European Journal of Biotechnology and Bioscience
www.biosciencejournals.com
ISSN: 2321-9122
Received: 25-06-2023, Accepted: 10-07-2023, Published: 26-07-2023
Volume 11, Issue 2, 2023, Page No. 7-11

Enzyme inhibitory and antioxidant properties of methanol and acetone extracts of three traditional medicinal plants

Pooja Bharti*, Anand Sagar, Shailja Chauhan
Department of Biosciences, Himachal Pradesh University, Shimla, Himachal Pradesh, India

Abstract
The present study was conducted to screen the enzyme inhibitory and antioxidant activities of methanol and acetone extracts of *Boenninghausenia albiflora*, *Broussonetia papyrifera* and *Xanthium strumarium*. Enzyme inhibitory activity was performed against enzymes alpha glucosidase and urease. Antioxidant activity was tested using DPPH assay and reducing power assay. All the three plants showed inhibition against enzyme alpha glucosidase and urease with maximum activity shown by methanol extract of *X. strumarium* (alpha glucosidase) and acetone extract of *B. papyrifera* (Urease). Also, methanol extract of *X. strumarium* showed maximum antioxidant activity with IC50 value (22.02 µg/ml) and EC50 value (271.61 µg/ml). No antioxidant activity was shown by *B. papyrifera*.

Keywords: Alpha glucosidase, urease, antioxidant, plant extract

Introduction
The wide chemical diversity and biological actions of natural products from plants have been considered the main source of treatment for various human diseases (Shengule *et al*., 2018) [1]. Diabetes mellitus is the most common metabolic disorder which is characterized by increase in blood glucose level for a longer duration (DeFronzo, 2004) [2]. The enzyme alpha glucosidase is involved in the digestion and absorption of carbohydrates in the small intestine. Hence, alpha glucosidase is the important therapeutic target to treat carbohydrate-mediated diseases (Chiasson, 2006; Elya *et al*., 2012) [3, 4]. Alpha glucosidase inhibitors slow down the digestion and absorption of carbohydrates. In this way, the post prandial glucose level is maintained at a lower level leading to a decreased insulin demand (Abbas *et al*., 2017) [5]. Another enzyme, urease from bacteria *Helicobacter pylori* is responsible for many diseases like gastritis, peptic ulcer, stomach cancer etc. (Mobley, 2001; Hassan, 2017; Mahbubul Hasan *et al*., 2018) [6, 7, 8]. There is need to find out natural potent enzyme inhibitors considering the toxicity and adverse side effects of synthetic drugs. In the living organisms, different reactive species like reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced by various metabolic processes and environmental stress. They induce the oxidative damage to biomolecules which leads to the occurrence of several health problems such as cancer, atherosclerosis, cardiovascular diseases, ageing and anti-inflammatory diseases (Braca *et al*., 2002) [9]. These reactive species are counteracted by natural antioxidants, which are known to possess beneficial health effects such as the potential to prevent diseases as compared to synthetic ones (Ana *et al*., 2014) [10]. Traditional medicinal system utilizes a large number of plants and their derived preparations for treating various health ailments. *Boenninghausenia albiflora* is used to treat vomiting, dysentery and as an antiseptic to treat cuts and wounds (Alam *et al*., 2011) [11]. *Broussonetia papyrifera* is used traditionally to treat prostatitis, impotence and ophthalmic disorders (Lee *et al*., 2001) [12]. *Xanthium strumarium* is used to cure allergic rhinitis, sinusitis, rheumatism, constipation, diarrhoea, leprosy etc. (Kamboj and Saluja, 2010) [13]. The present study was undertaken to evaluate the different biological activities of *B. albiflora*, *B. papyrifera* and *X. strumarium* because of little information known about alpha glucosidase inhibitory, urease inhibitory and antioxidant activities of these three plants.

Materials and methods
Reagents
All the reagents used were of analytical grade. Methanol, Acetone, Dimethyl sulfoxide (DMSO), Alpha glucosidase from *Saccharomyces cerevisiae*, p-Nitrophenyl-α-D-glucopyranoside (PNPG), Acarbose, Urease from *Canavalia ensiformis* and Thiourea were procured from Sigma-Aldrich, India. Butylated hydroxytoluene (BHT), 2,2-diphenyl-1-picrylhydrazyl (DPPH), di-Potassium hydrogen phosphate, Potassium dihydrogen phosphate, di-Sodium hydrogen phosphate, Sodium dihydrogen phosphate, Sodium carbonate, Sodium hypochlorite and Urea were procured from HiMedia, India. Ferric chloride, L-Ascorbic acid, Potassium ferricyanide, Phenol, Sodium hydroxide, Sodium nitroprusside and Trichloroacetic acid were obtained from CDH, India.

Extract preparation
Plant samples were collected from Mandi district of Himachal Pradesh and identification was done by Associate Prof. Suresh Kumar (Department of Biosciences, Himachal Pradesh University, Shimla). Collected plant parts (root of *B. albiflora*, leaves of *B. papyrifera* and fruit of *X. strumarium*) were washed properly with tap water and surface sterilized with 0.1 % mercuric chloride. The material was then dried in shade. Dried samples were crushed and extracts were prepared with two solvents (methanol and acetone) using Soxhlet apparatus. Extracts were evaporated to dryness and stock was prepared using DMSO and stored in refrigerator until further use.

Enzyme Inhibitory Activity

1. Alpha glucosidase inhibitory assay

Enzyme inhibitory activity was assessed using a previously described method (Mugaranja and Kulal, 2020) [14] with some modification. Different concentrations (5, 10, 15, 20 and 25 µg/ml) of plant extracts (0.25 ml) were mixed with 0.25 ml of enzyme alpha glucosidase (0.02 units/ml) and incubated at 37℃ for 20 min. The substrate, 0.25 ml of PNPG (10mM) was added to the mixture and incubated at 37 ℃. After 30 min. 0.5 ml of Na_2Co_3 (200mM) was added to stop the reaction. The absorbance was taken at 405 nm. IC50 was calculated from graph of percent inhibition activity and concentration of plant extracts. Acarbose was taken as reference standard. Reaction mixture containing buffer instead of plant extract was taken as negative control.

2. Urease inhibitory assay

Urease inhibitory activity of plant extracts was determined following a previously described method (Paulo *et al*., 2011) [15] with slight modifications. Reaction mixture containing 20 µl of urease enzyme solution (units/ml), 80 µl sodium phosphate buffer (100 mM, pH 7.4), 100 µl of test sample in different concentrations (2, 4, 6, 8, 10 µg/ml) was incubated for 10 min. Thereafter, 100 µl of urea (25mM) was added and reaction mixture was incubated for 30 min. 180 µl of phenol reagent (1 % w/v phenol and .005% sodium nitroprusside) and 280 µl of alkaline reagent (0.5% w/v NaOH and 0.075% active chloride NaOCl) was added to reaction mixture. The change in absorbance was measured at 636 nm after 50 min. Thiourea was used as reference standard. The negative control was prepared by using all the reagents except test sample.

Antioxidant Activity

1. DPPH radical scavenging assay

The antioxidant activity of plant extracts was measured using DPPH as described by Blois, 1958 [16] with some modification. Solutions with different extract concentrations (5-100 µg/ml) were prepared. 1 ml of different concentrations of the extract solution was mixed with 1:1 (v/v) of DPPH solution (0.1 mM). The mixture was shaken and left to stand for 30 min. in the dark at room temperature. The reduction of the DPPH-radical was measured by continuous monitoring the decrease in absorbance at 517 nm. BHT was taken as reference standard. The extract concentration providing 50% inhibition (IC50) was calculated from the graph of percent scavenging activity against extract concentration. The negative control was prepared by using all the reagents except test sample.

2. Reducing power assay

Method described by Oyaizu, 1986 [17] with slight modification was used to assess the reducing power of plant extracts. Various concentrations (20, 40, 60, 80, and 100 µg/ml) of all plant extracts (200 µl) were mixed with 500 µl of 200 mM potassium phosphate buffer (pH 6.6) and 500 µl of 1% potassium ferricyanide. The mixture was incubated at 50 ℃ for 20 min. After that 500 µl of 10 % trichloroacetic acid (w/v) was added and the mixture was centrifuged at 3000 rpm for 10 min. The upper layer (500 µl) was mixed with 500 µl deionized water and 100 µl of 0.1 % (w/v) of ferric chloride, and the absorbance was measured at 700 nm. Higher absorbance indicates higher reducing power. EC50 was calculated from the graph of absorbance at 700 nm against extract concentration. BHT was taken as reference standard. Reaction mixture containing buffer instead of plant extract was taken as negative control.

Statistical analysis

All the experiments were performed in triplicates and results were expressed as mean ± SD. The results were analyzed by one-way ANOVA and results were considered to be statistically significant with a 95% confidence level ($P<0.05$).

Results and conclusion

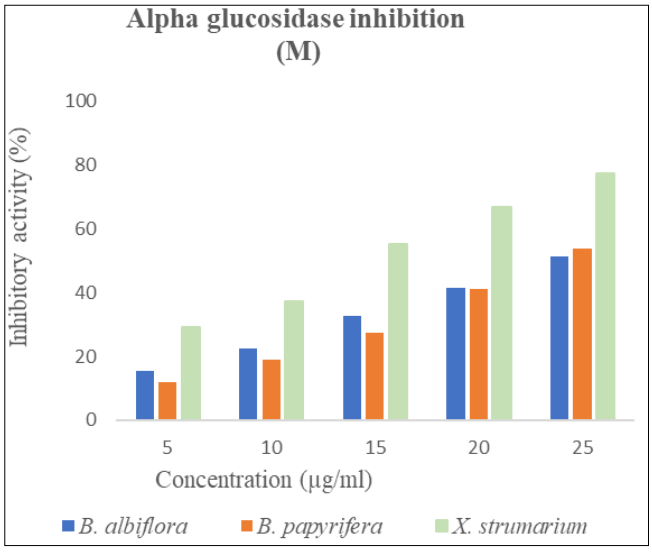
Enzyme inhibitory activity

X. strumarium showed maximum inhibition against alpha glucosidase and IC50 (µg/ml) was found to be 13.76±0.59 and 14.35±0.53 for methanol and acetone extracts respectively (Table 1). IC50 of standard acarbose was found to be 1.59±0.09 mg/ml. All the three plants were found to be active against enzyme urease. Maximum inhibition was shown by methanol extract of *X. strumarium* (IC50 4.30±0.14 µg/ml) and acetone extract of *B. papyrifera* (IC50 2.09±0.69 µg/ml). IC50 of standard thiourea was found to be 11.09±0.81 µg/ml, which is more than that of all plant extracts. Figure 1 presents the alpha glucosidase inhibitory activity of methanol and acetone extracts of *B. albiflora*, *B. papyrifera* and *X. strumarium* at different concentrations. Figure 2 presents the urease inhibitory activity of methanol and acetone extracts of three considered plants. It is clear from the graphs that with the increase in concentration of plant extract, inhibitory activity goes on increasing. Methanol extract was more active than acetone extract in most cases for enzyme inhibition.

Table 1: IC50 (µg/ml) of three plant samples for alpha glucosidase and urease inhibition.

Sample		IC50 * (Alpha glucosidase)	IC50 * (Urease)
B. albiflora	M	24.57±0.18	4.98±0.19
	A	32.09±1.10	6.76±0.27
B. papyrifera	M	24.14±0.47	4.99±0.28
	A	26.01±0.40	2.09±0.69
X. strumarium	M	13.76±0.59	4.30±0.14
	A	14.35±0.53	6.85±0.19

*Values are expressed as mean ± SEM. M-Methanol extract, A-Acetone extract.



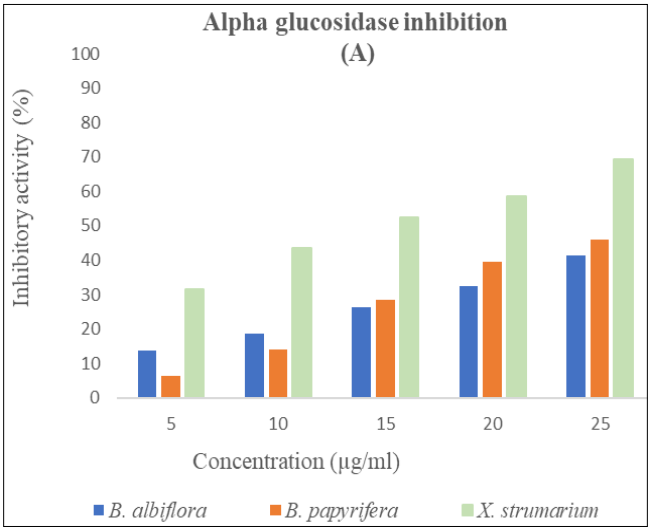
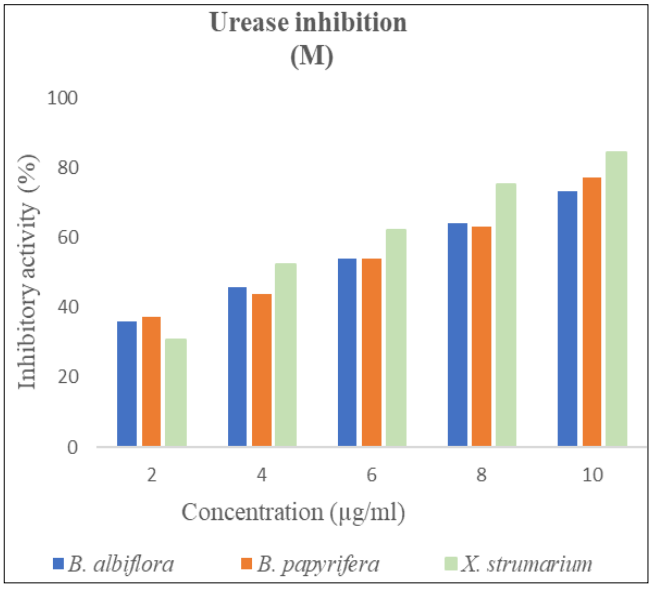


Fig 1: Alpha glucosidase inhibitory activity of methanol (M) and acetone (A) extracts of *B. albiflora*, *B. papyrifera* and *X. strumarium*.



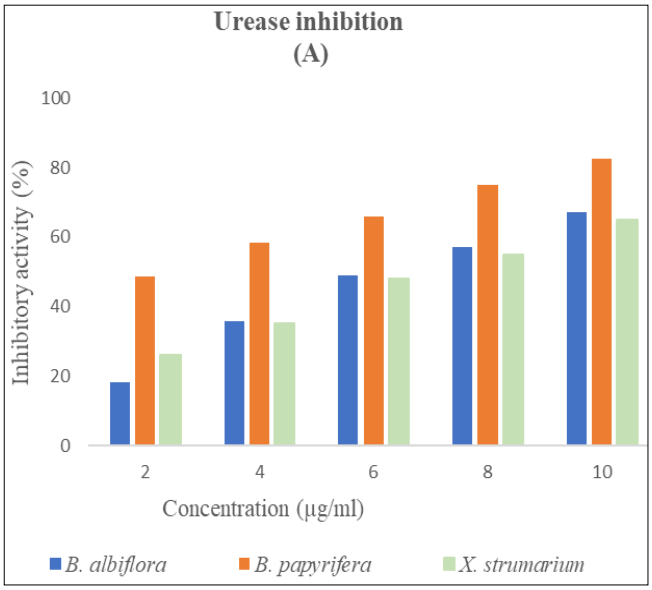


Fig 2: Urease inhibitory activity of methanol (M) and acetone (A) extracts of *B. albiflora*, *B. papyrifera* and *X. strumarium.*

Antioxidant activity

B. albiflora and *X. strumarium* were found to possess antioxidant activity. Maximum DPPH radical scavenging activity was exhibited by methanol extract of *X. strumarium* (57.13±1.31), which was better than standard BHT (44.41±1.38) at the concentration of 25 µg/ml (Table 2). At the concentration of 100 µg/ml, decreasing order of reducing power (absorbance) was found to be BHT> *X. strumarium*> *B. albiflora* for methanol and acetone extracts. DPPH scavenging activity of *X. strumarium* fruit is supported by the studies of Malpani *et al*., 2019 [18]. Antioxidant activity of plant extracts is dose dependent as it is evident from figure 3 which represents antioxidant activity in terms of DPPH scavenging activity and absorbance of methanol and acetone extracts of two plants at different concentrations. No antioxidant activity was found in *B. papyrifera*. Methanol extract showed more antioxidant activity than acetone extract in the present investigation which is in agreement with the results of other reports (Burli and Khade, 2007; Ahmad *et al*., 2020) [19, 20]. The antioxidant activity may be attributed to the different antioxidant components present in medicinal plants. The excellent natural sources of antioxidants or the major contributor to the antioxidant activity of medicinal plants are the phenolic compounds (Wong *et al*., 2006; Hossain *et al*., 2021) [21, 22].

Table 2: Antioxidant activity* of methanol and acetone extracts of two plants understudy.

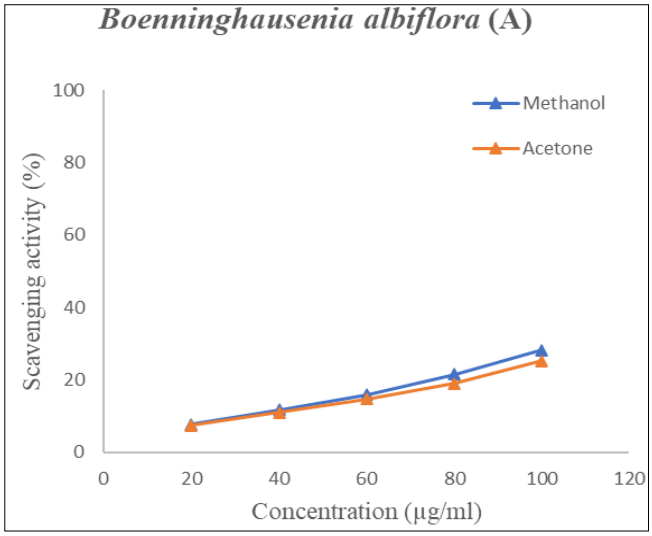
Sample	DPPH scavenging activity (25 µg/ml)		Absorbance (700 nm) (100 µg/ml)	
	M	A	M	A
B. albiflora	8.77±0.81	8.30±0.65	0.051±0.00	0.041±0.01
X. strumarium	57.13±1.31	17.87±0.49	0.195±0.01	0.077±0.01
BHT	44.41±1.38		0.455±0.01	

*Values are expressed as mean ± SD. M-Methanol extract, A-Acetone extract.

Table 3: IC50 and EC50 (µg/ml) values of two plants understudy.

Sample	IC50 (DPPH scavenging activity)		EC50 (reducing power)	
	M	A	M	A
B. albiflora	190.03	223.80	946.92	1649.00
X. strumarium	22.02	72.58	271.61	736.05
BHT	28.68		108.43	

M-Methanol extract, A-Acetone extract.



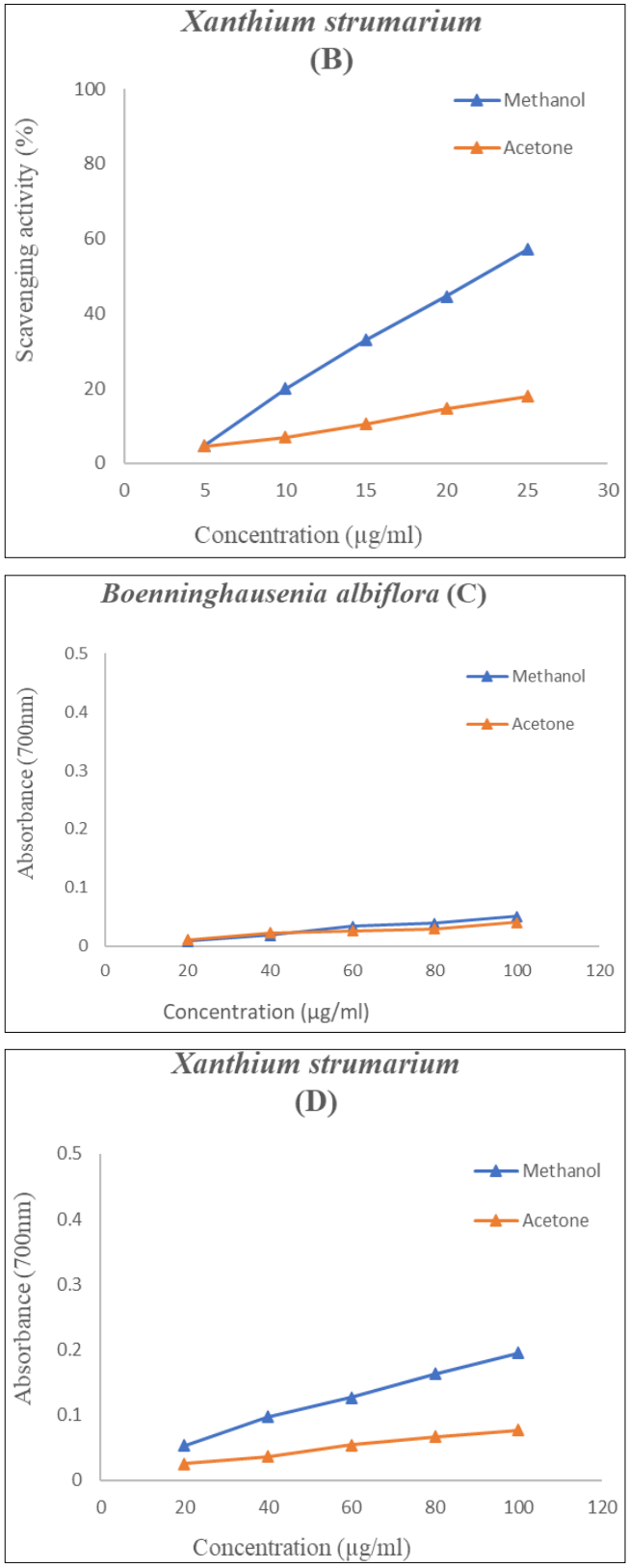


Fig 2: Antioxidant activity in terms of DPPH scavenging activity (A, B) and reducing power (C, D) of methanol and acetone extracts of *B. albiflora* and *X. strumarium.*

Conclusion

The observations of present work revealed that methanol and acetone extracts of *B. albiflora*, *B. papyrifera* and *X. strumarium* were found to have considerable enzyme inhibitory and antioxidant activities. This will form a basis for the further isolation, identification and characterization of natural enzyme inhibitors and antioxidants, and their further use in the food industries and human health.

References

1. Shengule SA, Mishra S, Joshi K, Apte K, Patil D, Kale P, Shah T, Deshpande M, *et al.* Anti-hyperglycemic and anti-hyperlipidaemic effect of *Arjunarishta* in high-fat fed animals. Journal of Ayurveda and Integrative Medicine,2018:9(1):45-52.
2. DeFronzo RA. Pathogenesis of type 2 diabetes mellitus. Medical Clinics of North America,2004:88(4):787-835.
3. Chiasson JL. Acarbose for the prevention of diabetes, hypertension, and cardiovascular disease in subjects with impaired glucose tolerance: The study to prevent non-insulin-dependent diabetes mellitus (stop-niddm) trial. Endocrine Practice,2006:12(1):25–30.
4. Elya B, Basah K, Mun'im A, Yuliastuti W, Bangun A, Septiana EK. Screening of α-glucosidase inhibitory activity from some plants of Apocynaceae, Clusiaceae, Euphorbiaceae, and Rubiaceae. Journal of Biomedicine and Biotechnology, 2012, 281078.
5. Abbas G, Al-Harrasi AS, Hussain H. α-Glucosidase enzyme inhibitors from natural products. In: Discovery and development of antidiabetic agents from natural products. Elsevier Inc. 2017, 251-269.
6. Mobley HLT. Urease. In-*Helicobacter pylori*: physiology and genetics. ASM press, Washington DC, 2001.
7. Hassan STS, Šudomová M. The development of urease inhibitors: What opportunities exist for better treatment of *Helicobacter pylori* infection in children? Children,2017:4(1):2.
8. Mahbubul Hasan SM, Chowdhury A, Keya CA. Urease: the ultimate therapeutic target for *Helicobacter pylori*. International Journal of Cell Science & Molecular Biology,2018:3(5):555625.
9. Braca A, Sortino C, Politi M, Morelli I, Mendez J. Antioxidant activity of flavonoids from *Licania licaniaeflora*. Journal of Ethnopharmacology,2002:79(3):379-381.
10. Ana A, Mariana O, Matevski V, Marin PD, Sonja DL. Antioxidant activity and total phenolic and flavonoid contents of *Salvia amplexicaulis* Lam. extracts. Archives of Biological Sciences,2014:66(1):307-316.
11. Alam MF, Safhi MM, Chopra AK. *In vitro* anti-malarial activity of aerial part of *Boenninghausenia albiflora*. Environment Conservation Journal,2011:12(3): 7-11.
12. Lee D, Bhat KPL, Fong HHS, Farnsworth NR, Pezzuto JM, Kinghorn AD. Aromatase inhibitors from *Broussonetia papyrifera*. Journal of Natural Products,2001:64(10):1286–1293.
13. Kamboj A, Saluja AK. Phytopharmacological review of *Xanthium strumarium* L. (Cocklebur). International Journal of Green Pharmacy,2010:4(3):129-139.
14. Mugaranja KP, Kulal A. Alpha glucosidase inhibition activity of phenolic fraction from *Simarouba glauca*: An *in-vitro*, *in-silico* and kinetic study. Heliyon,2020:6:e04392.
15. Paulo L, Oleastro M, Gallardo E, Queiroz JA, Domingues F. Anti-*Helicobacter pylori* and urease inhibitory activities of resveratrol and red wine. Food Research International,2011:44(4):964–969.

16. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature,1958:181:1199–1200.
17. Oyaizu M. Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine. The Japanese Journal of Nutrition and Dietetics,1986:44:307–315.
18. Malpani MO, Rajput PR, Chinchole KV, Kapse SS, Ambarkar, KS. Phytochemical screening and antioxidant activity of extracts of *Xanthium strumarium*, *Chrysanthemum* and their mixture. Rasayan Journal of Chemistry,2019:12(4):1901-1908.
19. Burli DA, Khade AB. A comprehensive review on *Butea monosperma* (Lam.) Kuntze. Pharmacognosy Reviews,2007:1(2):94-99.
20. Ahmad M, Mohammad N, Maswood AA, Aziz MA, Alam MA, Hossain MS, *et al*. Comparison of antioxidant role of methanol, acetone and water extracts of *Andrographis paniculata* Nees. Journal of Medicinal Plants Research,2020:14(8):428-437.
21. Wong CC, Li HB, Cheng KW, Chen F. A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. Food Chemistry,2006:97(4):705-711.
22. Hossain MA, Arafat MY, Alam M, Hossain MM. Effect of solvent types on the antioxidant activity and total flavonoids of some Bangladeshi legumes. Food Research,2021:5(4):329-335.