INTRODUCTION

Plants are integral part of the medicinal system from ancient time. Many local and tribal groups all over the world still depend completely on natural remedies based mainly on plants. According to an official report by the World Health Organization approximately 80% of the world populations still rely on phytotherapy [1]. However, owing to the lack of scientific validation and proper documentation of the processing of such plants for medicinal purposes, they are yet to attain their status. Hence, researchers from all over the world are now interested in plant-based drug discovery research. 25% of the small drug molecule approved in 2014 are natural product based. The biological properties of plants can be attributed to their chemical constituents. Privileged chemical structures from nature are chosen as backbone for many new chemical entities developed for targeting various diseases [2].

Plants of the genus Garcinia are reported to possess antibacterial [3,4], antifungal [5], antioxidant [6], anti-inflammatory [7], and anticancer [8,9] activity. Many important chemical constituents are reported to be isolated from plants of these genus such as garcinol, isogarcinol, alpha mangosteen, beta mangosteen, gambogenic acid [10-12]. Garcinia morella is a lesser explored plant of this genus and mainly found in northeastern region of India, Southern region of China, and Sri Lanka. It is commonly called as Kuji Thekera in Assam. The fruit of this plant is used in traditional medicine system for treatment of inflammatory disorders, bowel syndromes, wound healing, and tumors.

Neuroblastoma is childhood tumor associated with the sympathetic nervous system. It is aggressive tumor with severe clinical complexities often leading to high rate of mortality. Neuroblastoma is characterized to have cellular heterogeneity and human neuroblastoma derived cell lines retain the heterogeneous character [13]. Even with the recent advancements of medical field, still, neuroblastoma accounts for approximately 15% of cancer deaths in younger children [14]. Hence, novel chemotherapeutic agents from natural sources are much sought for treatment of such form of deadly cancer.

In our previous report, we demonstrated the anticancer activity of G. morella fruit on T-cell murine lymphoma in in vitro and in vivo condition [9]. Since there are no earlier reports of anticancer activity of G. morella fruit on neuroblastoma, we have taken up this study to support our claim of G. morella fruit to a be a rich source of anticancer compounds. Moreover, Garcinol isolated from plants of Garcinia family are reported to have remarkable anticancer efficacy, but there are no reports highlighting its effect on neuroblastoma. Therefore, we embarked on this preliminary study to establish the anticancer potential of Garcinol on neuroblastoma cell line SH-SY5Y.

METHODS

Collection and identification of plant samples

G. morella fruits were collected from Sorbhog, Patchala district, Assam, India (N26.33’37E091.00’99) during the month of February, 2012. The plant sample was identified and authenticated by taxonomist at Northeast Indian Ayurvedic Research Institute (Government of India) Guwahati, Assam, India. The fruit sample was preserved and herbarium was prepared and deposited in drug Discovery Laboratory, Institute of Advanced study in Science and Technology, Guwahati with voucher no IASST/BCCS/HNO112/2012.

Preparation of extract

The fruit samples were finely cut into small pieces and air dried. The dried pericarps were grounded to coarse powder and extracted at room temperature by continuous stirring in methanol for 3 consecutive days. The extract was filtered and the solvent was evaporated by rotator evaporator at 45°C. The dried extract obtained was further aseptically air dried and stored in closed container at 4°C.
Phytochemical analysis
Methanol extract of G. morella was phytochemically investigated by standard protocols [15].

Fractionation of G. morella crude extract
1 kg of G. morella crude methanol extract was loaded to a silica gel column and eluted with solvents in increasing polarity such as hexane: Ethyl acetate, ethyl acetate, chloroform, and methanol: Chloroform. The fractions were collected separately and rotor evaporated. The dried samples were stored in tight containers at 4°C for biological assays.

Identification of garcinol in chloroform fraction
The chloroform fraction was further fractionated in flash chromatography. 1.2 g chloroform fraction was loaded in 12 g silica gel column and eluted with hexane: Ethyl acetate gradient. The eluent of each peak was separately collected with regular monitoring by thin-layer chromatography. A yellow powder obtained in subfraction 23 was identified by determination of melting point of the compound.

Anticancer activity

Chemicals
Dulbecco’s modified eagle’s medium (DMEM), fetal bovine serum (FBS), trypsin ethylenediaminetetraacetic acid (EDTA), phosphate buffered saline, pentsrep, dimethyl sulfoxide (DMSO), and 3-(4,5-dimethyl thiazol–2–yl)–2,5-diphenyl tetrazolium bromide (MTT) were procured from Sigma Aldrich Co., St. Louis USA. Solvents Methanol, Chloroform, Hexane, and Ethylacetate were purchased from Merck Ltd., Mumbai, India. Garcinol was procured from Santa Cruz Biotechnology Inc.

Cell culture
Neuroblastoma cell line (SH-SYSY) was obtained from National centre for cell science, Pune. SH-SYSY cells were maintained in DMEM with 10% FBS and 1% Pen strep in humidified atmosphere of 5% CO₂ incubator at 37°C. The cells were grown in T-25 flask till confluent then they were dissociated from the flask by trypsin EDTA. The dissociated cells were counted using cell counter. All the cytotoxic assays were carried out using 96 well culture plates (Tarsons, India Pvt. Ltd., Kolkata, India).

Drug preparation
Stock solution of Garcinia morella fruit chloroform fraction (GFCH) (100 mg/ml) was prepared in 100% DMSO. Different dilutions of GFCH were made in incomplete sterile DMEM. Garcinol was dissolved in 100% DMSO at a concentration of 10 mm. The stock solution was further diluted in incomplete DMEM for experiments.

Evaluation of cytotoxicity of the test samples by MTT assay
Anticancer activity of chloroform fraction GFCH and garcinol on neuroblastoma cell line via apoptotic pathway [25]. From our results, we can interpret that GFCH has a higher cytotoxic potential (IC₅₀ 5.3 µg/ml). Clarke root extract was found to induce dose dependent activity against neuroblastoma cell line at 30 and 100 µg/ml respectively[24]. Similarly, IC₅₀ doses of both GFCH and Garincol were found to decrease upon increasing the exposure time. Thus, at the longest duration of the treatment period (72 h.), GFCH and garcinol induced the highest activity.

DISCUSSION
According to American cancer society 2009-2013, Neuroblastoma is the third most popular type of cancer detected in children and adolescents. Neuroblastoma is reported to be resistant to chemotherapeutic drugs and display traits of recurrence [21]. Thus, research towards the identification of novel anti-neuroblastoma agents and their subsequent development into drugs is still recommended to improve the patient condition.

Recent studies have revealed the capability of various plant based products in inhibiting the proliferation of several cancer cell lines including neuroblastoma [22-24]. Plants of Garcinia family are cited to have anticancer activity against different types of cancer cell lines. But to our knowledge, the activity of G. morella has never been investigated against neuroblastoma. Thereby, in the present study, for the first time, we reported the remarkable efficacy of G. morella fruit against neuroblastoma.

In similar studies, the n-Hexane fraction of Nardostachys jatamansi was reported to have exhibited 54% and 91% inhibition against neuroblastoma cell line at 30 and 100 µg/ml respectively[24]. Similarly, Sousseina lappa Clarke root extract was found to induce dose dependent activity against neuroblastoma cell line via apoptotic pathway [25]. From our results, we can interpret that GFCH has a higher cytotoxic potential (IC₅₀ 5.3 µg/ml at 24 h) on neuroblastoma than the other previously reported plant crude extracts. In our previous study, we have demonstrated the apoptosis inducing the effect of G. morella fruit extract on Dalton’s ascites lymphoma cells by activation of caspases [9]. In the present study, we can hypothesise the involvement of apoptotic markers in bestowing cytotoxic potential to G. morella fruit against neuroblastoma.
Polyisoprenylated benzophenones, Garcinol is isolated from different species of Garcinia family especially from *G. indica* and *G. mangostana*. Many studies have reported the significant anticancer activity of Garcinol on a diverse range of cancer cell lines. Garcinol is reported to have dose dependent activity against prostate cancer cell lines PC-3, LNCaP and DU-145 [26]. The type of cancer on which cytotoxic effect of garcinol is tested are breast cancer [27], Burkitt lymphoma [28], Colon cancer [29], esophageal cancer [27], hepatocellular carcinoma [30]. We assume that garcinol exhibits anticancer activity against neuroblastoma through triggering the apoptotic cascade.

CONCLUSION
The results of this study indicate that *G. morella* fruit can be a potential anticancer source against neuroblastoma. More importantly, garcinol the main bioactive molecule of *G. morella* chloroform fraction was found to be highly effective against this cancer. Thereby, our study instigates new research in this direction which may lead to the development of a novel drug for treatment of neuroblastoma.

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AUTHOR CONTRIBUTION
Ms. Choudhury is the main author of this research work including study design, experimental setup and writing of the manuscript. She performed all the experiments. Mr. Kandimalla contributed towards study design and compilation of data. Dr. Kotoky and Dr. Bharali supervised the design of experiments and assessment of results.

REFERENCES


