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Preliminary Phytochemical Analysis and Antioxidant Effect of Fruit Extract of *Neoalsomitra clavigera* Hutch

H. Lalhlenmawia[1*], V. Lalremruata[1], S.C. Mandal[2], Lalduhsanga Pachuau[1], K.Thanzami[1]

[1]Department of Pharmacy, Regional Institute of Paramedical and Nursing Sciences (RIPANS), Aizawl, Mizoram, India-796017.
[2]Department of Pharmaceutical Technology, Jadavpur University, Kolkata, West Bengal, India-700032.

ABSTRACT

The fruits of *Neoalsomitra clavigera* Hutch., locally known as 'Lalruanga Dawibur', have been used traditionally by the Mizo people of Mizoram, North East India for the treatment of various ailments including diabetes and stomach ulcer. The present study was undertaken to report the analysis of phytoconstituents present in successive extracts of the fruit extracts of *Neoalsomitra clavigera* and the antioxidant effect of methanolic extract of the plant. Its phytochemical analysis showed the presence of alkaloids, carbohydrates, proteins and amino acids, phenolic compounds and tannins, flavonoids, sterol and terpenoids. Free radical scavenging activity of the methanolic extract of the plant at different concentrations were determined with 2,2-diphenyl-1-picrylhydrazyl (DPPH). The phenolic content and total flavonoid concentration of the extract were determined using Folin-Ciocalteu reagent method and aluminium chloride method respectively. Reducing power of the extract was also determined. The methanolic extract exhibited radical scavenging activity with an IC_{50}:5.16 g/ml. The total phenolic compounds determined using Folin-Ciocalteu reagent was found to be 0.73 mg/g dry weight expressed as gallic acid equivalents (GAE) and the total flavonoid concentrations detected was 0.215 mg quercetin equivalents (QE)/g dry weight. Increasing the concentration of the extracts resulted in an increased ferric reducing antioxidant power for the methanolic extract tested. The methanolic extract of *Neoalsomitra clavigera* was found to have an antioxidant activity which may be due to the presence of flavonoids and phenolic compounds. Further investigation may be needed to indicate the mechanism of action of its antioxidant property.

KEYWORDS: *Neoalsomitra clavigera* Hutch., Lalruanga Dawibur, phytochemical constituents, antioxidant activity

***Corresponding Author-**
H.Lalhlenmawia
Assistant Professor,
Department of Pharmacy, Regional Institute of Paramedical and Nursing Sciences,
Zemabawk-796017, Aizawl, Mizoram, India.
Email: hlenmawia@gmail.com
Mob. Ph. 9862480044.

INTRODUCTION

Neoalsomitra clavigera Hutch. (family:Cucurbitaceae), locally known as 'Lalruanga Dawibur' by the Mizo people of Mizoram, North East India, is a woody climber, glabrous or pubescent and it is an endemic plant.[1] In other parts of India, they are found mainly in the hilly region of Arunachal Pradesh, Assam, Meghalaya, Sikkim and West Bengal at an altitude of around 3000-4000 ft. The dried ripe fruit is very bitter and can be stored for a very long time without deterioration and without losing its bitter properties. The dried fruits of *Neoalsomitra clavigera* Hutch., are used by the tribal people of Mizoram as one of the most effective medicine for the treatment of stomachache and as anti-diarrheal. It has also been reported that the fruit is used for the treatment of fever and diabetes.[2]

MATERIALS AND METHODS

COLLECTION AND PROCESSING OF PLANT MATERIALS

The ripened fruits of *Neoalsomitra clavigera* Hutch., were collected from Saitual area, Mizoram, North East India during the month of April 2012. Authentication was done at the Botanical Survey of India, Eastern Circle, Shillong, Meghalaya, India. Samples were then air dried at room temperature after which they were grinded to powder form.

EXTRACTION

The powdered fruits were extracted successively by cold maceration with different solvents of increasing polarity starting from *n*-hexane, chloroform and methanol, each macerated for 7 days. Each extracts were concentrated and dried under vacuum at 45˚C using rotary vacuum evaporator. The extracts were then collected and stored at room temperature.

PRELIMINARY PHYTOCHEMICAL SCREENING

It involves testing of different extracts of *Neoalsomitra clavigera* for their contents of different classes of compounds. The methods used for detection of various phytochemicals were followed by qualitative chemical test to give general idea regarding the nature of constituents present in crude drug.[3] The qualitative chemical tests for various phytoconstituents were carried out for alkaloids, glycosides, carbohydrates, proteins and amino acids, phytosterols, phenolic compounds and tannins, flavonoids and saponins, fixed oils and fats, gum and mucilage on all the extracts of *Neoalsomitra clavigera.*

DETERMINATION OF ANTIOXIDANT ACTIVITY

DPPH free radical scavenging activity

DPPH free radical scavenging activity of the extract was determined by employing the method of Blois[4]. Gallic acid was used as reference standard. To 1 ml 0.1 mM DPPH solution in methanol, 1 ml methanolic extract of *Neoalsomitra clavigera* and Gallic acid prepared in various concentrations (50,100, 150, 200, 250 g/ml) were added. Absorbance reading was then measured at 517 nm using UV Spectrophotometer after 30 m. 0.01 mM solution of DPPH in methanol was used as control. The percent inhibition observed was then calculated using the following formula:

$$\% \text{ Inhibition} = [(\text{Control Abs} - \text{Test Abs})/\text{Control Abs}] \times 100$$

The concentration in g/ml required for 50% inhibition of the DPPH radical absorbance at 517 nm was then calculated from a graph by plotting the inhibition percentage against extract concentrations.

Determination of total phenolic compounds

Total Phenolic content of the extract was determined by employing Folin-Ciocalteu method (Gutfinger, 1981).[4] To 5.0 ml extract, 5.0 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent was added. After 3 m, 1.0 ml of saturated sodium carbonate solution was added and the mixture was shaken in a vortex mixture and allowed to stand for 1 h. Absorbance reading was then measured at 725 nm in an UV-Vis spectrophotometer. From the calibration curve of Gallic acid prepared in different concentrations (100-600µg/ml), amount of phenolic compound was determined and expressed as mg GAE (Gallic acid equivalents)/g dry extract.

Determination of total flavonoid content

The total flavonoid content of the extract was determined by the aluminium chloride method.[5] To 0.5 ml of 2% aluminium chloride ($AlCl_3$), ethanol was mixed with the same volume of extract (1 mg/ml). After 1 h, absorbance reading was taken at 415 nm against blank. A standard curve was prepared with Quercetin at different concentrations (0-50 mg/ml). From the calibration curve of reference standard, the total flavonoid content was determined and expressed as milligrams of quercetin equivalents (QE/g of dry extract).

Determination of reducing power of extract

The reducing power of extract was determined by the method of Oyaizu et al.(1986)[6] using Gallic acid as standard. To 1 ml of extracts prepared at different concentrations (50, 100, 150, 200, 250 µg/ml), 2.5

ml 0.2 M, 6.6 pH phosphate buffer and 2.5 ml 1% potassium ferricyanide was added. The mixture was then incubated at 500°C for 20 m and then 2.5 ml 10% trichloroacetic acid was then added which was centrifuged at 1500 for 10 m. Then 0.5 ml, 1% of $FeCl_3$ was finally added to all the mixtures and properly mixed. Absorbance reading was measured at 700 nm. Here, the increased absorbance of reaction mixture indicate an increase in reducing power.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

Table 1: Phytochemical test for various extracts of *Neoalsomitra clavigera* Wall

Phytochemicals		Hexane[1] Extract	Chloroform[1] Extract	Methanol[1] Extract
Alkaloids	Mayer's Test	-	+	+
	Wagner's Test	-	+	+
	Hager's Test	-	+	+
	Dragendorff's Tes	-	+	+
Glycosides	Borntrager Test	-	-	-
	Legal's Test	-	-	-
	Keller Killiani's Test	-	-	-
Carbohydrates	Molisch's Test	-	-	+
	Fehling's Test	-	-	+
	Barfoed's Test	-	-	+
	Benedict's Test	-	-	+
Phenolic compound and tannins	Ferric Chloride Test	-	+	+
	Gelatin Test	-	+	+
	Lead acetate Test	-	+	+
Proteins and Amino acids	Millon's Test	-	+	+
	Biuret Test	-	+	+
	Ninhydrin Test	-	+	+
Flavonoids	Mixed with few drops of Liquid ammonia	-	+	+
	Residue dissolve in alkali	-	+	+
	Treated with 10% NaOH	-	+	+
Sterol and Terpenoids	Liebermann-Burchard's Test	+	+	+
	Salkwoski Reaction	+	+	+
Saponin	Foam or Froth Test	-	-	-

+= Present, - = Absent

1 – After cold maceration for 72 hrs at room temperature (27 ± 2˚C)

Since many modern allopathic medicines have been developed from the traditional knowledge, the ethnomedicinal use and hence, the probable pharmacological and medicinal activity of the fruits of *Neoalsomitra clavigera* (Wall.) Hutch. cannot be ignored. In order to assess and hence, isolate the probable compound that may have pharmacological activity, it is necessary to extract the crude drugs using different solvents having different polarity and qualitatively determine the different phytochemical constituents of the different extracts.

All the extracts were viscous in nature and have a characteristic bitter smell. The n-hexane extract is very oily and gave positive result only for the sterol and terpenoid test. In fact, all the extracts gave positive test for sterols as well as terpenoids. The terpenoid present could be a cucurbitane-type triterpenoid called cucurbitacins, a highly bitter compound and a characteristic group of constituents of the family Cucurbitaceae which explains the bitter taste of the fruit infusion. There have been many reports that cucurbitane type triterpenoids present in most species of Cucurbitaceae family exhibit antidiabetic, anticancer, antioxidant, antiobesity, antiviral, hepatoprotective and antiulcer activity. It is seen from the literature survey that some of the scientifically proven biological activities of the chemical compound present in most species of Cucurbitaceae family conform to the traditional usage of *Neoalsomitra clavigera*.[7] It was found that glycosides and saponins were absent in all the extracts. Since all the extracts give negative results for glycosides, the presence of cucurbitane type tritepene glycosides, whose presence is quite common in Cucurbitaceae family, can be ruled out. Flavonoids were present in the methanol and chloroform extracts while saponins absent in all the extracts.

Antioxidants are useful for combating degenerative diseases such as cancer, cardiovascular diseases, brain dysfunction and cataracts.[8] DPPH is a free radical which is stable at room temperature and the method is often employed to determine the antioxidant activity of many plant extracts. The concentration in g/ml required for 50% inhibition of the DPPH radical absorbance at 517 nm is called IC_{50} and lower IC_{50} values indicates higher antiradical activity. The IC_{50} of methanolic extract of *Neoalsomitra clavigera* exhibited 5.16 g/ml compared to standard gallic acid which exhibited 3.36 g/ml.

Phenolic compounds like flavonoids are known to possess a broad range of antioxidant activity. As phenolic compounds and flavonoids have been detected in the extract, they may be responsible for its antioxidant activities. The total phenolic compounds detected in the methanolic extract of *Neoalsomitra clavigera* was found to be 0.73 mg/g dry weight expressed as gallic acid equivalents (GAE) and total flavonoid concentrations detected was 0.215 mg quercetin equivalents (QE)/g dry weight.

The reducing power of the extract increase as the concentration increases suggesting that some compounds in the extract may be able to terminate radical chain reactions. However, it was lower when compared to standard gallic acid.

The study suggested that methanolic extract of *Neoalsomitra clavigera* possess an antioxidant activity which may prove to be helpful in preventing or slowing the progress of diseases related to various oxidative stress. Further investigation on isolation and identification of compound(s) responsible for its antioxidant activity in the plant may lead to chemical entities with potential for clinical use.

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