Comparative Analysis of in Vitro Antioxidant and Cytotoxic Activity of Unripe and Ripe Fruits of *Solanum sisymbriifolium*

Farhana Momen[1], Rashu Barua[1, 2], Md. Golam Kabir[1*]

[1]Department of Biochemistry and Molecular Biology, University of Chittagong
Chittagong-4331, Bangladesh.

[2]Department of Molecular Biochemistry, Nagoya University Graduate School of Medicine
Nagoya-466-0065, Japan

[*]*Corresponding author's email: Kabir [AT] cu.ac.bd*

ABSTRACT— ***Solanum sisymbriifolium Lam. (Solanaceae), commonly referred to as Kantikari (Bengali), sticky nightshade, or litchi tomato. Despite, the traditional use of this plant for several ailments, very little is known about the phytochemical and pharmacological content of the edible ripe berries of this medicinal plant. Ethanol and petroleum ether extract of the ripe and unripe fruits of Solanum sisymbriifolium were examined by using standard analytical methods to determine and compare their bioactive chemical constituents, total phenolic content, antioxidant, and cytotoxic potential. Quantitative phytochemical screening showed that the presence of medicinally active secondary metabolites like alkaloids, flavonoids, and saponins were detected in both ripe and unripe fruits. Antioxidant activity and total phenolic content were found to be more pronounced in ripe fruit extract, whereas unripe fruit extracts showed moderate cytotoxic activity. DPPH free radical scavenging method demonstrated that IC_{50} value of ethanol and petroleum ether extract of ripe fruits were 194.40µg/ml and 436µg/ml, and for unripe fruit the values were 689.82µg/ml and 1411.23µg/ml. In brine shrimp lethality bioassay, the LC_{50} value of unripe fruits was 230.6559µg/ml for ethanol extract and 314.9227µg/ml for petroleum ether extract. The LC_{50} value of the ethanol extract of ripe fruits was 456.1021µg/ml and for petroleum ether extract was 1,017.6848µg/ml. The results of this study showed that the litchi tomato not only has the potential to be a rich source of an edible compound due to the presence of profound phytochemical constituents but also can be used as a source of pharmacological references.***

Keywords— *Solanum sisymbriifolium*, Phenolic content, Antioxidant, Cytotoxicity.

---

1. INTRODUCTION

The popularity of medicinal plants as relief from numerous illnesses is rapidly increasing across the world despite the immense advancement of conventional chemical drugs. Countless side effects are often encountered with these drugs while treating and managing diseases, whereas medicinal herbs and natural products can become a source of hope to meet the healthcare needs due to the lower side effects in comparison to the prescription drugs. In today's world, soaring concern exists over the escalation in oxidative stress-related disorders such as cancer, cardiovascular disease, Alzheimer's, autoimmune disease, atherosclerosis, diabetes, multiple sclerosis [1]. When free radicals with an unpaired electron overwhelm the antioxidant protective system, a condition known as oxidation stress develops which leads to the disintegration of cell membranes and cell compounds including proteins, lipids, and nucleic acids [2]. It results from the imbalance between the productions of free radicles (reactive oxygen and nitrogen species) and the antioxidant defense system. Numerous studies with plant phytochemicals showed that the phytochemicals with antioxidant activity helps in converting the free radicals to less reactive species [3] [4]. Many researches have been focused on abilities of different plants to induce antioxidant effects due to the concerns about the safety of synthetic antioxidants. Various plant extracts contain antioxidant compounds that protect cells against the damaging effects of reactive oxygen species (ROS), such as peroxides, superoxide, hydroxyl radical, singlet oxygen, and alpha-oxygen [5]. Several studies have shown the inverse relationship between a human diet containing antioxidant-rich food and the incidence of human diseases [6], [7].

Researches on the screening of extracts and isolated constituents of medicinal plants used traditionally to treat cancer has intensified intending to find out potential cytotoxicity for cancer chemotherapy [8], [9]. Proper scientific evaluation of many traditionally used plants in Bangladesh for the cytotoxic effect would carry enormous potential and promise as part of pharmaceutical products [10].

Since ancient times both cultivated and wild species of the Solanaceae family have played an essential role in human nutrition and health [11]. Nevertheless, there are wild species with the great significance of this family that has not been reported much on their pharmacological activities. *Solanum sisymbriifolium* (Litchi Tomato) is one of the wild plants which is a viscid and very prickly erect spiny shrub commonly known as Kantikari (Bengali), Kanta begun, Sticky nightshade (English). It originates in South America, where it is used for cooking and medicinal purposes. It is commonly found throughout Bangladesh. This plant has been used as a trap crop for the management of potato cyst nematode (PCN) [12]. The chemical constituents previously reported being found in the roots of *S. sisymbriifolium* were cuscohygrine and solacaproine [13]. Solasodine (molecular formula: C27H43NO2), a glycoalkaloid used in the synthesis of corticosteroids, sex hormones, and a large component of oral contraceptives, has been isolated from this plant [14]. The edible fruits are globose-ovoid and shiny red when ripe, and comparable to cherries and gooseberries in taste. The berries contain sisymbriifolin (a neolignan) and carpesterol (a rare C30 sterol) [15]. Traditionally the root of the plant has been used in indigenous medicine for the treatment of hypertension, hysteria, diarrhea, respiratory tract infections, and various central nervous system (CNS) disorders such as epilepsy and depression [16]. These activities of *Solanum sisymbriifolium* may be due to the presence of a diversified groups of phytochemicals and compounds, and many of which possess antioxidant and cytotoxic activity. As we know, fruits synthesize a wide array of phytochemicals at different ripening stages that are reported to have health-promoting effects in humans. Therefore, to establish its traditional uses, the present investigations were carried out to study and compare the phytochemical screening and pharmacological activities of ethanol and pet ether extract from both ripe and unripe fruits of *S. sisymbriifolium* available in Bangladesh.

1. MATERIALS AND METHODS

2.1 Sample Collection and Extraction:

The fresh unripe and ripe fruits of *Solanum sisymbriifolium* were collected from the hilly areas of Khulsi, Chittagong, a district of Bangladesh. The study was carried out in the Department of Biochemistry and Molecular Biology, and the species was identified by Dr. Sheikh Bokhtear Uddin, Professor, Department of Botany. The collected fruits were washed and sun-dried for consecutive days. The dried unripe and ripe fruits were ground to a coarse powder with mortar and pestle separately. The dried powder of unripe and ripe fruits was macerated in ethanol and pet ether in a separate conical flask for 5 days at room temperature (25±1)^{0}C with occasional stirring. After completion of the extraction, the liquid was filtered using a sterilized Whatman No.1 filter paper. Then the filtrate was concentrated under reduced pressure below 50^{0}C through a cyclone rotary evaporator (RE 200, Bibby sterling, UK). The concentrated extracts were collected in a petri dish for complete evaporation of ethanol and pet ether. The whole process was repeated three times and finally, 15 gm Green-colored concentrated plant extracts of unripe fruits and 15 gm red-colored plant extract of ripe fruits were obtained (yield 2.22 % w/w). The green and red-colored crude extracts were preserved in the refrigerator at 4^{0}C.

2.2 In *Vitro* Assay of Antioxidant Activity of Ethanol and Pet ether Extract of Unripe and Ripe Fruits of *Solanum sisymbriifolium:*

The antioxidant activity of ethanol and pet ether extract from unripe and ripe fruits of *Solanum sisymbriifolium* and the standard antioxidant ascorbic acid were assessed based on the free radical scavenging effect of the stable 2, 2- diphenyl-1- picrylhydrazyl (DPPH)-free radical activity according to the method described by [17]. Briefly, Ascorbic acid and the ethanol and pet ether extracts (20, 40, 60, 80, 100, 200, 400, and 800 mg/ml) of ripe and unripe fruits of *Solanum sisymbriifolium* were prepared in methanol. DPPH (Sigma, USA) solutions (0.004%) were prepared in methanol and 5ml of this was mixed with the same volume of extracts and positive control solution separately. These solution mixtures were kept in dark for 30 min and the absorbance of the mixture was determined at 517 nm using UV-Visible Spectrophotometer (UV-1601 Shimadzu, Japan). Ascorbic acid was acted as a positive control and low absorbance of the reaction mixture indicated higher free radical-scavenging activity. The scavenging activity against DPPH was calculated using the following equation:

Scavenging activity (%) = [(A - B) / A] x 100, Here, *A* is the absorbance of the (DPPH solution without the sample), *B* is the absorbance of DPPH solution in the presence of the sample (extract/ ascorbic acid).

Then, % scavenging activity or % inhibition was plotted against log concentration and IC_{50} (Inhibition concentration 50) was calculated from the graph by linear regression analysis with Microsoft Office Excel 2007.

2.3 In Vitro Assay of Cytotoxicity of *Solanum sisymbriifolium* Unripe and Ripe Fruits:

Cytotoxic activity of ethanol and pet ether extract from *Solanum sisymbriifolium* were determined by Brine-Shrimp Lethality Bioassay as described by [17]. 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 and pet ether extract with different concentrations (20, 40, 60, 80 100, 200, 400, 600, 800 and 1000µg/ml) were prepared by dissolving them in DMSO (not more than 50µl in 5 ml solution) along with seawater. A vial of 30µl DMSO (Sigma, USA) was used as negative control and standard gallic acid (Sigma, USA) was used as a positive control. In each experimental and control vial, 10 Nauplius

were transferred with Pasteur pipette and were tested in triplicate. The test tubes were maintained under illumination. After 24 hours have elapsed, survivors were counted with the aid of a 3x magnifying glass. The LC_{50} values were calculated from Probit Chart using computer software "BioStat-2009".

2.4 Phytochemical Screening:

Phytochemical study was performed through conducting preliminary phytochemical group tests using unripe and ripe fruits of *S. sisymbriifolium*. The presence of alkaloids, flavonoids, and saponins was carried out according to the methods of Harbone (1973) as described by Edeoga et al. (2005), Boham, and kocipaiabyazan (1994) and Obadoni and Ocuko (2001) respectively. [18] [19] [20] [21]

2.5 Total Phenolic Content Determination:

Total phenolic content (TPC) of the ripe and unripe fruits of *Solanum sisymbriifolium* was determined by Folin–Ciocalteu reagent (FCR) [22]. Briefly, 5 ml freshly prepared dilute Folin–Ciocalteu Reagent and 4 ml of 7.5% sodium carbonate were added to 1 ml of dilute plant extract (200mg/ml) to prepare the reaction mixture. These mixtures were kept in dark for 1h at ambient conditions to complete the reaction. The absorbance was measured by using a spectrophotometer (Shimadzu UV PC-1600) at 765 nm. For standard reference, gallic acid was used and the results were presented as dry weight basis with µg gallic acid equivalent (GAE)/mg of sample.

3. RESULT

3.1 Qualitative Phytochemical screening

The phytochemical screening was carried out to analyze the presence of alkaloids, flavonoids, and saponins in both the ripe and unripe fruits of *Solanum sisymbriifolium*. Although, the mentioned phytochemical characters were present in both ripe and unripe fruits, the investigation revealed that unripe fruits are a rich source of alkaloids and saponins, whereas flavonoids is predominantly present in Ripe fruits (Table 1).

Table 1: Phytochemical Screening of unripe and ripe fruits of *Solanum sisymbriifolium*

Sl. No.	Phytochemicals	*Solanum sisymbriifolium*	
		Unripe Fruits	Ripe Fruits
1.	Alkaloids	++	+
2.	Flavonoids	+	++
3.	Saponins	++	+

3.2 Total Phenolic Content

Total phenolic content (TPC) of the fruit extracts were determined according to the method described by Folin–Ciocalteu reagent (FCR), expressed as Gallic acid equivalent phenol compounds and was reported as Gallic acid equivalents (GAE) by reference to a standard curve ($y = 0.013x + 0.106$; $r2 = 0.998$) (data not shown). Ethanol and pet ether extracts of ripe fruits showed higher TPC content which were 62.15 and 57.83 µg GAE/mg of dry weight compared to the ethanol and pet ether extracts of unripe fruits which was 48.75 and 46.28 µg GAE/mg of dry weight, respectively (table 2).

Table 2: Total phenolic content of ethanol and pet ether extracts of unripe and ripe fruits of *Solanum sisymbriifolium*

Name of samples	Total phenolic content (µg/gm equivalent of Gallic acid)
Ethanol extract of unripe fruits of *Solanum sisymbriifolium*	48.75±0.30
Ethanol extract of ripe fruits of *Solanum sisymbriifolium*	62.15±0.31
Pet ether extract of unripe fruits of *Solanum sisymbriifolium*	46.28±0.50
Pet ether extract of ripe fruits of *Solanum sisymbriifolium*	57.83±0.26

Values are means (n=3) ±SD. Here, all the values are statistically significant at the 5% level.

3.3 In vitro Assay of Antioxidant Activity

A widely used in-vitro mechanism for screening the antioxidant activity of plant extract is DPPH radical scavenging due to their hydrogen donating ability [23]. In this assay, the violet/purple-colored DPPH solution was reduced to the yellow-colored stable diamagnetic molecule, diphenylpicryl hydrazine, by the addition of an antioxidant compound [24] [25].

Here, the DPPH scavenging effect of the ripe and unripe fruit extract was compared with ascorbic acid as a standard antioxidant. Both the ethanol and pet ether extract of ripe and unripe fruits of *Solanum sisymbriifolium* have shown dose-dependent activity in comparison with Ascorbic acid reference. The antioxidant activity of the fruit extracts was measured by the decrease of absorbance at 520nm. The increased concentration of both the fruit extracts and the standard showed an increase in antioxidant activity by decreasing the free radicals in the mixture [26].

Eight different concentrations were used in this study and at concentration of 800 µg/mL, the value for ripe fruit's ethanol extract amounted to 74.13%, while a similar concentration of green unripe fruit ethanol extract showed a far lower antioxidant activity (53.47%) (Fig 1). As DPPH scavenging activity %), fully mature ripe fruit's pet ether extract's activity (60.59%) was greater than unripe green fruits pet ether extract's (47.57%) (Fig 1). The IC_{50} value of ethanol extract of ripe fruits, ethanol extract of unripe fruits, Pet ether extract of ripe fruits, Pet ether extract of unripe fruits, and Ascorbic acid were found 194.40µg/ml, 689.82µg/ml, 436µg/ml, 1411.23µg/ml, and 1.13µg/ml respectively (Supplement table 1).

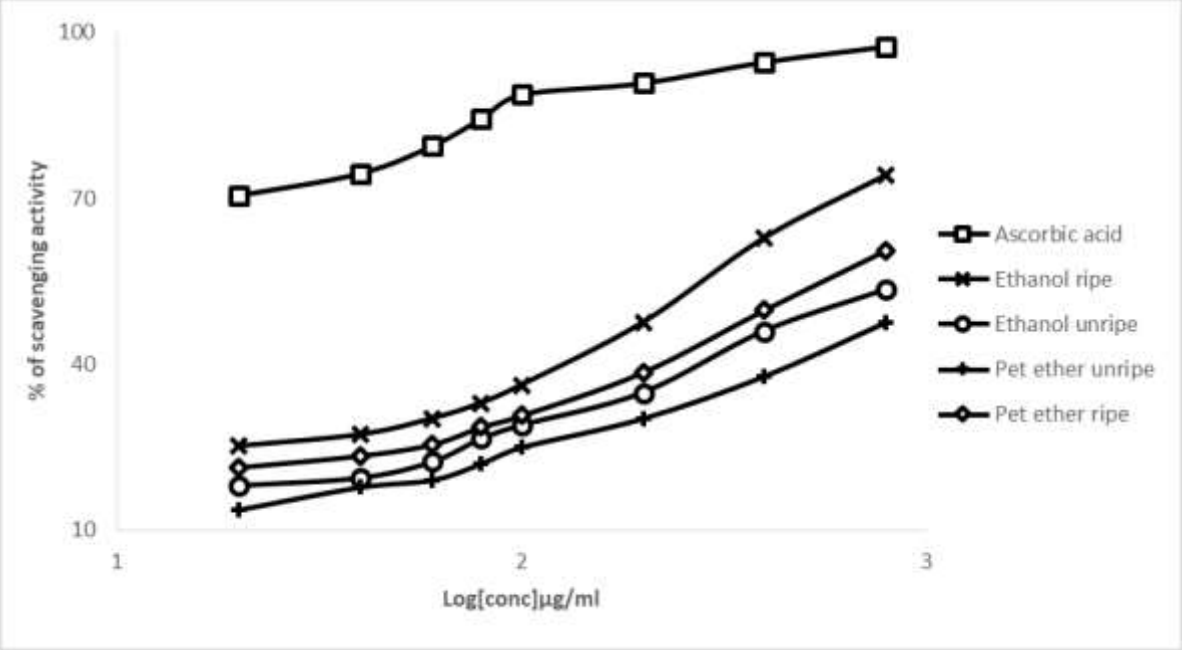

Fig. 1: Comparative % of scavenging activities Ethanol ripe (IC_{50} 194.40µg/ml), Ethanol unripe (IC_{50} 689.82µg/ml), Pet ether ripe (IC_{50} 436µg/ml), Pet ether unripe (IC_{50} 1411.23µg/ml) and Ascorbic acid (IC_{50} 1.13µg/ml)

3.4 In vitro Assay of Cytotoxic Activity:

Cytotoxic activity of ethanol and pet ether extracts of unripe and ripe fruits of *Solanum sisymbriifolium* was assessed using the Brine Shrimp Lethality Bioassay. The results showed that percentage lethality of brine shrimp at ten different concentrations (20 to 1000 µg/ml) of ethanol and pet ether extracts of unripe and ripe fruits exhibited lethality in a dose-dependent manner. Ethanol extract of unripe fruits showed 0,10,20,20,30,40,50,80,80 and 100% mortality of brine shrimp at 20, 40, 60, 80 100, 200, 400, 600, 800 and 1000 µg/ml concentrations (Fig 2), respectively. The LC_{50} value of ethanol extract of unripe fruits was found 230.6559 µg/ml, with 95% confidence limit where the lower and upper limits were 158.2663 and 346.7523 µg/ml (Supplement table 2). However, pet ether extract of unripe fruits showed 0,10,10,10,20,40,50,70,70 and 90% mortality of brine shrimp at 20, 40, 60, 80 100, 200, 400, 600, 800 and 1000 µg/ml concentrations, respectively (Fig 2). The corresponding LC_{50} value was found 314.9227 µg/ml (Supplement table 2), with 95% confidence limit where the lower and upper limits were 214.8288 and 496.0832 µg/ml.

Ethanol extract of ripe fruits showed 0,0,10,10,20,20,40,50,70 and 80% mortality of brine shrimp at 20, 40, 60, 80 100, 200, 400, 600, 800 and 1000 µg/ml concentrations (Fig 2), respectively. The LC_{50} value of this extract was found 456.1021 µg/ml (Supplement Table. 2), with 95% confidence limit where the lower and upper limits were 306.8122 and 783.8993 µg/ml. Pet ether extract of ripe fruits showed 0,0,10,10,10,20,30,40,40 and 50% mortality of brine shrimp at 20, 40, 60, 80 100, 200, 400, 600, 800 and 1000µg/ml concentrations (Fig 2), respectively and the LC_{50} value of the corresponding extract was found 1,017.6848 µg/ml (Supplement Table. 1), with 95% confidence limit where the lower and upper limits were 543.9804 and 4,319.4246 µg/ml.

The LC_{50} value of Gallic acid was found 4.40 µg/ml (Supplement Table. 1) with 95% confidence limit where the lower and upper limits were 0 and 10.331 µg/ml.

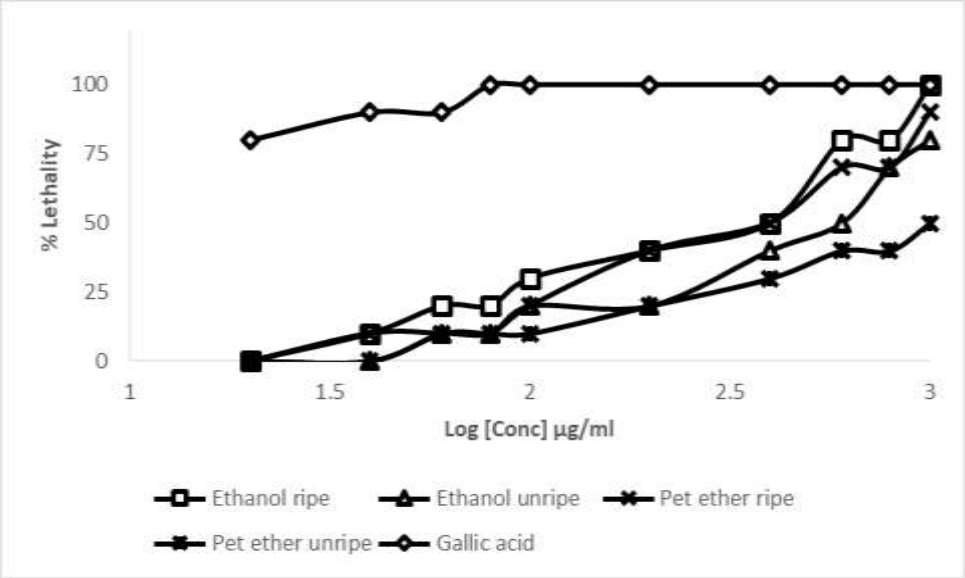

Fig. 2: Comparative % of lethality ethanol and Pet ether extract of unripe and ripe fruits of *Solanum sisymbriifolium* with Gallic acid (positive control)

4. DISCUSSION

4.1 Phytochemical Screening

The phytochemical screening revealed the presence of medicinally active constituents', alkaloids, flavonoids, and saponins in both ripe and unripe fruits of *Solanum sisymbriifolium*. These findings are in correlation with the previous phytochemical screening of the extracts which showed that *Solanum sisymbriifolium* possess alkaloids, flavonoids, saponins, and other secondary metabolites [27]. These secondary metabolites are accountable to produce definite pharmacological actions in the human body which can lead to the development of novel and safe medicinal agents [28] [29].

4.2 Total Phenolic Content

The current study assessed the Total Phenolic Content (TPC) in the extract of ethanol and pet ether of unripe and ripe fruits of *S. sisymbriifolium*. Results showed that ethanol extract of ripe fruits contained higher phenolic content (62.15µg GAE/mg) than the unripe extracts and the TPC value of pet ether extracts of matured red fruits was in close proximity of its ethanol extract (57.83 µg GAE/mg). Phenolic is a large class of chemical compounds, and in plants, they exist as secondary metabolites [30]. These are produced in plant cells through secondary metabolism and function in plant reproduction and growth, and had substantial health potential area of research and medical use [31]. Total phenolic content of plant extract is determined as an approach to get the quantitative data for compounds that have the antioxidant potential. Therefore, the nutritional and medicinal value of phenolic compounds provides enormous health benefits and serves as defense mechanisms to counteract reactive oxygen species (ROS) [32].

4.3 Antioxidant Activity:

Natural phenolic compounds such as flavonoids have redox properties that allow them to act as reducing agents and thus, show antioxidant property. In addition, they have the potential to metallic chelating [33]. The IC_{50} value obtained for ethanol and pet ether fruit extracts and the standard reference ascorbic acid indicate that the efficacy of both the fruits extracts is lower than the ascorbic acid. Researchers have shown that plants synthesize various kinds of secondary metabolites that act as antioxidants to thrive in hostile conditions situations [34] [35] [36]. The present study reveals that the ripe fruits of *S. sisymbriifolium* possess greater antioxidant activity compared to the activity exhibited by the fruits immature green stage which may be due to the presence of higher polyphenols and flavonoids in the ripened fruits extracts [37]. This phenomenon can be explained by the existence of higher concentration of flavonoids (demonstrated by the phytochemical screening) in ripe fruits of *S. sisymbriifolium* compare to the unripe one. Several studies have proved that, flavonoids, present in fruits and vegetables are one of the major groups where the free radical scavenging activity is attributed by a broad spectrum of biological activities and are responsible for diminishing free radicals and lipid peroxidation [38] [39].

4.4 Cytotoxic Activity:

Ethanol and pet ether extract of unripe fruits has shown moderate cytotoxic activity compared to the respective ripe fruits extract, in comparison with the positive control (Gallic acid). This result indicates that the fruits of *Solanum sisymbriifolium* in the immature developmental stage possess a higher amount of potent bioactive compounds, which might be useful as anticancer, antitumor, antimicrobial, and other pharmacological activities [40]. Thus, the unripe fruit extracts might be considered for therapeutic and clinical potentials for the treatment of many life-threatening diseases such as cancer [41]. The predominant presence of alkaloids and saponins in unripe fruits could be responsible for the observed bioactivity. Further investigations of the active principles reside in the unripe fruits of *Solanum sisymbriifolium* are necessary as a varied array of pharmacological activities are indicated by the potent cytotoxic activity of plant extracts.

5. CONCLUSION

On the basis of our research results, it can be stated that the unripe fruits extract of *Solanum sisymbriifolium* demonstrated a moderate level of phenolic content and free radical scavenging activity but showed potent cytotoxic effects, whereas ripe fruits exhibited reasonable cytotoxic effect with higher total phenolic content and greater values of antioxidant activity. Therefore, the ripe fruits of *Solanum sisymbriifolium* have a significant potential to use as a natural antioxidant agent and to include in food systems to maintain food quality. Furthermore, the demonstrated cytotoxic effects of the unripe green fruits might be subjected to clinical and therapeutic trials for life-threatening diseases such as cancer. However, further studies are required to explicate detail medicinal values of fruits of *Solanum sisymbriifolium* for possible therapeutic utilization.

6. CONFLICT OF INTEREST

The authors declared no conflict of interest.

7. FINANCIAL SUPPORT

No funding was received for the study.

8. ACKNOWLEDGEMENT

Authors are thankful to Dr. Sheikh Bokhtear Uddin, Professor, Department of Botany, University of Chittagong for identifying the plant.

9. REFERENCES

1. Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M., & Telser, J. Free radicals and antioxidants in normal physiological functions and human disease. *The international journal of biochemistry & cell biology*; *39*(1): 44–84, 2007.

2. Rahman, K. Studies on free radicals, antioxidants, and co-factors. *Clinical interventions in aging*, *2*(2): 219. 2007.

3. Barros, L., Cabrita, L., Boas, M. V., Carvalho, A. M., & Ferreira, I. C. Chemical, biochemical and electrochemical assays to evaluate phytochemicals and antioxidant activity of wild plants. *Food Chemistry, 127*(4): 1600-1608, 2011.

4. Choi, C. W., Kim, S. C., Hwang, S. S., Choi, B. K., Ahn, H. J., Lee, M. Y., ... & Kim, S. K. Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. *Plant science*, *163*(6): 1161-116, 2002.

5. Kähkönen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J. P., Pihlaja, K., Kujala, T. S., & Heinonen, M. Antioxidant activity of plant extracts containing phenolic compounds. *Journal of agricultural and food chemistry, 47*(10): 3954-3962, 1999.

6. Pandey, K. B., & Rizvi, S. I. Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative medicine a cellular longevity, 2*(5); 270-278, 2009.

7. Duthie, G. G., Duthie, S. J., & Kyle, J. A. Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. *Nutrition research reviews*; *13*(1): 79-106, 2000.

8. KIMURA, Y. New anticancer agents: in vitro and in vivo evaluation of the antitumor and anti-metastatic actions of various compounds isolated from medicinal plants. *In Vivo*, *19*(1): 37-60, 2005.

9. Balunas, M. J., & Kinghorn, A. D. Drug discovery from medicinal plants. *Life sciences*, *78*(5): 431-441, 2005.

10. Rahman, S., Hasnat, A., Hasan, C. M., Rashid, M. A., & Ilias, M. Pharmacological evaluation of Bangladeshi medicinal plants—a review. *Pharmaceutical biology*; *39*(1): 1-6, 2001.

11. Svobodová B, Kubáň V. Solanaceae: A family well-known and still surprising. Bentham Science Publishers; 2018 Nov 15.

12. Varandas, R., Egas, C., & Conceição, I. Luci. Potato cyst nematodes: New solutions to an old problem. *Crop protection*; *137:* 105303, 2020.

13. Ferro, E. A., Alvarenga, N. L., Ibarrola, D. A., Hellión-Ibarrola, M. C., & Ravelo, A. G. A new steroidal saponin from Solanum sisymbriifolium roots. *Fitoterapia*; *76*(6): 577-579, 2005.

14. Weissenberg, M. Isolation of solasodine and other steroidal alkaloids and sapogenins by direct hydrolysis-extraction of Solanum plants or glycosides therefrom. *Phytochemistry*; *58*(3): 501-508, 2001.

15. Chakravarty, A. K., Mukhopadhyay, S., Saha, S., & Pakrashi, S. C. A neolignan and sterols in fruits of Solanum sisymbri [i] folium (collected from India). *Phytochemistry*; 41(3): 935-939, 1996

16. Hellión-Ibarrola, M. C., Montalbetti, Y., Heinichen, O., Alvarenga, N., Figueredo, A., & Ferro, E. A. Isolation of hypotensive compounds from Solanum sisymbriifolium Lam. *Journal of ethnopharmacology*; *70*(3): 301-307, 2000.

17. Barua, R., Sultana, S., Talukder, M. E. U., Chakma, K., Hasan, C. M. M., & Islam, M. S. Antioxidant and cytotoxic activity of crude flavonoid fraction from the fruits of hybrid variety of Momordica charantia (Bitter gourd). *Journal of Pharmaceutical Research International*; 4(7): 778-786, 2014.

18. Harborne, J.B., Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 2nd edition, Chapman and Hall Publishers, New York; pp: 85, 1973.

19. Edeoga, H.O., Okwu, D.E and Mbaebie, B.O. Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotechnol; 4(7): 685-688, 2005.

20. Boham, B.A., & Kocipai-Abyazan, R. Flavonoids and condensed tannins from leaves of Hawaiian vaccinium vaticulatum and V. calycinium. Pacific sci; 48: 458-463. 1974.

21. Obadoni, B.O. and Ochuko, P.O. Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. Global J. Pure and Appl. Sci; 8:203-208, 2001

22. Mills, D.F. Symposia from the XII International congress on Nutrition Prog. Clin. Biol. Res; 77: 165 – 71, 1981.

23. Ozturk M, Ozturk FA, Duru ME, Topcu G. Antioxidant activity of stem and root extracts of Rhubarb (Rheum ribes): An edible medicinal plant. Food Chem; 103:623–630, 2007.

24. Marxen K, Vanselow KH, Lippemeier S, Hintze R, Ruser A, Hansen UP. Determination of DPPH radical oxidation caused by methanolic extracts of some microalgal species by linear regression analysis of spectrophotometric measurements. Sensors; 7:2080-2095, 2007.

25. Lee YR, Woo KS, Kim KJ, Son JR, Jeong HS. Antioxidant Activities of Ethanol Extracts from Germinated Specialty Rough Rice. Food Sci Biotechnol; 16:765- 770, 2007.

26. Apak, R., Özyürek, M., Güçlü, K., & Çapanoğlu, E. Antioxidant activity/capacity measurement. 1. Classification, physicochemical principles, mechanisms, and electron transfer (ET)-based assays. *Journal of agricultural and food chemistry*; *64*(5): 997-1027, 2016.

27. Gupta, V. K., Simlai, A., Tiwari, M., Bhattacharya, K., & Roy, A. Phytochemical contents, antimicrobial and antioxidative activities of Solanum sisymbriifolium. *Journal of Applied Pharmaceutical Science*; *4*(3): 75, 2014.

28. Prakash V, Saxena S, Gupta S, Singh SK. Preliminary phytochemical screening and biological activities of Adina cardifolia. J Microb Biochem Technol. 2015; 7:33–38.

29. Osman ME, Yassen HH, Deng Deng J, Hussein MS. General Phytochemical Screening and Antioxidant Activity of Some Sudanese Medicinal Plants. J Forest Prod Ind. 2014;3:292–295.

30. Zhang, H. F., Yang, X. H., & Wang, Y. Microwave assisted extraction of secondary metabolites from plants: Current status and future directions. *Trends in Food Science & Technology*; *22*(12):672-688, 2011.

31. Gorelick, J., & Bernstein, N. Elicitation: an underutilized tool in the development of medicinal plants as a source of therapeutic secondary metabolites. In *Advances in agronomy*; 124: 201-230. Academic Press, 2014.

32. Mohammedi, Z., & Atik, F. Impact of solvent extraction type on total polyphenols content and biological activity from Tamarix aphylla (L.) Karst. International journal of Pharma and Bio science; 2(1): 609-615, 2011

33. Rice-evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB. The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Radic Res.; 22(4):375-383, 1995.

34. Simlai, A., & Roy, A. Analysis of and correlation between phytochemical and antimicrobial constituents of Ceriops decandra, a medicinal mangrove plant, from Indian Sundarban estuary. *Journal of Medicinal Plants Research*; *6*(32): 4755-4765, 2012.

35. Shulaev, V., Cortes, D., Miller, G., & Mittler, R. Metabolomics for plant stress response. *Physiologia plantarum*; *132*(2): 199-208, 2008.

36. Reyes, L. F., & Cisneros-Zevallos, L. Wounding stress increases the phenolic content and antioxidant capacity of purple-flesh potatoes (Solanum tuberosum L.). *Journal of Agricultural and Food Chemistry*; *51*(18): 5296-5300, 2003.

37. Pourmorad, F., Hosseinimehr, S. J., & Shahabimajd, N. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *African journal of biotechnology*; *5*(11): 1142-1145, 2006.

38. Akhila S, Bindu A, Bindu K, Aleykutty N. Comparative evaluation of extracts of Citrus limon burm peel for antioxidant activity. J. Young Pharmacists, 1(2):136, 2009
39. Bernardi APM, López-Alarcón C, Aspée A, Rech SB, Von Poser GL, Bridi R, Dutrafilho CS, Lissi E. Antioxidant Activity in Southern Brazil Hypericum species. J. Chil. Chem. Soc. 53(4):1658-1662, 2008.

40. More, G. K. A review of the ethnopharmacology, phytochemistry and pharmacological relevance of the South African weed Solanum sisymbriifolium Lam. (Solanaceae). Environment, Development and Sustainability; 21(1), 37-50, 2019.

41. Kaur S, Michael H, Arora S, Härkönen PL, Kumar S. The in vitro cytotoxic and apoptotic activity of Triphala—an Indian herbal drug. J Ethnopharmacol;97(1):15-20, 2005.

10. Supplement

Table 1: DPPH free radical scavenging activity of ethanol and pet ether extracts of unripe and ripe fruits of *Solanum sisymbriifolium*

NAME OF SAMPLE	IC_{50}	Log IC_{50}
Ascorbic acid	1.13µg/ml	0.0540
Ethanol extract of *Solanum sisymbriifolium* unripe fruits	689.82µg/ml	2.84
Ethanol extract of *Solanum sisymbriifolium* ripe fruits	194.40µg/ml	2.29
Pet ether extract of *Solanum sisymbriifolium* unripe fruits	1411.23µg/ml	3.15
Pet ether extract of *Solanum sisymbriifolium* ripe fruits	436µg/ml	2.64

Table 2: LC_{50} Values of ethanol and pet ether extracts of unripe and ripe fruits of *Solanum sisymbriifolium*

NAME OF SAMPLE	LC_{50}
Gallic Acid	4.40 µg/ml
Ethanol extract of *Solanum sisymbriifolium* unripe fruits	230.6559 µg/ml
Ethanol extract of *Solanum sisymbriifolium* ripe fruits	456.1021 µg/ml
Pet ether extract of *Solanum sisymbriifolium* unripe fruits	314.9227 µg/ml
Pet ether extract of *Solanum sisymbriifolium* ripe fruits	1,017.6848 µg/ml