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In vitro Assessment of Cytotoxic Activity of Hybrid Variety of *Momordica charantia* (Bitter Gourd)

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ABSTRACT

Momordica charantia (Family: Cucurbitaceae), commonly referred to as Bitter Gourd, Karela, and balsam pear, is a very familiar vegetable of Bangladesh and has long been used in traditional medicine to cure numerous illnesses. Three different extracts of a selected hybrid variety of *M. charantia* fruit from Bangladesh were prepared and screened to assess cytotoxic activity using the brine shrimp lethality test (BST). Gallic acid was used as a positive control. The LC_{50} value of standard gallic acid, ethanol, ethyl acetate, and petroleum ether extract from the fruits of *Momordica charantia* L. found 4.40 µg/ml, 125.89 µg/ml, 146.49 µg/ml, and 194.2 µg/ml, respectively. The ethanol extract demonstrated a prominent cytotoxic activity in comparison with the moderate cytotoxic activity of ethyl acetate and petroleum ether extract. The study results support the predominant presence of cytotoxic bioactive compounds in the more polar solvent of the fruit extract which can be beneficial for our health because of the numerous pharmacological activities of these compounds.

Keywords: *Momordica charantia*, Cytotoxicity, Fruit extract.

INTRODUCTION

In today's world, elevated concern exists over the development of different types of ailments and chronic diseases, and countless side effects are often encountered with chemical drugs while treating and managing diseases. Furthermore, excessive and careless use of commercial antimicrobial drugs to treat various diseases has developed multiple drug tolerance or resistance in the biochemical and physiological mechanisms of humans [1]. As a source of hope to meet global healthcare needs, natural medicines, green health products, food supplements are receiving mounting attention and importance due to no toxic or adverse effects compared to prescription drugs. For centuries, many countries have adopted herbs and other dietary supplements as traditional treatments against different ailments. Studies have revealed the efficacy of various plant components that can affect tumor initiation and progression [2]. Determination of cytotoxic activity in medicinal plant to extract bioactive constituents has increased to be used in the treatment of cancer [2,3]. Plant extracts are pharmacologically evaluated to develop safe and innovative drugs. For designing novel drugs, natural compounds existing in medicinal plants such as alkaloids, tannins, carbohydrates, terpenoids, steroids, flavonoids are identified and isolated from plants, and these have been proved to be responsible for exerting definite pharmacological actions in the human body [4,5].

Due to the unique biophysical setting, Bangladesh is blessed with a plethora of medicinal plants but is still left to fully explore the growing recognition of its importance. Traditional medicine-based healthcare is centered on an old and new experience and clinical data; many of them have no foundation of any kind for their value. Traditionally, these plants' use is based solely on their positive effects on the patients [6].

There is ample evidence that natural products derived from plants, microorganisms, and marine organisms make up a large proportion of the total repertoire of available anticancer drugs [7,8]. This phenomenon has led to the search for the potential cytotoxic standard for cancer chemotherapy based on plant's medicinal potency [9,10]. As a result of such efforts, several essential molecules such as vincristine, taxol, camptothecin, combretastatin, podophyllotoxin, and many more have been isolated from plants and are currently successfully being practiced in cancer treatment [11].

Momordica charantia Linn (Family: Cucurbitaceae), also known as Bitter Cucumber or Bitter Melon, as all parts of the plant, including the fruit taste very bitter, is an annual herb cultivated extensively as a tropical vegetable throughout Bangladesh [12]. The fruit is consumed throughout the world in every stage between maturing. The whole plant of *M. charantia* also has been traditionally used. For instance, the extract of this plant is used as a stimulant for digestion, dyspepsia, constipation, and more significantly for treating diabetes [13]. A recent study confirmed that the extract of this plant increases insulin sensitivity and can reduce escalated blood sugar levels [14]. The plant is also used for preventing and treating malaria,

jaundice, piles, and diabetes. Researches have shown its efficacy as anthelmintic, purgative, antimalarial and eczema, dysmenorrhea, gout, jaundice, abdominal pain, fever, and leprosy piles, pneumonia, rheumatism, scabies and in various cancers [15,16]. Its large-spectrum antibacterial property can effectively fight against several infections. Likewise, the extracts of leaf, fruit, and even the whole plant of *M. charantia* are routinely used to treat wounds, parasites (e.g., worms), measles, and hepatitis [17]. Extensive research on *M. charantia* discovered several medicinal properties such as hypoglycemic, anti-bacterial, anti-viral, anti-tumor, anti-oxidant, antimutagenic, hepatoprotective, immunomodulation, and anti-inflammatory activities, as well as anti-ulcerogenic and immune-modulatory activities [18-20]. Inhibitory effects of *M. charantia* proteins (α- and β-momorcharin) have been confirmed against the human immune deficiency virus (HIV) [21]. A combination of different bioactive constituents such as steroids, flavonoids, tannis, and saponins, of *M. charantia* might be responsible for all these activities, and the presence of these secondary metabolites was confirmed by the preliminary phytochemical screening [22].

In the present study, we performed an in vitro cytotoxic screening of ethanol, ethyl acetate, and petroleum ether extracts of a hybrid variety of *M. charantia* fruits in order to investigate potential antitumor activity in this variety. Brine Shrimp Lethality Assay was used to determine active compounds in the plant extracts that have cytotoxic potentiality and the lethality concentration (LC_{50}) to further enrich the medicinal value of the fruits of this promising herbal plant. In this method, *in vivo* lethality in a simple zoological organism (Brine shrimp nauplii) was used as a favorable monitor for screening the existence of antitumor bioactive natural compounds in the hybrid variety of *M. charantia* fruits, essential for the isolation of biologically active compounds for the development of drugs [23,24].

MATERIAL AND METHODS

Sample Collection and Extraction:

Hybrid variety of *Momordica charantia L.*, "TIA" was collected in the month of April-May, 2011, from the local market of Chittagong, a district of Bangladesh, and the study was carried out in the Department of Biochemistry and Molecular Biology, University of Chittagong (CU), Bangladesh. The variety was identified by Dr. Sheikh Bokhtear Uddin, Associate Professor, Department of Botany, and CU. After washing the sample, the seeds were separated, and the fruit part (pericarp) was air-dried for 5 days to make them suitable for grinding. Finally, the dried fruits were ground to a fine powder with an electrical grinder. Then the powdered material was stored in an air-tight container and reserved at 25 °C. The dried sample powder was macerated in ethanol, ethyl acetate, and pet ether in a separate conical flask for 5 days at room temperature (25±1) °C with occasional stirring. Afterward, the extracted liquid was filtered using a sterilized Whatman No.1 filter paper. The filtrate was then concentrated through a cyclone rotary evaporator (RE 200, Bibby sterling, UK), under reduced pressure below 500C. After that, the concentrated extracts were collected in a petri dish for complete evaporation of ethanol, ethyl acetate, and pet ether. The whole process was repeated three times. Finally, the semisolid mass of the three different crude extracts was obtained and preserved in the refrigerator at 4 °C.

In vitro Assay of Cytotoxicity of *Momordica charantia L.* Fruits:

Brine shrimp lethality bioassay was conducted to investigate the cytotoxic activity of ethanol, ethyl acetate, and pet ether extract of *Momordica charantia L.* fruit [25]. This cytotoxic activity determinant assay represents an inexpensive, fast, and simple bioassay for testing plant extracts bioactivity, which in most cases predictive of cytotoxic, pesticidal, and anti-tumor properties [26]. Cysts (eggs) of Brine shrimp were collected from the Institute of Marine Science and Fisheries, University of Chittagong, Bangladesh, and the test samples of ethanol, ethyl acetate, and pet ether extract with ten different concentrations (20, 40, 60, 80, 100, 200, 400, 600, 800 and 1000 µg/ml) were prepared in DMSO (not more than 50 µl in 5 ml solution) along with seawater. As a negative control, 30 µl DMSO (Sigma, USA) was used, and standard as a positive control, gallic acid (Sigma, USA) was used. 10 Nauplius were transferred in each experimental and control vial with a Pasteur pipette. After 24 hours, survivors were counted with the aid of a 3X magnifying glass, and at this whole time, the test tubes were maintained under illumination. Finally, LC_{50} (lethal concentration 50) value was obtained through regression analysis with plotting probits (% lethality) against corresponding log concentration of crude ethanol, ethyl acetate, and pet ether extracts of *Momordica charantia L.* fruit by using "Microsoft Excel 2010".

RESULT

In vitro Assay of Cytotoxic Activity:

To assess the cytotoxic activity of ethanol, ethyl acetate, and pet ether extracts of *Momordica charantia L.* fruit, we performed Brine Shrimp Lethality Bioassay. This method showed that ethanol, ethyl acetate, and pet ether extracts of the samples exhibited percentage lethality of brine shrimp in a dose-dependent manner at ten different concentrations (20 to 1000 µg/ml). Ethanol extract of the sample showed 20, 30, 30, 40, 40, 50, 60, 70, 90 and 100% mortality of brine shrimp at 20, 40, 60, 80 100, 200, 400, 600, 800 and 1000 µg/ml concentrations (Fig.1), respectively. The LC_{50} value was found at 125.892 µg/ml (Supplement Table.1). Ethyl acetate fruits extract showed 10, 30, 30, 40, 40, 50, 60, 70, 80 and 100% mortality of brine shrimp at 20, 40, 60, 80 100, 200, 400, 600, 800 and 1000 µg/ml concentrations (Fig.1), respectively. The LC_{50} value of this extract was found 146.498 µg/ml (Supplement Table. 1), However, pet ether fruits extract showed 10, 10, 20, 30, 30, 40, 60, 70, 80 and 100% mortality of brine shrimp at 20, 40, 60, 80 100, 200, 400, 600, 800 and 1000 µg/ml concentrations, respectively (Fig.1). The corresponding LC_{50} value was found 194.2 µg/ml (Supplement Table 1).

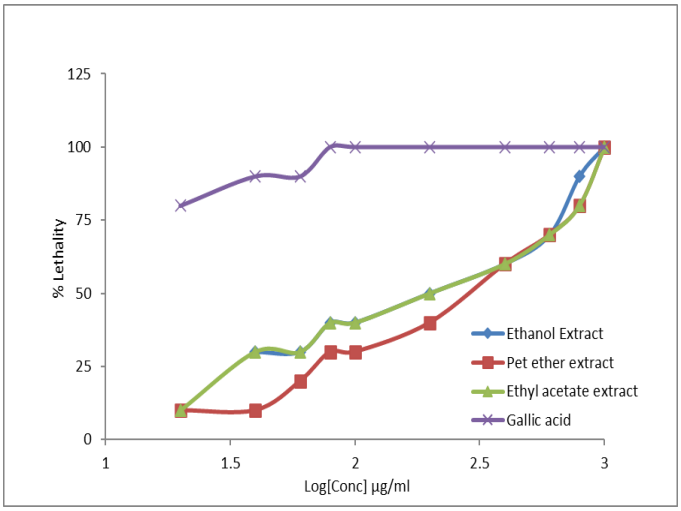

Fig. 1: Comparative analysis of lethality in ethanol, ethyl acetate and pet ether extracts of *Momordica charantia* L. fruit. Gallic acid was used as positive control

Table 1: LC_{50} values of ethanol, ethyl acetate and pet ether extract of *Momordica charantia* L. fruit with gallic acid (positive control)

Name of sample	LC_{50}
Gallic Acid	4.40 µg/ml
Ethanol extract of *Momordica charantia L.* fruit	125.89 µg/ml
Ethyl acetate extract of Momordica *charantia L.* fruit	146.49 µg/ml
Pet ether extract of *Momordica charantia L.* fruit	194.2 µg/ml

DISCUSSION

The result of the Brine Shrimp Bioassay revealed that the extract from ethanol, ethyl acetate, and petroleum ether of *Momordica charantia* fruit exhibited substantial cytotoxic activity compared to the gallic acid as a positive control. Three different solvents of varied polarity have been used in this study to extract the diverse and available bioactive constituents and to determine the most potent lethal extract against brine shrimp. Ethanol extract of the fruits found to be predominant for demonstrating cytotoxic activity than the ethyl acetate and petroleum ether extracts. This phenomenon is probably due to the effectiveness of ethanol to dissolve active compounds in cells and thus, it was easier to extract the intracellular ingredients from plant materials by penetrating the cell membrane [27]. (*Momordica charantia Linn*.) had been known to contain several active compounds such as anthocyanin, triterpenoids, saponins, tannins, flavones, polyphenols, and so on that can be easily extracted by using organic solvents (ethanol, methanol, n-hexane) [28]. The study also revealed that solvent polarity changes alter its ability, and an appropriate solvent can selectively extract more significant constituents from natural sources [29]. Thus, the results support that polar solvent like ethanol is a better solvent system than the intermediate-polar solvents like petroleum ether and ethyl-acetate used in extracting bioactive compounds from the fruit of *Momordica charantia L.* With the surge of the polarity of the solvent, the presence of the polyphenols in the extract also has increased. These active components of the fruits have been reported to act as anti-tumor agents primarily by deterring tumor cell proliferation, differentiation promoting a normal cell, influencing energy metabolism, inducing tumor cell apoptosis, and suppressing tumor cells metastasis [30]. Some of these bioactive compounds, specifically triterpenoids, saponins are shown to have an inhibitory action on carcinogenesis [31]. Superior hepatic GST and –SH levels, significantly reduced tumor burden in DMBA-induced papilloma genesis, has been validated by the whole fruit extracts of *M. charantia*, and no skin papillomas were observed in the groups during the entire experimental period [32]. Momordin (a protein purified from *M. charantia*) was related to anti-CD5 monoclonal antibodies and presented a better performance on human T cell leukemia Jurkat than the other anti-CD5-based immunoconjugates [33].

A previous report of Brine Shrimp Lethality Study revealed that the LC_{50} values from extracts of ethanol, ethyl acetate, and n-hexane of *M. charantia* fruit and fruit juice were 294,440 µg/mL; 445,090 µg/mL; 937,728 µg/mL and 484,269 µg/ml, respectively [34]. These results are correlated with the current study, where the ethanolic fruit extract showed the highest cytotoxicity. Furthermore, several researches revealed that ethanol extract of *M. charantia* L. fruit possesses higher cytotoxic activities compared to other tested extracts [35,36]. This finding can be well established by the superior cytotoxic effect of the Ethanol fruit extract (FE) against lung cancer (A549), chronic myeloid leukemia (K562), T cell leukemia, and breast cancer (MCF-7), comparing with the other extracts [37]. Though the range of LC_{50} values varies between the studies which can be concluded by stating that there could have a deviation in the cytotoxic activity of plants of the same species depending on many features including environmental factors, geographical location, maturity, sample collection for experiments under different circumstances, soil condition or may be due to the differential gene expression of this plant under stressed conditions.

Moreover, this variation in biological activity could exist within the same variety. For instance, a quantitative and qualitative study between the two varieties of *M. charantia* L. (GOJNEE and TIA) fruit demonstrated that although both varieties confirmed the presence of similar types of phytochemicals, the content of alkaloids, flavonoids, and saponins were lower in the TIA variety [38], which might further explain the reason behind the less cytotoxicity of the current sample extracts in comparison to the standard gallic acid. The study of Barua *et al*. 2020 also showed that the phytochemical screening of *M. charantia* L. pet ether fruit extract does not contain alkaloids. These findings further strengthen the statement that the cytotoxic active compounds of the current sample are better extracted with polar solvent rather than the non-polar ones.

CONCLUSION

A cytotoxic determinant analysis of the test plants' ethanol, ethyl acetate, and pet ether extracts revealed lethal and insecticidal components in the extract and implored the practical use in future pest control and pharmaceutical endeavors. Despite the inadequate elucidation of the brine shrimp lethality assay, it is very convenient to assess the bioactivity of the plant extracts. Based on the result of our findings, it can be concluded that the cytotoxic response of ethanol extract of 'TIA' variety of *M. Charantia* is greater than the ethyl acetate and pet ether fruit extract, which could be potentially subjected to clinical trials for anticancer treatment. Though, most studies on bioactive components have not yet been demonstrated their effects on humans. Thus, clinical research is a prerequisite to fully utilize its application in the health sector under the premise of ensuring safety.

Conflict of interest

The authors declare no conflict of interests

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