www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(7): 04-07

www.thepharmajournal.com
Received: 12-05-2018
Accepted: 15-06-2018

Anita Devi Thokchom
Department of Life Science and Bioinformatics, Assam University, Silchar, Assam, India

Manabendra Dutta Choudhury
Department of Life Science and Bioinformatics, Assam University, Silchar, Assam, India

Correspondence
Anita Devi Thokchom
Department of Life Science and Bioinformatics, Assam University, Silchar, Assam, India

Determination of phenolics and flavonoids contents of the ethyl acetate and methanol bark and leaves extract of Engelhardtia spicata Lechen ex Blume

Anita Devi Thokchom and Manabendra Dutta Choudhury

Abstract
Engelhardtia spicata Lechen ex Blume is a traditional medicinal plant which got several phytochemical constituents. The present study was to determine the presence of phenolics contents and flavonoids contents in the ethyl acetate and methanol bark and leaves extract of the of Engelhardtia spicata Lechen ex Blume. Methanol leaves (240.50±8.43) posses the higher values in the total phenolics contents and ethyl acetate leaves (140.86±2.55) got the highest flavonoids contents.

Keywords: Engelhardtia spicata Lechen ex Blume, phenolics, flavonoids, ethyl acetate, methanol

1. Introduction
Medicinal plants consist of natural products and extractions of these natural products are based on the traditional medicinal system. Phytomedicinal system by the old experience depends on their medicinal and its nutritional properties [1]. The natural products are the active phytochemical compounds or constituents in medicinal plants known as secondary metabolites which have the protective and preventive properties to cure various diseases and help in the physiological action on the body of human being. Phenolics are the largest group of phytochemicals that account for most of the antioxidant activity in plants or plant products [2]. Flavonoids, colouring pigments present in plants act as protective antioxidants at various level [3]. Natural antioxidant obtained from plants are found as phenolic compounds viz, phenolic acids, flavonoids and tocopherols etc [4]. Phenolic compounds are the most secondary plant metabolites which help in the target of pharmacological activity due to its property of having excellent ability of protection from free radical scavenging, oxidative enzymes, hydrolytic inhibition and anti-inflammatory [5].
Engelhardtia spicata Lechen ex Blume commonly known as Great Malay bean (English) and Linphop (Manipuri) belonging to the family Juglandaceae. A widely found species in the primary hilly area forest up to 2500 metres. It is found in Butan, China, India, Indonesia, Malaysia, Nepal, and Vietnam. But in India, it is distributed in Arunachal Pradesh, Assam, Manipur, and Uttarakhand. It is a deciduous tree, straight bole with grey-brown woolly shoots; leaves imparipinnate, ovate-oblong; flowers are unisexual, pendulous spikes; stamens sessile; fruit is up to 3.5 cm long, 1-seeded, 3-winged nutlet, not splitting open. Fruit juice is used in the treatment of abdominal pain [6]. The bark is used against diarrhea [7] and piscidal [8]. Oleanolic acid and engelhardtione is the compound isolated from Engelhardtia spicata lechen ex Blume [9]. In spite of its various biological activities, there are fewer reports on this plant regarding the phytoconstituents present in this plant. Therefore, it is an immense need to establish the presence of phenolic contents as well as flavonoid contents of the ethyl acetate and methanol bark and leaf extract of Engelhardtia spicata lechen ex Blume in order to find out the important ingredients which will help in the future drug targeting programs.

Systemic classification of *Engelhardtia spicata* Lechen ex Blume
Kingdom: Plantae
Division: Tracheophyta
Class: Magnoliopsida
Family: Juglandaceae
Order: Juglandales
Genus: Engelhardtia
Species: spicata

Scientific name: *Engelhardtia spicata* Lechen ex Blume

Fig 1: Bark of *Engelhardtia spicata* Lechen ex Blume

Fig 2: Leaves of Engelhardtia spicta Lechen ex Blume

2. Material and methods

2.1. Collection of plant materials

The fresh barks and leaves of Engelhardtia spicata Lechen ex Blume were collected from Heirok, Manipur. The plant was identified at Botanical Survey of India, Kolkata with the Voucher specimens No. TAD-01 and submitted to the Assam University Herbarium, Silchar, Assam.

2.2. Preparation of plant materials and crude extraction method

The collected *Engelhardtia spicata* Lechen ex Blume barks and leaves were rinsed with tap water followed by distilled water to remove the dirt on the surfaces of the plants. Barks and leaves were cut into small pieces, air dried for about 72 hours (5 days) and then ground into fine powder. About 250 gm of the air-dried powder barks of *Engelhartdia spicata* Lechen ex Blume were soaked according to the polarity basis of hexane, ethyl acetate, butanol and methanol at room temperature yielding four extracts but out of these, only ethyl acetate and methanol were taken for the further study and crude extract were obtained by rotary evaporator under reduced pressure.

2.3. Determination of phenolics contents in plant extracts [10]

In plant extract, the total phenolic content was determined by using Folin-Ciocalteu colourimetric method with slight modification based on oxidation-reduction reaction. Various concentration of gallic acid solutions in methanol (1, 10, 20, 40, 60 and 100mg/mL) were prepared. In a 20 ml test tube, 1 mL gallic acid of each concentration was added and to that 5 ml of Folin-ciocalteu reagent (10%) and at 10 minutes 4mL of 7% Na_2CO_3 were added to get a total volume of 10 mL. The blue coloured mixture was shaken well and incubated for 30 minutes at 4^0C in a water bath. The absorbance was measured against blank at 760 nm. All experimental data were carried out in triplicate manner. In different concentration of gallic acid, different average absorbance values were obtained and these values were used to plot the calibration curve. The total phenolic content of the extracts was expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g). The total phenolic contents in all samples were calculated using the formula:

C= c X V/m

Where,

C=Total phenolic content mg GAE/g dry extract

C=Gallic acid concentration obtained from calibration curve in mg/mL

V= Extract volume in mL

M= Extract mass in gram

2.4. Determination of flavonoids contents in plant extracts [11]

Total flavonoids content was measured by the method of $AlCl_3$ colorimetric assay. An aliquot (1ml) of extracts or standard solution of catechin (1, 10, 20, 40, 60 and 100µg/mL) was added to 10mL volumetric flask containing 4mL of distilled water. To the flask was added 0.3 mL of 5% $NaNO_2$. After 5 minutes, 0.3 mL of 10% aluminium chloride ($AlCl_3$) was added. At 6 minutes, 2 mL of 1M sodium hydroxide (NaOH) was added and total volume was made upto 10mL with distilled water and solution were mixed well and after 15 minutes the absorbance was measured at 510 nm. The total flavonoids were calculated from the calibration curve of catechin (10-250 µg/mL) plotted by using the same procedure and total flavonoids were expressed as catechin equivalents in milligrams per 100 gram sample.

3. Result and discussion

3.1. Total phenolics and flavonoids contents

The total phenolics and total flavonoids contents of ethyl acetate bark, ethyl acetate leaves, methanol bark extract, methanol leaves extract of *Engelhardtia spicata* Lechen ex Blume is calculated from the regression equation of the calibration curve(R2 =0.992, y = 0.010x + 0.017), expressed in GAE(Gallic acid equivalent) as milligrams per gram of the extract (GAE mg/g extract) and for flavonoids contents was calculated from the regression equation of calibration curve (R2 =0.983, y = 0.001x + 0.026), expressed in CTE (Catechin

equivalent) as milligrams per gram of extract (CTEmg/g extract). The phenolics content of ethyl acetate bark extract (159.12 ± 4.78), ethyl acetate leaves extract (219.36 ± 1.86), methanol bark extract (173.62 ± 0.38) and methanol leaves extract (240.50 ± 8.43) respectively. Among the four extract methanol leaves extract got the highest amount of percentages followed by ethyl leaves extract, methanol bark extract and ethyl acetate bark extract respectively. And the flavonoids contents among the four extract the highest content was found in ethyl acetate leaves extract (140.86±2.55) followed by methanol leaves (129.03±3.35), methanol bark extract (116.18±1.55) and ethyl acetate bark (104.01±4.75) respectively shown in Table No.1. Another study of total phenolics and condensed tannins on dry matter basis percentages of *Engelhadtia spicata* Lechen ex Blume was found as 13.8702± 2.02 and 0.5144 ± 0.19 respectively [12].

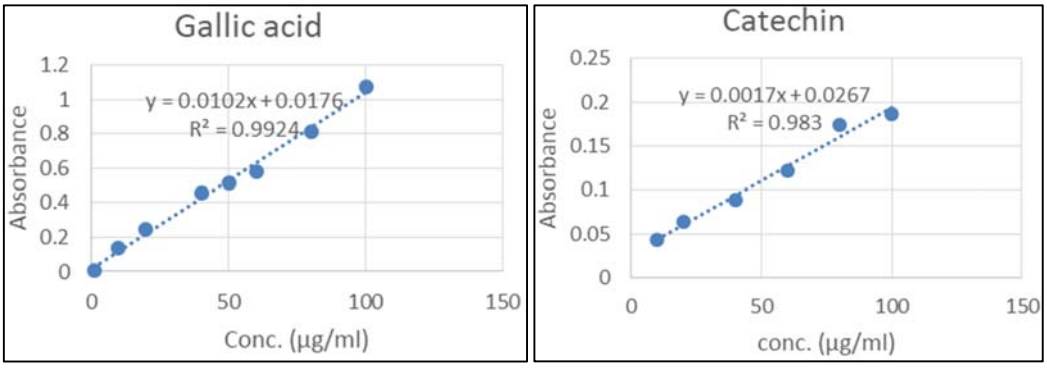

Fig 3: Standard curve of the gallic acid and catechin

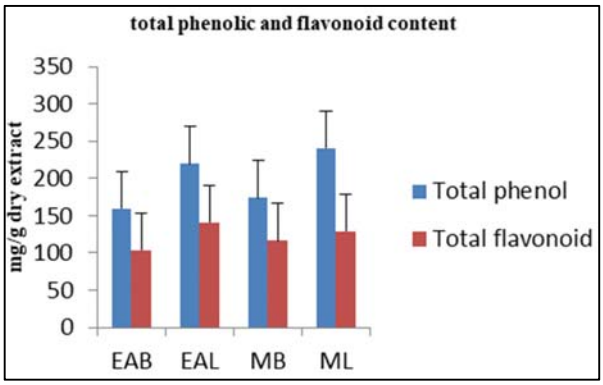

Fig 4: Ethyl acetate and methanol bark and leaves extract of *Engelhardtia spicata* Lechen ex Blume.

Table 1: Total phenolic and flavonoid contents of different crude extract of *Engelhardtia spicata* Lechen ex Blume.

Plant extract	Total phenolic content	Total flavonoid content
Ethyl acetate bark	159.12 ± 4.78	104.01 ± 4.75
Ethyl acetate leaves	219.36 ±1.86	140.86 ± 2.55
Methanol bark	173.62 ± 0.38	116.18 ±1.55
Methanol leaves	240.50 ± 8.43	129.03 ± 3.35
Gallic acid	176.4 ± 0.39	
Catechin		120.32 ± 1.12
Each value is expressed as mean ± standard deviation (n = 3).		

4. Conclusions

The above study suggested that *Engelhardtia spicata* Lechen ex Blume detected the presence of total phenolic and total flavonoid contents. This is the first report on finding out such a new work regarding this plant. Therefore, it is also most promising to find out the natural products having the therapeutic properties which will provide an important role in the development of novel drugs which will help in the treatment of various diseases.

5. Acknowledgements

The authors are thankful to the DBT-BIF Bioinformatics Centre and the Ethnobotanany Lab, Assam University, Silchar, for providing infrastructural support in the present study.

6. Conflict of interest

There is no conflict

7. References

1. Shil S, Choudhury MD, Das S. Indigenous knowledge of medicinal plants used by the Reang tribe of Tripura state of India. J Ethnopharmacol. 2014; 152(1): 135-141.
2. Okpuzar J, Ogbunugafor H, Kareem GK, Igwo- Ezikpe MN. *In vitro* investigation of antioxidant phenolics compounds in extract of Senne alata. Res J Phytochem. 2009; 3:68-76.
3. Shahidi F, Wanasundara PKJPD. Plenolic antioxidants. Critical reviews in Food Science and Nutrition. 1992; 32:67-103.
4. Lin D, Xiao M, Zhao J, Li Z, Xing B, Li X et al. An

overview of plant phenolic compounds and their importance in human Nutrition and management of Type 2 Diabetes. Molecules. 2016; 21(10):1374.
5. Frenkel E. Nutritional benefits of flavonoids. In: International conference on food factors: chemictry and cancer prevention, Hamamatsu, Japan. Abstracts, 1995, C6-2.
6. Burlakoti C, Kunwar RM. Folk herbal medicines of Mahakali watershed area, Nepal. Medicinal Plants in Nepal: An Anthology of Contemporary Research. 2008, 187-193.
7. Malik ZA, Bhat JA, Ballabha R, Bussmann RW. Ethnomedicinal plants traditionally used in health care practices by inhabitants of Western Himalaya. Journal of Ethnopharmacology. 2015; 172:133-144.
8. Tangjitman K, Wongsawad C, Kamwong K, Sukko T, Trisonthi C. Ethnomedicinal plants used for digestive system disorders by the Karen of northern Thailand. Journal of Ethnobiology and ethnomedicine. 2015, 11-27.
9. Kunwar RP, shrestha KP, Bussman RW. Traditional herbal medicine in Far-west Nepal: a pharmacological appraisal. Journal of Ethnobiology and Ethnomedicine. 2010; 6:35.
10. Waterhouse AL. Determination of total phenolics. Current protocols in food analytical chemistry. Scientific research. 2002; 11:11-11.18.
11. Kim DO, Jeong SW, Lee CY. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food chemistry. 2003; 81:321-326.
12. Buragohain R. Tree foliages fed to dairy animals in Mizoram: Traditional medicinal uses, screening and quantification of phytochemicals. International Journal of current Microbiology and Applied Sciences. 2017; 6(10):61-71.