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Phytochemical analysis and DPPH scavenging activity of *Combretum punctatum* var. squamosum (Combretaceae), an ethnomedicinal plant of Mizoram.

Kawl Thanzami*[1], Bibhuti Bhusan Kakoti[2], Christopher VL Famkima[1], H Lalhlenmawia[1],

***Corresponding author:**

Kawl Thanzami

[1]Regional Institute of Paramedical and Nursing Sciences India.
[2]Dibrugarh University, India.

Abstract

The main objective of the study is to determine the phytochemical constituents and the DPPH scavenging activity of the aqueous extract ofCombretum punctatum var. squamosum. The phytochemical screening revealed that the crude aqueous extract contained phenols, flavonoids, phytosterols and diterpenes while alkaloids, glycosides, tannins, carbohydrates, proteins and amino acids were found to be absent. The total phenol and flavonoid content in the crude aqueous extract was found to be comparatively high, i.e. 111 mg/g GAE and 120.99 mg/g QE respectively. It was also found that the % DPPH scavenging activity was very high and comparable to the standard used. The IC50 value of the aqueous extract was found to be 1.76 mg/ml which is lower but comparable to the IC50 value of the standard.

Keywords: Ethnomedicinal plant, Leihruisen, Phytochemical analysis, Total phenol, Total flavonoid, DPPH

Introduction

The search for natural biologically active compound is one of the most promising niches in the scientific world today; and the use of natural products with therapeutic properties is still one of the most popular systems of medicine especially in Asian countries like China, Japan, India and Pakistan and has also gained interest in many developed countries nowadays. Even though synthetic medicines are still preferred by many for pharmacological treatment, one cannot deny the involvement of natural products on the development of such medicines. Many of the modern allopathic medicines are obtained directly or indirectly, by chemical modification, from natural sources, especially from plants.

Mizoram, one of the north-eastern states of India, is situated at the Indo-Burma mega biodiversity hotspot region and is included among the 8 hottest biodiversity hotspots in the world in terms of five different factors by Nature [1]. The region has variety of ethnomedicinal plants which are not yet exploited for scientific study; in which the plant taken for study, known as Leihruisen by the Mizos, is also one of them. *Combretum punctatum* var. *squamosum* (Roxb. Ex. G. Don) M.G. Gangop. & Charab., belonging to the family Combretaceae is a deciduous climber found mainly in South-east Asian countries like India, Burma, Bangladesh, Bhutan, Nepal, Philipines Thailand, Vietnam and China [2]. Traditionally, the juice of the fresh leaves is applied to wounds and cuts to stop bleeding; the broth of boiled leaves are taken as medicine for diarrhea and cholera [3,4].

Materials and Methods

Collection and extraction of plant materials

The leaves were collected from Khanpui village, Aizawl District, Mizoram, NE India during October-December, 2011 and were identified at Botanical Survey of India, Kolkata. The air-dried and powdered leaves of *Combretum punctatum* var. *squamosum* was extracted by cold maceration with distilled water after defatting with petroleum ether to give crude aqueous extract. The extract was concentrated and dried using lyophilliser to yield free flowing powder.

Phytochemical analysis

Preliminary phytochemical analysis of the crude aqueous extract of *Combretum punctatum* var. *squamosum* was carried out. The presence of phytochemical constituents like alkaloids, carbohydrates, glycosides, phytosterols, flavonoids, phenols, triterpenoids, tannins, proteins and amino acids were qualitatively analyzed [5,-7].

Determination of total phenols

The amount of total phenolics in extracts was determined according to the Folin-Ciocalteu procedure. Samples (3ml) were introduced into the test tubes. 0.15ml of Folin-Ciocalteu reagent and 0.3ml of

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sodium carbonate (7%) were added. The test tubes were mixed and allowed to stand for 30 minutes. Absorption at 725nm was measured. The total phenolic content was expressed as gallic acid equivalent (GAE) in mg per gram tissue as calculated from standard gallic acid graph [8,9].

Determination of total flavonoids

Total flavanoids content of the plant extracts was determined according to a modified colorimetric method of Gorinstein *et.al*, 2007. Plant extract (1.5ml) was taken and 75µl of 5% $NaNO_2$ solution was added .After 6 minute, 150µl of 10% $AlCl_3.H_2O$ solution was added. After 5 minutes, 0.5ml of 1M NaOH was added and total volume was made upto 2.5ml by distilled water. The solution was mixed well and absorbance was measured immediately at 510nm using UV-visible spectrophotometer. The total flavonoid content was calculated using standard Quercetin calibration curve. The results were expressed as milligrams of quercetin equivalent (QE) per gram extract [10].

DPPH scavenging activity

DPPH stable free radical scavenging activity was determined by the method of Blois. Plant extract 3 ml added to 1ml of 0.1 mM solution of DPPH in methanol. After 30 min incubation at 37 C absorbance was measured at 517 nm against control using a spectrophotometer. Ascorbic acid and Butylated hydroxyl anisole (BHA) were used as the reference materials. The percentage of inhibition was calculated by comparing the absorbance values of the test samples with those of the controls (not treated with extract). The inhibition percentage (I) was calculated as radical scavenging activity as follows [11-13].

I= (Abs control – Abs sample) / Abs control X 100

The IC50 value of both the standard substance, BHA and aqueous extract of *Combretum punctatum* var. *squamosum* was also calculated.

Results

Phytochemical analysis

The aqueous extract of the leaves of *Combretum punctatum* var. *squamosum* was screened for the presence of various bioactive phytochemical compounds using specific qualitative tests. The analysis revealed the presence of flavonoids, phenols, terpenes and phytosterols but showed that alkaloids, carbohydrates and proteins were absent and the results are as shown in table no. 1.

Table 1: Phytochemical analysis of the aqueous extract of the leaves of *Combretum punctatum* var. *squamosum*

Sl. no	TESTS	RESULT
1	Test for Alkaloids	-
2	Test for Carbohydrates	-
3	Test for Glycosides	-
4	Legal's Test	-
5	Test for Saponins	-
6	Test for Phyto-sterols	+
7	Test for Phenols	+
8	Test for Tannins	-
9	Test for Flavanoids	+
10	Test for Proteins and Amino acids	-
11	Test for terpenes	+

(+) Present (-) Absent

Determination of total phenols

The total phenolic content in the aqueous extract of the leaves of *Combretum punctatum* var. *squamosum* was calculated as 111 mg/g of gallic acid equivalent (GAE) using the gallic acid standard graph (Figure 1, $R^2 = 0.991$).

Determination of total flavonoids

From the quercetin standard graph (Figure 2, $R^2 = 0.996$), the total flavonoid content in the aqueous extract of the leaves of *Combretum punctatum* var. *squamosum* was found as 120.99 mg/g of quercetin equivalent (QE).

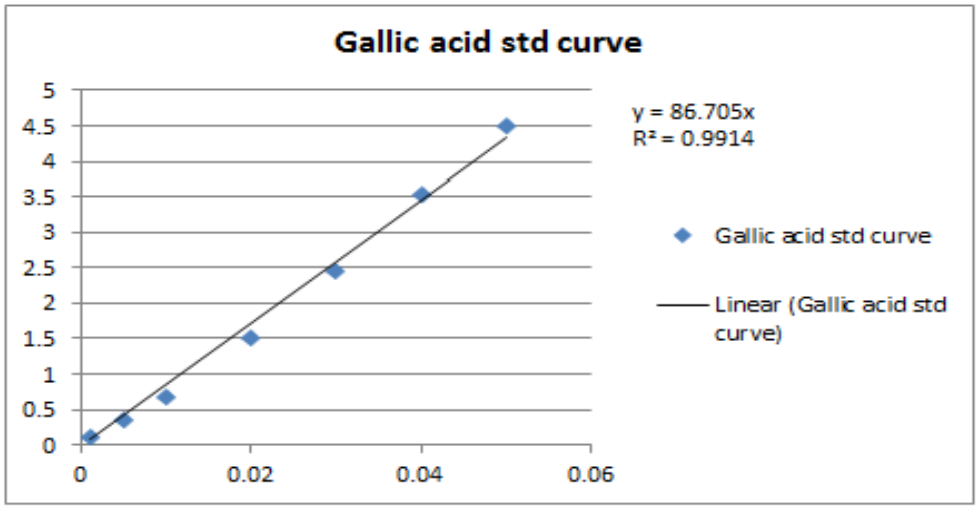

Figure 1: Standard curve for determination of gallic acid equivalents for total phenol assay

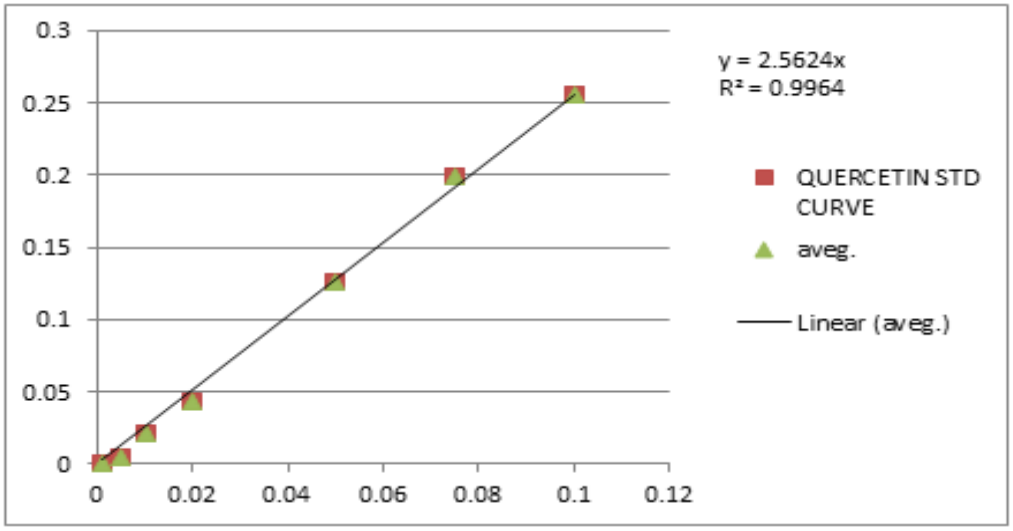

Figure 2: Standard curve for determination of Quercetin equivalents for total flavonoid assay

DPPH scavenging activity

The free radical scavenging activity of the aqueous extract of *Combretum punctatum* var squamosum was determined by determining its ability to scavenge DPPH free radical at room temperature, which is often employed to determine the antioxidant activity of many plant extracts. The concentration of the extract to scavange 50% of the DPPH radical is called IC50 and lower IC50 values indicates higher antiradical activity. IC_{50} was calculated from the graph by plotting the % inhibition in Y-axis and concentration in X-axis (Figure 3). The IC50 of the extract of *Combretum punctatum* var. *squamosum* exhibited 1.76 mg/ml as compared to standard (BHA) which exhibited 1.21 mg/ml.

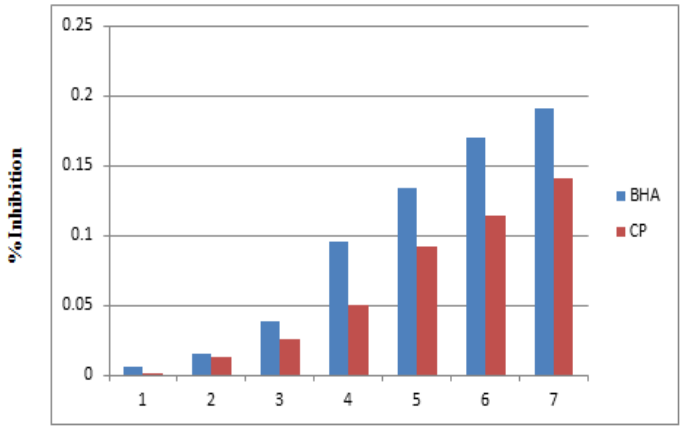

Figure 3: DPPH scavenging activity of BHA and *Combretum punctatum* var. *squamosum*

Discussion

Traditional knowledge plays a very important role in the discovery of many important and life-saving drugs; hence, taking traditional knowledge into account is of utmost importance while searching for new drug candidates from medicinal plants. Moreover, many of the secondary plant metabolites are known to be directly responsible for different activities such as antioxidant, antimicrobial, antifungal and anticancer [14-16]. In fact, many modern medicines have been obtained directly or indirectly from plant's secondary metabolites. Hence, the first and foremost step is to determine the phytochemical constituents of any plant taken for study.

Phytochemical analysis of the crude aqueous extract of the leaves *of Combretum punctatum* var. *squamosum* was found to contain flavonoids and phenolic compound in addition to terpenes and phytosterols. Previous phytochemical studies carried out from the genus *Combretum* have also demonstrated the occurrence of many classes of constituents, including triterpenes, flavonoids, lignans and non-protein amino acids, among others [17]. Oleanolic acid, quadranoside and arjunolic acid which are triterpenoid compounds; methyl gallate, a phenolic compound; stigmasta-4,25(26)dien-3-one and β-sitosterol, which are phytosterols; β-daucosterol which is a glycoside of β-sitosterol; octadecanoic acid, a saturated fatty acid and vitexin, an apigenin flavone glucoside have been isolated from *Combretum punctatum* var*. squamosum* [18].

The presence of phenolic compounds account for the majority of antioxidant activity in plants, in which the antioxidant properties are mainly because of their redox potential, which allow them to act as reducing agents, hydrogen donators, metal chelators and singlet oxygen quenchers [19]. Moreover, studies have shown that the degree of glycosylation significantly affects the antioxidant properties of the compounds, for example, aglycons of quercetin and myricetin were more active than their glycosides [20]. Hence, it can be concluded that the aqueous extract of *Combretum punctatum* var. *squamosum* may also exhibit strong anti-oxidant property as the phenol content is comparable to green tea (128.7 ± 1.7 mg/g), which is considered to be have a very good antioxidant property [21].

Flavonoids are naturally occuring phenolic compounds which largely include anthoxanthins (flavones, flavonols, flavanones, flavanols, chalcones and isoflavones), anthocyanins, leucoxanthins and flavonoidal alkaloids [22]. and are found in a variety of plant materials [23]. Due to high flavonoid content (120.99mg/g QE) of the aqueous extract of *Combretum punctatum* var. *squamosum*, the plant may be a suitable candidate for investigating several pharmacological activities like anti ulcer, anti-ageing, anti bacterial, anti oxidant, anti fungal, anti-inflammatory, anti diabetic, anti-hepatotoxic, anti allergic anti cacer,anti tumor and vasodilator properties as these properties have been shown to be associated with flavonoidal compounds [24].

The presence of terpenoids in the aqueous extract may also be one of the reasons that the plant has been used as therapeutic agent by the Mizos as terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer, and also to have antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, antiinflammatory, and immunomodulatory properties [25-28]. The presence of phytosterols in the aqueous extract is an added advantage since phytosterols and plant sterols, by competing with with dietary and biliary cholesterol for intestinal absorption, have long been known to lower serum cholesterol concentrations, thereby decrease the risk of various heart conditions.

Further works are underway to explore this ethnomedicinal plant in terms of isolation of pure compound(s) from the crude extract, *in vitro* and *in vivo* anti-oxidant activity, anti-inflammatory activity and anti-cancer activity studies.

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Conflict of Interest

The authors declare no conflict of interest.

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