Journal of Applied Pharmacy

OPEN ACCESS Freely available online

Research Article

Investigation of Antioxidant and Cytotoxic Activity of Methanol Extracts of *Oroxylum indicum* and *Begonia roxburghii*

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ABSTRACT

Oroxylum indicum (*O. indicum*) and *Begonia roxburghii* (*B. roxburghii*) are medicinal plants widely used as drugs or component of drugs for treatment of various diseases. In this study, the methanol extracts of the leaf and stem of both plants were evaluated for their phytochemicals, antioxidant and cytotoxic activities. To measure *in-vitro* antioxidant potential, six methods were used such as DPPH free radical scavenging assay, superoxide radical scavenging activity, total phenolic content, total flavonoid content, total antioxidant content, and total antioxidant capacity determination assays. *O. indicum* stem and *B. roxburghii* leaves, showed strong antioxidant potential with IC_{50} values of 19.66 and 22.35 µg/mL, respectively in DPPH test and high superoxide radical scavenging activity with IC_{50} values of 19.57 and 26.96 µg/mL, respectively in SRS test. Moreover, both extracts showed 98.75 and 100.58 TPC (GAE) mg of gallic acid per gram of dried extract. At the highest concentration, in TAC *B. roxburghii* extract produced strong antioxidant potential represented by 98.58 mg of ascorbic acid per gram of dried extract whereas TACT of *O. indicum* found to be very high (150.56 mM of $FeSO_4$ per gram of dried extract). *In-vitro* cytotoxic potential of both extracts by MTT assay at different concentrations against MOLT-4 & HeLa cell lines respectively revealed that, at the highest concentration of 25 mg/mL, leaf extracts of both plants exhibited 90.58% and 80.66% of the cell death. Based on these preliminary findings of this study, further work could be the isolation of pure compounds those may be used as drugs for treating cancer and diseases caused by free radicals.

Keywords: DPPH scavenging; TPC; TFC; TAC; SRS; TACT; MTT assay

INTRODUCTION

Free radicals are responsible for causing a number of diseases such as cancer, cardiovascular diseases, neural disorder, Alzheimer's disease, Parkinson's disease, arteriosclerosis, ischemia, cancer, AIDS, diabetes, autoimmune disorders and aging [1] and are made throughout metabolism or by ecological contaminants, radioactivity, substances, pollutants and lead to cell membrane breakdown, membrane protein impairment and DNA mutation, which can cause diseases [2–5]. Medicinal preparations are prepared from various parts of plants such as leaves, stems, roots and flowers which comprise various chemicals and desired constituents [6]. Natural antioxidants have drawn profound interest as they can scavenge free radicals. Plant's secondary metabolites such as phenolics, flavonoids, tannins and proanthocyanidins have been found effective in reducing morbidity and mortality linked to degenerative diseases [7].

Cancer is a multistage process comprising of initiation, promotion and progression, thus it urges medicines that can interfere at different phases for the prevention and intervention of cancer [8]. Many discovered phytochemicals have been effective in the treatment of cancer and these provided leads for the development of novel anticancer drugs like paclitaxel [9]. However, due to poor selectivity and severe side effects of the currently available anticancer drugs, development of safer and more effecting chemotherapeutic agents are in demand [10].

Previously, different parts of *O. indicum* plant exhibited anti-inflammatory, antiulcer, antioxidant, anticancer, antimutagenic, cytotoxic, antiarthritic, immunostimulant, antiproliferative, and hepatoprotective activities in various *in vivo* and *in vitro* test models [11]. Since antioxidant activity using stem and cytotoxic activity against MOLT-4 had not been conducted on *O. indicum* and no antioxidant and cytotoxic potential were investigated on *B. roxburghii* previously, this study was focused on these two particular plant parts.

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Received: September 09, 2020; **Accepted:** March 18, 2021; **Published:** March 25, 2021

Citation: Akter R, Chowdhury R (2021) Investigation of Antioxidant and Cytotoxic Activity of Methanol Extracts of *Oroxylum indicum* and *Begonia roxburghii*. J Appl Pharm. 13:284.

MATERIALS AND METHODS

Collection and authentication of plant materials

In order to investigate *in-vitro* antioxidant and cytotoxic potential, plants were collected from Sylhet, Bangladesh and were identified as *O. indicum* (ACCESSION NO: DACB- 43774) and *B. roxburghii* (ACCESSION NO: DACB-73890) by the National Herbarium of Bangladesh (NHB), Mirpur.

Extraction procedure

Extraction of plant materials were performed by maceration process and the powdered leaves and stems were soaked in 900 mL of methanol in two separate beakers for five days at room temperature (22-25°C) and agitated occasionally. The solvent was removed by rotary evaporator and obtained extracts. These extracts were used for further investigation for antioxidant and cytotoxic properties [12].

Phytochemical screening of leaf and stem extract: To assess the qualitative chemical composition, phytochemical screening was performed on the leaf and stem extracts of both plants by various test methods. For the detection of alkaloids, flavonoids, carbohydrates, tannin, phenols, glycosides phytosterols, steroids and saponin different tests were performed. For example, Hager's test, Mayer's test, Wagner's test, Molisch's test Fehling's test, Ferric chloride test, potassium dichromate test, Borntrager's test, Libermann Burchard's test, Froth test and Salkowski test [13–16].

***In-vitro* antioxidant potential**: To determine the antioxidant activity of both extracts, DPPH (1, 1-diphenyl-2-picryl hydrazyl) free radical scavenging, superoxide radical scavenging (SRS) activity, Total phenolic content (TPC), Total flavonoid content (TFC), total antioxidant content (TACT) using FRAP assay, and total antioxidant capacity (TAC) determination assay methods were used [17].

Determination of DPPH (1, 1-diphenyl-2-picryl hydrazyl) free radical scavenging assay: Using L-ascorbic acid as standard, DPPH free radical scavenging assay of the extracts were determined. To prepare 0.004% (w/v) DPPH solution 2 mg of DPPH was dissolved in 50 mL methanol. The absorbance of solutions and control DPPH was measured at 517 nm against blank (methanol) using a spectrophotometer [18,19].

Determination of superoxide radical scavenging activity: Superoxide radical scavenging activity of both plant extracts was determined by the method described by Zhishen et al., [20]. Extract solutions were prepared using 0.05 M phosphate buffer of pH 7.8. Superoxide anion radicals were generated by illuminating the reaction mixtures containing different concentrations of both plant extracts using 20 W fluorescent lamps at 25°C for 40 minutes. Blue formazan was produced due to reduction of NBT by photochemically reduced riboflavin generated superoxide, O^{2-}. BHT (Butylated hydroxytoluene) was used as a standard and the absorbance of the standard and sample solutions was measured against blank at 560 nm [21].

Determination of total phenolic content: TPC of the both plant extracts were determined by using Folin-Ciocalteu method. The reaction was neutralized with sodium carbonate and the extracts were oxidized with Folin-Ciocalteu reagent. The absorbance of the standard and sample solutions was measured against blank at 765 nm by using spectrophotometer [22,23].

Determination of total flavonoid content: TFC of the extracts were determined by using this method where quercetin was used as a standard, potassium acetate and aluminum chloride were used as reagents. The absorbance of standard and sample were measured against blank at 415 nm by using spectrophotometer and TFC was expressed as Quercetin Equivalent (QE) per gram extract [23,24].

Determination of total antioxidant content: To measure total antioxidant content of each plant extract the FRAP (Ferric reduced capacity of plasma) assay described by Gohari et al., [25] was used. FRAP reagent was prepared by adding acetate buffer (pH 3.6), TPTZ (2, 4, 6-tripyridyl-s-triazine) solution and $FeCl_3.6H_2O$ solution in 10:1:1 ratio. $FeSO_4.7H_2O$ solution was used as standard. Certain volume of distilled water was added to certain volume of FRAP solution and incubated for 5 minutes at 37°C. After that different concentrations of plant extracts were added to the incubated FRAP solution and further incubated for 10 minutes at 37°C. Finally, the absorbance of the reaction mixture and the different concentration of $FeSO_4.7H_2O$ solution were measured at 593 nm.

Determination of total antioxidant capacity: Ammonium molybdate, L-ascorbic Acid (standard), sodium triphosphate, concentrated sulfuric acid were used as reagents for the determination of TAC. Finally, the absorbance of the standard and sample were calculated against blank measured at 695 nm using spectrophotometer [23,26].

Cytotoxicity evaluation by MTT assay

MOLT-4 (human acute lymphoblastic leukemia, T cell) cell line was used for *O. indicum* and HeLa cell (cervical cancer cell) line for *B. roxburghii*.

Cell culture: The MTT colorimetric assay was performed by using celltiter 96® non-radioactive cell proliferation assay kit (Promega, USA). Cells were seeded onto 96 well plates and incubated at 37°C and 5% of CO2 atmosphere. This cell line was cultured and maintained in DMEM (Dulbecco's Modified Eagles Medium) supplemented with 1% penicillin-streptomycin, 0.2% Gentamicin and 10% fetal bovine serum (FBS).

MTT colorimetric assay: The cytotoxic activity of *O. indicum* and *B. roxburghii* were performed by MTT assay on MOLT-4 and HeLa cell line following the method of the Centre for Advanced Research in Sciences (CARS), Dhaka. A number of 1×104 cells were seeded onto 96 well plates and allowed to adhere for 24 h. After 24 h of incubation, cells were treated with varying concentrations (0.025-25 mg/mL) of the extracts. After 48 h of incubation, the cells were examined for cytotoxicity using celltiter 96® non-radioactive cell proliferation assay kit (Promega, USA). This was followed by measuring absorbance at 570 nm using a 96 well plate reader. Cycloheximide, a standard cytotoxic compound, served as the positive control.

RESULTS

Phytochemical screening of the extracts

Phytochemical screening of methanolic extract of *O. indicum* and *B. roxburghii* showed the presence of flavonoids, phenols/phenolic compounds, tannins, glycosides and carbohydrates, (Table 1).

Other phytochemicals such as alkaloids, sterols, steroids, saponins and phytosterols were present either in *O. indicum* or in *B. roxburghii* extracts (Table 1).

DPPH free radical scavenging activity of the extracts

With an increase in concentration of *O. indicum* and *B. roxburghii* from 50 to 1200 µg/mL, % of inhibition of free radicals increased (Table 2). In DPPH test, at the highest concentration of 1200 µg/mL, *O. indicum* and *B. roxburghii* showed notable % of free radical scavenging; 91.23% and 90.60%, respectively. The standard ascorbic acid exhibited 99.23% of free radical scavenging at the same concentration. IC_{50} values of *O. indicum* and *B. roxburghii* were 19.66 and 22.35 µg/mL, respectively whereas IC_{50} value of the standard was 15.61 µg/mL.

Superoxide radical scavenging activity of the extracts

Superoxide radical scavenging activity with the both plant extracts was found to be concentration dependent (Table 3). The highest concentration of *O. indicum* and *B. roxburghii* showed the highest scavenging of superoxide radical scavenging which was 95.23% and 94.30%, respectively whereas the standard, BHT showed 99.43% superoxide radical scavenging. The IC_{50} values of *O. indicum* and *B. roxburghii* were 19.57 and 26.96 µg/mL, respectively and the IC_{50} value of *O. indicum* was comparable to that of standard, BHT (17.01 µg/mL).

TPC, TAC, TACT and TFC of the extracts

TPC assay revealed that at the concentration of 1200 µg/mL, *B. roxburghii* and *O. indicum* extracts showed the strong presence of 100.58 mg and 98.75 mg of gallic acid per gram of dried extract (Table 4). The findings of TAC assay showed notable antioxidant activity for *B. roxburghii* extract which was expressed as 98.58 mg of ascorbic acid per gram of dried extract whereas *O. indicum* extract exhibited moderate antioxidant capacity (68.67 mg of ascorbic acid per gram of dried extract) at the highest concentration of 1200 µg/mL. TACT assay findings showed remarkable total antioxidant content in *O. indicum* extract at the highest concentration which was 150.56 mM of $FeSO_4$ per gram of dried extract and at the highest concentration *B. roxburghii* showed the total antioxidant content of 95.13 mM of $FeSO_4$ per gram of dried extract (Table 4). However, the TFC assay showed 41.17 and 41.42 mg of quercetin per gram of *O. indicum* and *B. roxburghii* dried extracts, respectively. In all antioxidant activity assessment methods, antioxidant potential was concentration dependent.

MTT assay employed cytotoxic activity of the extracts

The result of cytotoxicity test revealed that at the highest concentration of 25 mg/mL, methanol extract of *O. indicum* leaves showed 90.58% of the cell growth inhibition against MOLT-4 cell line and *B. roxburghii* leaves showed 80.66% of the cell growth inhibition against HeLa cell line (Table 5).

DISCUSSION

Literature search revealed that antioxidant activity of *O. indicum* using SRS, TPC, TFC, TAC, TACT antioxidant assay methods had not been conducted before using stem extract though DPPH free radical scavenging was done before [27,28]. DPPH test was used in this study to compare with previous findings. On the other hand, no antioxidant properties and cytotoxic activity were investigated previously on *B. roxburghii* using cancer cell lines. Thus, this study was focused to explore the selected activities.

Phytochemical screening of both plant extracts showed the presence of different classes of phytochemical such as flavonoids, phenols, tannins and glycosides. It is noteworthy that *B. roxburghii* indicated

Table 1: Phytochemical screening of methanol extracts of *O. indicum* and *B. roxburghii*

Alkaloids	-	+	+++
Flavonoids	+	+	++
Phenols/phenolic compounds	+	+	+
Glycosides	+++	+++	+
Tannins	++	++	+++
Carbohydrates	+	+	+
Phytosterols	+	+	-
Resins	-	-	+
Steroids	+	-	+
Saponins	+	-	-

(+), (++) and (+++) indicate the existence of phytocompounds in a single method, two methods and three methods, respectively.

Table 2: DPPH free radical scavenging potential of methanol extracts of *O. indicum* and *B. roxburghii*.

Standard (L-ascorbic acid) and sample conc. (µg/mL)	**% of Inhibition by standard (L-ascorbic acid)**	**% of Inhibition of** O. indicum	**% of Inhibition of** B. roxburghii
50	35.38	35.38	30.18
100	55.32	55.32	55.56
200	64.10	62.10	65.36
400	81.43	77.56	80.26
800	90.45	86.45	89.50
1200	99.23	91.23	90.60
IC_{50} value (µg/mL)	15.61	19.66	22.35

Table 3: Superoxide radical scavenging activity of methanol extracts of *O. indicum* and *B. roxburghii*.

Standard (BHT) and sample conc. (µg/mL)	% of Inhibition by standard (BHT)	% of Inhibition of *O. indicum*	% of Inhibition of *B. roxburghii*
50	15.23	18.38	31.89
100	60.25	60.32	57.42
200	80.25	77.10	65.81
400	85.43	80.56	75.26
800	91.45	89.45	89.50
1200	99.43	95.23	94.30
IC_{50} value (µg/mL)	17.01	19.57	26.96

Table 4: Antioxidant activity of methanol extracts of *O. indicum* and *B. roxburghii* in TPC, TFC, TAC and TACT assay

Sample conc. (µg/mL)	TPC (GAE) mg of gallic acid/gram of dried extract		TFC (QE) mg of quercetin/ gram of dried extract		TAC (AAE) mg of ascorbic acid/ gram of dried extract		TACT mM of $FeSO_4$/gram of dried extract	
	O. indicum	*B. roxburghii*	*O. indicum*	*B. roxburghii*	*O. indicum*	*B. roxburghii*	*O. indicum*	*B. roxburghii*
200	41.67	40.17	24.67	24.67	67.25	54.59	18.03	10.12
400	64.08	64.25	27.50	27.41	68.17	67.25	50.47	35.20
800	90.92	90.92	35.83	35.58	68.33	84.16	85.57	61.04
1200	98.75	100.58	41.17	41.42	68.67	98.58	150.56	95.13

Table 5: Cytotoxic activity of methanol extracts of *O. indicum* and *B. roxburghii*

Sample conc. (mg/mL)	*O. indicum* % of inhibition of MOLT-4 cell	*B. roxburghii* % of inhibition of HeLa cell
0.025	5.26	10.15
0.25	25.13	15.43
2.5	40.19	30.23
25	90.58	80.66

the strong presence of alkaloids, tannins and flavonoid (Table 1).

In DPPH test of *O. indicum* stem at the highest concentration of 1200 µg/mL, the extract showed notable % of free radical scavengers which was 91.23% and *B. roxburghii* leaf produced 90.60% of free radical scavenging. Thus, it was evident that antioxidant activity of both extracts using DPPH test was comparable to that of standard (99.23% of free radical scavenging). Comparing IC_{50} values of both plant extracts, it was evident that *O. indicum* (IC_{50}=19.66 µg/mL) had higher antioxidant potential than that of *B. roxburghii* (IC_{50}=22.35) (Table 2). Moreover, ethanol extract of *O. indicum* leaf showed significant antioxidant activity (IC_{50}=24.22 µg/mL) in the previous study [11]. Though the plant parts are different but the antioxidant potential are very similar, which supports our findings.

SRS assay result evident that *O. indicum* had higher superoxide radical scavenging potential than that of *B. roxburghii*. It is noteworthy to mention that superoxide radical scavenging activity of *O. indicum* was close to the standard compound, BHT. Researchers have reported that superoxide radical scavenging potential of plant extracts or compounds is crucial as it provides first line defense against oxidative stress. The mechanism behind this protection involves the conversion of superoxide anions into oxygen and hydrogen peroxide by superoxide dismutase and hydrogen peroxide is further converted into water and oxygen by catalase in the human body [21].

TPC assay findings revealed that both plant extracts showed strong antioxidant potential of 100.58 mg and 98.75 mg of gallic acid per gram of dried extract of *B. roxburghii* and *O. indicum*, respectively (Table 4). Findings of previous study using pet. Ether extract/ methanol of bark/leaf and methanol extract of seed showed lower antioxidant activity compared to this study findings in terms of TPC quantification [29]

In TAC test, *B. roxburghii* had 98.58 mg of ascorbic acid per gram of dried extract which was much higher than that of *O. indicum* (68.67 mg of ascorbic acid per gram of dried extract) (Table 4). This higher antioxidant potential of *B. roxburghii* leaf may be attributed due to the strong presence of flavonoids and tannins in this extract (Table 1).

In TACT determination using FRAP assay, in presence of antioxidants TPTZ-Fe^{3+} complex is reduced to TPTZ- Fe^{2+} and an intense blue color is developed with an absorption maximum at 593 nm [30]. FRAP assay has been reported as a sensitive method to measure antioxidant potential of plant homogenates and plant products [25]. *O. indicum* showed much higher antioxidant content (150.56 mM of $FeSO_4$ per gram of dried extract) than that of *B. roxburghii* (96.13 mM of $FeSO_4$ per gram of dried extract.

In phytochemical screening flavonoids, phenols and phenolic compounds were present. These compounds might be responsible for the presence of strong antioxidant potential in both extracts [31,32].

In MTT test, at the highest concentration of 25 mg/mL, *O. indicum* and *B. roxburghii* leaf extracts showed 90.58% and 80.66% of the cell death, respectively (Figure 1). In previous cytotoxicity screening, 70.41% of cell growth inhibition was found against MCF7 breast cancer cells using n-butanol extract of *O. indicum* root bark [33]. In

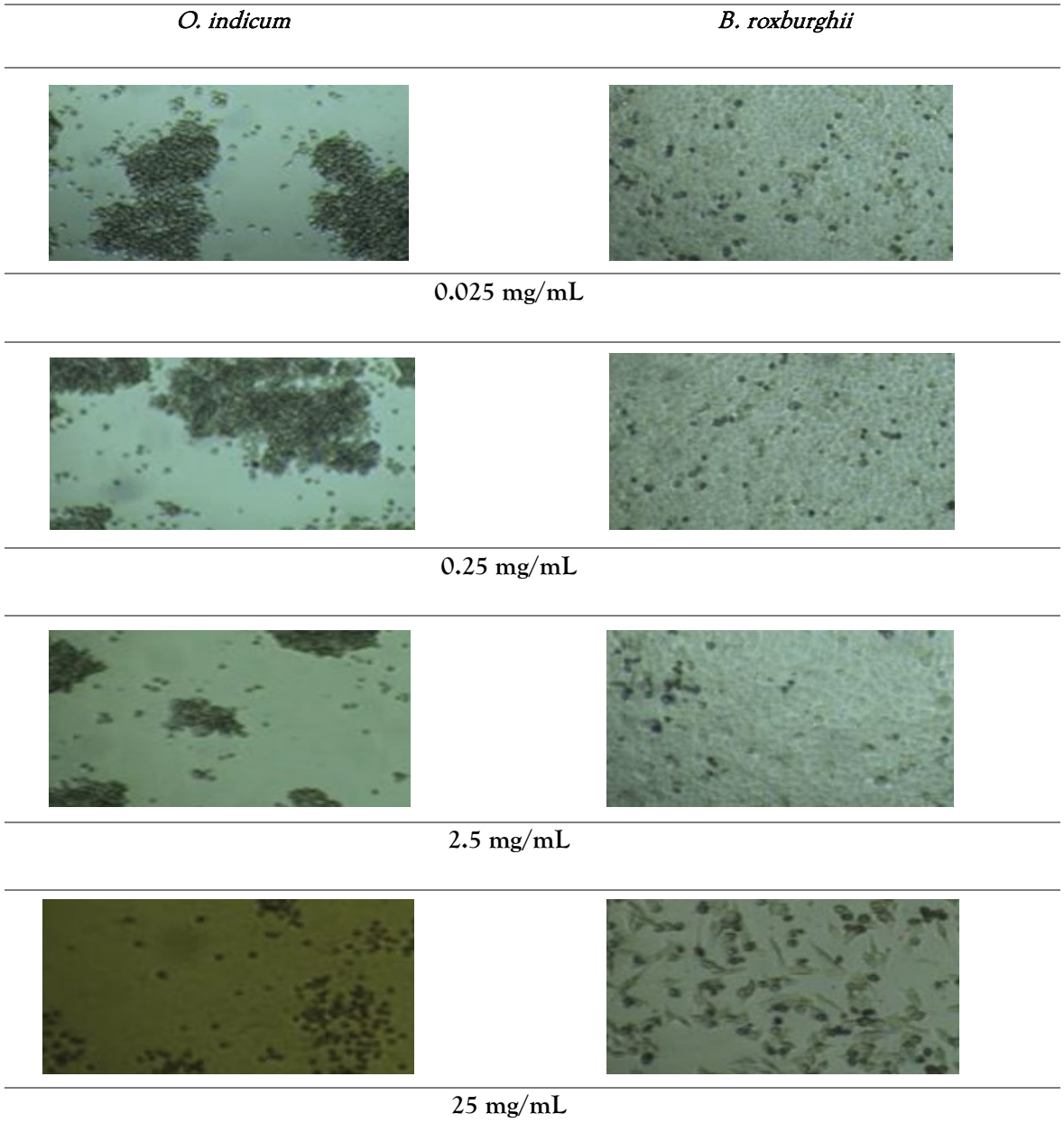

Figure 1: Viability of cancer cells at various concentrations of methanol extract of *O. indicum* and *B. roxburghii* leaf after incubation for at least 48 hours, as a positive control cycloheximide was used.

another study, PE extract of *O. indicum* stem showed IC_{50} =112.3 µg/mL against HeLa cells [34]. At conc. of 25-30 µM methanol extract of the fruits of *O. indicum* caused a 50% inhibition of HL-60 cells [11].

CONCLUSION

Study findings inferred that both plants showed strong antioxidant potential in all methods except TFC. Also, notable cytotoxic potential was observed in *O. indicum* and *B. roxburghii*. It can be concluded that these plants may be used as the remedies of cancer and free radical induced diseases. However, further studies are warranted for the isolation of phytochemical compounds and *in-vivo* experiment for the understanding of mechanism action of antioxidant and cytotoxic potential of them.

ACKNOWLEDGEMENT

We would like to express our most sincere gratitude Centre for Advanced Research in Sciences (CARS), which supported in the cytotoxicity activity. Additionally, we would provide gratefulness to the Department of Pharmacy, Brac University for accommodating the research facilities.

CONFLICT OF INTERESTS

All contributing authors declare no conflicts of interest.

REFERENCES

1. Alam N, Bristi NJ. Rafiquzzaman MD. Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. Saudi Pharm J. 2013;21(2):143-52.
2. Koushal P, calderon OH, Akter R, Daim A. *In vitro* Screening for phytochemicals and antioxidant activities of *Syngonium podophyllum* L .: An Incredible Therapeutic Plant. 2017;10:1267-1277.
3. Hossain S, Uddin MS, Kabir T, Begum M, Koushal P, Calderon OH, et al. *In vitro* screening for phytochemicals and antioxidant activities of

syngonium podophyllum l.: an incredible therapeutic plant. 2017;10:1267-1277.

4. Sharmin S, Kabir TM, Islam NM, Jamiruddin MR, Rahman I , Rahman A. Evaluation of Antioxidant, Thrombolytic and Cytotoxic Potentials of Methanolic Extract of *Aporosa wallichii* Hook. f. Leaves: An Unexplored Phytomedicine. 2018;8:51-56.
5. Uddin MS, Hossain MS, Kabir MT, Rahman I, Tewari D, Jamiruddin MR, et al. Phytochemical Screening and Antioxidant Profile of *Syngonium podophyllum* Schott Stems: A Fecund Phytopharmakon. 2018;8:120-128.
6. Begum Y, Sen P, Bulbul I, Nasrin F. Evaluation of Analgesic and Anti-inflammatory Potentials of the Leaf and Root Extracts of *Vanda roxburghii* (Roxb). 2018;5:1-8.
7. Gülçin I. Antioxidant activity of food constituents : an overview. Arch Toxicol. 2012;86(3):345-91.
8. Hassan R, Hussein F, Hawraa M, Akram H, Ahmad K, Ahmad D, et al. Antioxidant, Cytotoxic Properties and Phytochemical Screening of Two Lebanese Medicinal Plants. Int Res J Pharm. 2013;4(5):132-36.
9. Thakkar KN, Prasad AK, Nayak J, Iyer S V. Antioxidant and *in vitro* cytotoxic activity of extracts of aerial parts of Cocculus hirsutus (L) using cell line cultures (breast cell line). J Phyto. 2014;3(6):395-399
10. Ogbole OO, Segun PA, Adeniji AJ. *In vitro* cytotoxic activity of medicinal plants from Nigeria ethnomedicine on Rhabdomyosarcoma cancer cell line and HPLC analysis of active extracts. Altern Med. 2017;17(1):494.
11. Chaudhary A, Harminder, Singh V. A Review on the taxonomy, ethnobotany, chemistry and pharmacology of *Oroxylum indicum* vent. Indian J Pharm Sci. 2011;73(5):483-490.
12. Alara OR, Abdurahman NH, Ukaegbu CI. Journal of Applied Research on Medicinal and Aromatic Plants Soxhlet extraction of phenolic compounds from Vernonia cinerea leaves and its antioxidant activity. 2018;11:12-17.
13. Soni A, Sosa S. Phytochemical Analysis and Free Radical Scavenging Potential of Herbal and Medicinal Plant Extracts. Journal of Pharmacognosy and Phytochemistry. 2013;2(4):22-29.
14. Kamal T, Muzammil A, Akintunde R, Rahma MS, Omar MN. Preliminary phytochemical screening test of Garcinia griffithii Plant. 2012.
15. Jaradat N, Hussen F, Ali A Al. Preliminary Phytochemical Screening , Quantitative Estimation of Total Flavonoids , Total Phenols and Antioxidant Activity of Ephedra alata. J. Mater. Environ. Sci. (2015);6(6):1771-1778.
16. Khalid S, Shahzad A, Basharat N, Abubakar M, Anwar P. Phytochemical Screening and Analysis of Selected Medicinal Plants in Phytochemistry & Biochemistry. J Phytochemistry Biochem. 2018;2(1):2–4.
17. Riaz M, Shahid M, Jamil A, Saqib M. *In vitro* antioxidant potential of selected aphrodisiac medicinal plants. J Biol Regul Homeost Agents. 2017;31(2):419-24.
18. Lin C, Yu C, Wu S, Yih K. DPPH Free-Radical Scavenging Activity , Total Phenolic Contents and Chemical Composition Analysis of Forty-Two Kinds of Essential Oils. Journal of Food and Drug Analysis. 2009;17(5):386-395.
19. Alam N, Bristi NJ. Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. Saudi Pharm J. 2013;21(2):143-52.
20. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry. 1999;64(4):555-559
21. Ekin S, Bayramoglu M, Goktasoglu A, Ozgokce F, Kiziltas H. Antioxidant activity of aqueous and ethanol extracts of crataegus meyeri pojark leaves and contents of vitamin, trace element. J. Chil. Chem. Soc. 2017;62(4).
22. Wolfe K, Wu X, Liu RH. Antioxidant activity of apple peels. Journal of Agricultural and Food Chemistry. 2003;51(3):609-614
23. Amudha M, Rani S. Evaluation of *In Vitro* Antioxidant Potential of Cordia retusa Indian J Pharm Sci. 2016;78(1):80-86
24. Ahmed ZS, Nowrin T, Hemayet Ho M, Nasrin T, Akter R. Metabolite Profiling of *Crotalaria verrucosa* Leaf Extract and Evaluation of its Antioxidant and Cytotoxic Potency. Research Journal of Phytochemistry. 2018;12(2):60-70.
25. Gohari AR, Hajimehdipour H, Saeidnia S, Ajani, Hadjiakhoondi A. 2011. Antioxidant activity of some medicinal species using FRAP assay. Journal of Medicinal Plants. 2011;10(7).
26. Ahmed ZS, Nowrin T, Hossain H, Nasrin T, Akter R. Metabolite Profiling of *Crotalaria verrucosa* Leaf Extract and Evaluation of its Antioxidant and Cytotoxic Potency. 1819.
27. Sannigrahi S, Mishra S, Sinhamahapatra P, Nayak A, Das R. *In vitro* Antioxidant Potential of Different Parts of *Oroxylum indicum*: A Comparative Study 2010. Indian J Pharm Sci. 2010;72(2):267-269.
28. Trang DHT, Son H Le, Trung PV. Investigation on the *in vitro* antioxidant capacity of methanol extract, fractions and flavones from *Oroxylum indicum* linn bark. Braz. J. Pharm. Sci. 2018;54(1).
29. Samatha T, Shyamsundarachary R, Srinivas P, Swamy NR. Quantification of total phenolic and total flavonoid contents in extracts of *Oroxylum indicum* L. Kurz. 2012;5:177-79.
30. Carlsen MH, Halvorsen BL, Holte K, Bøhn SK, Dragland S, Sampson L, et al. The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide, 2010.
31. Lee MT, Lin WC, Yu B, Lee TT. Antioxidant capacity of phytochemicals and their potential effects on oxidative status in animals - A review. 2017;30:299-308.
32. Altemimi A, Lakhssassi N, Baharlouei A, Watson D, Lightfoot D. Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. 2017;6:42.
33. Dhru B, Bhatt D, Jethva K, Zaveri M. A review on the taxonomy, ethnobotany, chemistry and pharmacology of *Oroxylum indicum* vent. 2016;38:18-21.
34. Moirangthem DS, Talukdar NC, Bora U, Kasoju N, Das RK. Differential effects of *Oroxylum indicum* bark extracts: Antioxidant, antimicrobial,cytotoxic and apoptotic study. 2013;65:83-95.