE-2, TNF- α, interleukin 1β, which hyper-production in hypothalamus region triggers the hypothalamus which results into fever [7]. Fever is mostly been treated with the synthetic drugs non-steroidal anti-infammatory drugs (NSAIDs) which have been reported to cause severe nephrotoxicity, Gastric and hepatotoxicity thus it has always been preferred some traditionally used medicinal plants having extreme evidences for the purpose of being used as folk medicines [8].

The free radicals generation from different metabolic pathways as well as sources of environment has found to correlates the molecular genesis of several diseases by interacting the biological systems with lipid peroxidation [9]. Plants are considered as rich repository in terms of antioxidants property: thus a good attention has made directing towards ethnomedicines because of their safety profile and reach in valuable chemical constituents such as alkaloids, glycosides, phenols, flavonoids, tannins, terpenoids, vitamins, and so many other chemical compounds leading to numerous pharmacological activities [10]. Recent research has suggested that ingestion of such natural antioxidants has been found to be associated with lowering the risk for causing many life threatening diseases [11].

2. MATERIALS AND METHODS

2.1 Collection of Plant

The entire plant *Dendrobium chrysanthum* were collected from Jowai, West Jaintia Hills District, Meghalaya and washed in running water, segregated from the grass and other extraneous material and the field data of the plant like its height, flower color and soil condition were noted in the note book.

2.2 Authentication of Plant

The selected plant/parts of the plants were collected in flowering and fruiting condition and deposited in the form of herbarium and will be submit to the for authentication.

2.3 Preparation of Extracts

The leaf of the plant were collected and washed thoroughly with water to remove unwanted and extraneous matter. This was further dried in shade. After complete drying it was powdered and passed through sieve no 60 and stored in an air tight container.

2.4 Solvent Extraction

The shade-dried powder of *Dendrobium chrysanthum* leaf was taken for the extraction procedure. About 500 gm of dried powder was extracted initially with methanol at the temperature of 40^{o}C to 45^{o}C by using Soxhlet apparatus. The extraction was continued for 72 hours.

2.5 *In-vitro* Antioxidant Activity

2.5.1 DPPH radical scavenging activity

The free radical scavenging activity of methanolic leaf extract of *Dendrobium chrysanthum* was measured by 2-2-diphenyl-2-picrylhydrazyl (DPPH). One ml of 0.1 m mol solution of DPPH in methanol was mixed with 0.3 ml of methanolic extract of *Dendrobium chrysanthum* in various concentration (20, 40, 80, 160 mcg/ml) during 30 min at room temperature and the absorbance was recorded at 517 nm using UV visible spectrophotometer. Each experiment was performed with appropriate blank [10]. A positive control without extract was set up in parallel. Ascorbic acid at various concentrations was included as a standard. The scavenging activity of the extract was estimated base on the percentage of DPPH radical scavenged (1%) using equation-

$$\% \text{ Inhibition} = [(A \text{ control} - A \text{ sample}) / A \text{ control}] \times 100$$

Where A sample is the absorbance of a sample solution, and A control is the absorbance of the control solution (containing all of the reagents, except the test sample) [12,13].

2.6 *In-vivo* Anti-pyretic Activity

The investigation was performed by taking healthy rats (100-120gm) weight and grouped (n=6) which were injected 10% yeast solution subcutaneously with 10/kg body weight for inducing hyperthermia. Prior to 24 hours of injection of yeast solution the rectal temperature of each animal were recorded. Thereafter, the rats from test group were treated orally with 1ml (500 mg/kg body weight) of methanolic extract of *Dendrobium chrysanthum*. Similarly for positive control group 1 ml (100 mg/kg body weight) aqueous solution of Paracetamol was given orally. Post treatment the rectal temperature of individual animal from each groups were measured and recorded at 1hour, 2 hours, 4 hours and 6 hours. Each reading was calculated as the mean of three readings [14,15].

2.7 Experimental Animals

The experiment was performed in healthy rats (70-100 gm). Animals are acclimatized for 15 days, and all the procedure were performed as per CPCSEA guidelines and IAEC of assam down town university as approved through (AdtU/IAEC/2017/03, Dated 11/11/2017).

2.8 Preparation of Plant Extract

The plant parts were dried and extracted using ethanol as a solvent, the extract was dried and LD50 value was reconstituted into doses of 150, 300 and 600mg/kg body weight which were used and the final optimal lethal dose was considered as 100mg and 200mg/kg body weight which also been reported by other investigators [13,14].

3. RESULTS AND DISCUSSION

3.1 Qualitative Phytochemical Estimation

The preliminary phytochemical screening of methanolic extract was examined by using various standards test reagent which shows the presence of alkaloids, phenolic compound, tannins, glycosides, flavonoids and carbohydrate on the basis of secondary metabolites as shown in (Table 1) [16].

3.2 *In-vivo* Test for Anti-pyretic Activity on Animals (Rats)

The *In-vivo* anti-pyretic activity was carried by inducing hyperthermia with the aids of Brewers yeast in rats which has been kept in fasting conditioned for 24 hours. After 18 hours the rectal temperature were measured initially for each animal of different groups. The methanolic extract of plant leaf of *Dendrobium chrysanthum* was administered by oral route and the rectal temperature were noted after 1 hour, 2 hour, 4 hour and 6 hours for both test as well as positive control group [14,15]. The recorded temperature obtained for *in-vivo* anti-pyretic activity was shown in the table below (Table 2).

Discussion: The obtained result from the experiment suggested that the methanolic extract of leaf of *Dendrobium chrysanthum* possesses anti-pyretic activity at the tested doses by inhibiting the pro inflammatory mediators

cytokines, TNF- α and prostaglandins. The anti-pyretic activity as observed can be attributed to the presence of flavonoids, glycosides which also been established by other researchers obtained by same kind of evaluation with a decrease rate in temperature with same rate, which upon comparison with such reported may attribute to the results of this investigation [15].

3.3 *In-vitro* Antioxidant Activity Study

In-vitro evaluation of methanolic extract of leaf of *Dendrobium chrysanthum* was performed by using DPPH radical scavenging activity method by comparing with a standard anti-oxidant ascorbic acid in various concentrations as shown in the table below (Table 3) [16].

Discussion: DPPH (2-2-diphenyl-2-picrylhydrazyl) is the most frequently used method for testing the free radicals scavenging activity in plant extracts. The DPPH radical scavenging is being co-related with the lipid peroxidation inhibition. The free radical characteristics are neutralized by antioxidants either with transferring either hydrogen atom or an electron into the DPPH. In this investigation, the effect of methanolic extract on the DPPH radical scavenging was concomitantly increasing with increase in the concentration of methanolic extracts of the fruits from 10 to 50 µg/ml [17,18]. The percentage of inhibition obtained were ranging from 17.22 at 10 µg/ml to 41.46 at 50 µg/ml with 42.28 as an IC_{50} (µg/ml) for the extract and for positive control (ascorbic acid) were 36.28 at 10µg/ml and 68.18 at 50 µg/ml with 28.64 as an IC_{50} (µg/ml). (Table 3) the obtained data suggest that the species *Dendrobium chrysanthum*, possess hydrogen donating capabilities for methanolic extract and does it undergoes scavenging of free radicals.

Table 1. Phytochemical screening of methanolic extract of *Dendrobium chrysanthum* leaf

Sl. No.	Constituents	Test	Methanolic Extract
1	Alkaloids	Mayers reagent	++
		Dragendroffs reagent	++
		Hagers reagent	++
		Wagners reagent	++
2	Flavonoids	Aqueous NaOH	++
		Borntragers reagent	++
3	Glycosides	Legal test	++
		Keller kiliani test	++
4	Phenols	Ferric chloride test	++
		Lead acetate test	++
		Molischs reagent	++
		Fehlings reagent	++
5	Carbohydrate	Benedict reagent	++
		Seliwanoffs reagent	--
		Foam test	--
6	Saponin and Triterpenoids	Haemolysis test	--
		Salkowaski test	--

Abbreviation: (--) absent and (++) Present

Table 2. Effect of methanolic leaf extract against Brewers yeast induced Pyrexia in rats

Test item	Dose (mg/kg)	Initial rectal temperature (^{0}C)	Rectal temperature (^{0}C) 18 hrs after Brewers yeast induction	Rectal temperature (^{0}C) after treatment with extract			
				1 hr	2 hr	4 hr	6 hr
Control	-	37.22	39.28	39.18	39.12	39.13	39.12
Extract	100	37.12	39.32	39.20	38.45	38.08	37.54
	200	37.05	39.20	39.04	38.35	38.00	37.28
Paracetamol	100	37.14	39.18	38.56	38.14	37.08	37.00

Table 3. Free radical scavenging activity (DPPH) of methanolic extracts of *Dendrobium chrysanthum* leaf

Test items	Concentration (µg/ml)	Absorbance	% Inhibition	IC_{50} (µg/ml)
Methanolic Extract	10	0.0728	17.22	42.28
	20	0.0654	28.18	
	30	0.0608	31.14	
	40	0.0502	34.26	
	50	0.0489	41.46	
Ascorbic acid	10	0.0886	36.28	28.64
	20	0.0782	44.45	
	30	0.0704	46.36	
	40	0.0686	55.62	
	50	0.0602	68.18	

4. CONCLUSION

This study concludes the ethnomedicinal purpose of using leaf of *Dendrobium chrysanthum* plant as anti-pyretic and anti-oxidant. The prospect of finding traditional herbal drugs which can be a better option for healthcare procurement and comparatively with lower risk of toxicity as that of synthetic products. The methanolic extract of *Dendrobium chrysanthum* leaf shows a promising anti-oxidant (DPPH radical scavenging activity) and anti-pyretic activity. These studies suggest that it is having a numerous source for active constituents and can decrease the hyperthermia and its complications; the anti-oxidant activity was estimated by comparing with a standard anti-oxidant Ascorbic acid. The obtained result confirmed that *Dendrobium chrysanthum* has a good anti-pyretic and anti-oxidant activity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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