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Research Article

SCIENTIFIC EVALUATION OF *Buddleja asiatica*, *Camellia sinensis*, AND *Polygala arillata* OF NEPAL

Kabin Chemjong, Bimala Subba*
Central Department of Chemistry, Tribhuvan University, Kirtipur, Kathmandu, Nepal
**Correspondence: bimalasubba@gmail.com*
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ABSTRACT

Diabetes is one of the most prevalent diseases in the world. Medicinal plants remain to play an important role in the amelioration of diabetes and its complications. In this study, *Buddleja asiatica, Camellia sinensis*, and *Polygala arillata* were investigated for α-amylase inhibition, antioxidant, and antimicrobial activities. Methanolic extracts of these three plants were investigated for their potential antioxidant activity using DPPH assay. Total phenolic and flavonoid contents of extracts were evaluated by the following standard methods. The α-amylase inhibition assay was also carried out by starch-iodine assay and DNS assay method. Methanolic extract of *B. asiatica, C. sinensis,* and *P. arillata* exhibits a total phenolic content of 116.47 ± 6.62, 157.17 ± 18.79, and 44.27 ± 2.97 mg GAE per gm in dry weight of extracts ($p < 0.0001$) and flavonoid levels 5.99 ± 1.00, 34.28 ± 2.63, and 37.07 ± 2.24 ($p < 0.0001$) mg QE per gm in dry weights of extracts respectively. The extract showed promising antioxidant activity against DPPH with the IC_{50} values of *C. sinensis* (19.15 ± 4.32 μg/mL), *B. asiatica* (26.86 ± 2.00 μg/mL), and the standard the quercetin (2.36 ± 0.13 μg/mL). The extract showed modest α-amylase inhibitory activity. Methanolic extract of *B. asiatica* and *C. sinensis* were found effective against *Staphylococcus aureus* with zone of inhibition of 12.5 mm and 17 mm respectively. Therefore, obtained results support the traditional uses of these plants and also showed α-amylase inhibition activities although there are no reports about it.

Keywords: 96-well plate microreaders, α-amylase inhibition assay, antimicrobial activity, DPPH assay, phytochemical screening, FT-IR spectra

INTRODUCTION

Buddleja asiatica belongs to the Buddleja genus. Buddleja genus of the family Scrophulariaceaec comprises about 100 species (El-Domiaty *et al*., 2009). It is native to the tropical lands of America, Africa, and Asian countries such as Nepal and India, and is commonly known as butterfly bush (Garg & Dengre, 1992). *B. asiatica* roots, leaves, and stems have been used as a traditional Chinese medicine for the treatment of fever, ache, diarrhea, and articular rheumatism (Khan *et al*., 2015). Pharmacologically, *B. asiatica* was screened for antihepatotoxic, antibacterial, hypotensive, anticancer, antifungal, and antimalarial activities (Ali *et al*., 2011). In Nepal, *B. asiatica* leaves and flowers are utilized as a religious offering to gods and goddesses, while crushed leaves are used as a fish poison and for beverage fermentation (Sai *et al*., 2019). More than 80 compounds including phenylpropanoids, flavonoids, phenylpropanoid esters, non-phenolic compounds, triterpene, saponins, iridoid glucosides, benzoates, triterpenoids, monoterpenes, acetogenins, steroids, shikimates as well as other trace elements were identified in the plant (Liu *et al*., 2008).

Camellia sinensis, the tea plant belongs to the *Camellia* genus. The genus has about 200 species, most of which are found in the Tibetan highlands, northern east India, Nepal, and southern China (Dufresne & Farnworth, 2001).

Camellia sinensis is commonly grown for beverage production, has great economic importance as well as health benefit or medicinal value. Compounds like catechins and other polyphenols in *C. sinensis* are credited for their medicinal properties like anticancer, anti-inflammatory, Alzheimer diseases, antidiabetic and antioxidant properties etc. (Dufresne & Farnworth, 2001).

Polygala arillata belongs to the Polygala genus. The genus has 500 species growing throughout the World and some of the species are listed in the CITES list as endangered species in Nepal (Joshi *et al.,* 2017). In Nepal, it is mainly distributed in hilly regions and reported from India (Sikkim), Myanmar, and the north of Vietnam. *Polygala arillata*, the extract of roots and stems of this plant is traditionally used to treat irregular menstruation, hepatitis, pneumonia etc. (Jiangsu New Medical College, 1977).

Type 2 diabetes is an important metabolic disorder that is characterized by a high glucose level in the blood (WHO, 2006). Diabetes is projected to affect 4% of Nepalese adults, or 696,900 people, out of a total adult population of 17,570,100 (World Health Organization, 2016). One of the effective methods to control blood glucose level in diabetic patient is to inhibit the carbohydrate hydrolyzing enzymes, i.e., α-amylase and alpha glucosidase by using synthetic drugs such as acarbose. The acarbose and other related drugs are associated with several side effects such as abdominal distention, flatulence, diarrhea and also are

expensive. (Deng *et al*., 2018). Many phytochemicals from plants are reported with potential digestive enzyme inhibiting properties with lesser side effects as the alternative of acarbose (Turba *et al*., 2020). It is necessary to evaluate both the antioxidant potential and the hypoglycemic activity of antidiabetic drugs to control the oxidative stress in many diabetic patients. In current research trend, the search for new antidiabetic agents has been focused on plants used in traditional medicine (Sabu & Kuttan, 2002). Literature survey revealed that *Polygala arillata* from Nepal has not been evaluated scientifically, but there are few reports from other countries (Xiong *et al.,* 2011; Radhamani & Britto, 2013; Radhamani & Britto, 2016). Hence, in this research report, the three medicinal plants such as *Buddleja asiatica, Camellia sinensis*, and *Polygala arillata* collected from the hilly region of Nepal were taken for α-amylase inhibition activity, antioxidant, and antimicrobial analysis.

MATERIALS AND METHODS

Materials and chemicals

The organic solvent ethanol, methanol, DMSO were of the commercially available analytical grade from Thermo Fisher scientific, India. Porcine pancreatic α-amylase enzyme, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 3,5-dinitrosalicyclic acid (DNS) reagent, ascorbic acid, gallic acid, quercetin, Mueller Hinton agar and Mueller Hinton broth were bought from Sigma-Aldrich, USA. Similarly, acarbose and neomycin standard drug were imported from Canvax Biotech Company, Germany. Microorganism *Salmonella typhi* ATCC14028 and *Staphylococcus aureus* ATCC 25923 stains were cultivated at Central Department of Chemistry Laboratory, Kirtipur, Nepal, and it was used for antimicrobial assay.

Collection, identification, and preparation of methanolic extracts

The collection of plants was done by ethnomedicinal approach from the eastern part of Nepal, specifically from Panchthar district, as shown in (Fig. 1). Then the different parts of a plant such as leaves of *C. sinensis* (3000-7000 feet), *B. asiatica*, (2000-6000 feet), and root of *P. arillata* (3000-6000 feet) were collected in plastic bags. The identification of the plants was verified at the National Herbarium and plant laboratories Department, Godavari, Nepal by an officer Tila Kumari Thapa and their Voucher specimen numbers are written in Table no.1.

Figure 1. Herbarium of study plants

Table 1. Voucher code of study plants

S.N	Scientific Name	Family	Parts use	Common name	Voucher code
a.	*Camellia sinensis* (L.) Kuntze	Theaceae	leaf	Chiya	001 K.C (KATH)
b.	*Buddleja asiatica* Lour.	Scrophulariaceae	leaf	Bhimsen patti	002 K.C (KATH)
c.	*Polygala arillata* Buch.-Ham.ex D. Don	Polygalaceae	root	Marcha	003 K.C (KATH)

The collected parts of the plant were cleaned, washed by tap water, and shade dried for 10-15 days. Dry powder of samples was prepared by grounding in mechanical grinder, packed in zip lock plastic bag and stored in cold place until use. The preparation of methanolic extract was done by cold percolation method using methanol as solvent. Plant extracts were prepared from weighed 40 gram in 120 mL of methanol in a ratio 1:3, and the solvent extract was obtained through filtration. The same procedure was repeated three times and dried extract in a vacuum rotatory evaporator in 150 rpm at 40 ºC. Then the extract was stored at 4°C.

Phytochemical screening

Phytochemical screening is the qualitative analysis of bioactive chemicals such as polyphenol, flavonoids, terpenes, polysaccharides, and other phytochemical constituents using appropriate reagents. The

phytochemical screening procedure used was a standard protocol by (Das *et al.*, 2018).

Total Phenolic content

The total phenolic content of plant extracts was determined by using folin ciocalteu reagent and a 96 well plate based colorimetric method (Ainsworth & Gillespie, 2007). In brief, 20 µL of different concentrations of standard gallic acid (10-80 µg/mL) was loaded on 96 well plates in triplicates manner. Similarly, 20 µL of 50% DMSO (control) and 20 µL plant extract samples of respective plants were loaded, and their initial reading was taken. After that, each well containing standard gallic acid, 50% DMSO, and plant extract sample were loaded with 100 µL of folin ciocalteu reagent (1:10) followed by 80 µL of sodium carbonate (1M) separately. Finally, it was incubated in a dark place for 15 minutes, and the absorbance was measured at 765 nm by using a microplate reader. In this way a standard curve for gallic acid was constructed, and the total phenolic content in the plant extract was calculated as milligram of gallic acid equivalent per gm of dry weight (mg GAE/g) by the following equation.

$$C = \frac{cV}{m}$$

Where C = total phenolic content in milligram per gallic acid equivalent (GAE)

c= concentration of gallic acid from the standard curve (µg/mL).

V = volume of the extract (mL).

m = mass of extract in gram (g)

Total flavonoid content

Flavonoids content of plant extracts was analyzed in the 96 well plate, which was a modification of the colorimetric method (Chang *et al.*, 2002). Initially, 20 µL of different concentrations of standard quercetin, 10-500 µg/mL, were loaded in plate as a triplicate manner. Similarly, along with 20 µL of plant extract (500 µg/mL) was also loaded in plate as a triplicate manner. Subsequently, 100 µL distilled water was added in each well to maintain the final volume of 120 µL, followed by 60 µL of ethanol, 10 µL 5% aluminum trichloride, 10 µL potassium acetate (0.5 M). Then, it was incubated in a dark place for 30 mins, and the absorbance was measured at 415 nm by using a microplate reader. Thus, a quercetin standard curve was constructed. The calculated total flavonoid content in extracts was expressed as milligram of quercetin equivalent to per gram of dry weight of extract by the following equation:

$$C = \frac{cV}{m}$$

Where, C = total flavonoid content in milligram to quercetin equivalent (QE)

c = concentration of quercetin from standard curve (µg/mL)

V = volume of extract in mL

m = weight of plant extract in gram (g).

Antioxidant (DPPH) assay

This assay was performed by using a standard protocol, (Subedi *et al.*, 2012), a slight modification was done. First of all, 100 µL of different concentration 40, 20, 10, 5, 2.5, 1.25, 0.625 and 0.3125 µg/mL of positive control quercetin, and extracts of different concentration 160, 80, 40, 20, 10, 5 µg/mL were loaded in a triplicate manner in 96 well plates respectively. Similarly, negative control 100 µL of 50 % DMSO was also loaded and recorded initial reading. Immediately, 100 µL of DPPH (0.1mM) was loaded in all wells of positive control, negative control, and sample extract respectively. Finally, it was kept in a dark place for 30 minutes thereafter absorbance was measured at 517 nm by using a microplate reader. A standard curve was obtained by plotting the graph between % inhibition and concentration of quercetin. Moreover, percentage inhibition was calculated by the following equation, and their fifty-percentage inhibitory concentration was measured.

$$\%\ \text{inhibition} = \frac{(A0 - At)}{A0} * 100$$

Where, A0 = Absorbance of control (i.e negative control)

At = Absorbance of test sample/ standard

In vitro α-amylase enzyme inhibition by starch-iodine assay

The α-amylase inhibition in plant extracts was analyzed by the iodine-starch method as given by the standard protocol of (Xiao *et al.*, 2006), where slight modification was done during the analysis. Initially, phosphate buffer solution at pH 6.9 for negative control (starch only 60 µL and starch with enzyme 40 µL), and for different concentrations of positive control acarbose and plant extracts 20 µL each were loaded in a triplicate manner on 96 well plates. Simultaneously followed by the addition of 20 µL of α-amylase 0.2 U in all the well of above, except in starch only. Furthermore, 20 µL of acarbose concentrations 625, 312.5, 156.25, 78.125, 39.06, 19.531, 9.765, 4.8828 µg/mL, and plant extract of 2500, 1250, 625, 312.5, 156.25 µg/mL were added, and immediately initial reading was taken at 620 nm. Then, the plate was incubated for 10 minutes at 37 ° C in an incubator. After incubation, 20 µL of 0.5% soluble starch substrate was loaded in all wells containing negative control, positive control, and plant extracts. Then, the second incubation of the plate was done for 15 minutes at 37 ºC. And after the second incubation, 20 µL of hydrochloric acid 0.1 M solution was loaded in all wells. Finally, 100 µL of 5 mM iodine solution was added, and optical density (O.D.) was measured at 620 nm by using a microplate reader. The percentage inhibition was calculated by using the following equation.

$$\%\ \text{relative enzyme activity} = \frac{(\text{Enzyme activity of test})}{(\text{Enzyme activity of control})} * 100$$

$$\%\ \text{inhibition in the } \alpha\text{-amylase activity} = (100 - \%\ \text{relative enzyme activity})$$

α-amylase inhibition by DNS assay

The analysis was done by using the protocol on slight modifications (Sudha *et al*., 2011). Initially, 200 µL of α-amylase of 2.5 U was taken and added with 200 µL of a fraction of plant extracts of different concentrations 2500, 1250, 625, 312.25, 156.125, 78.25, 48.06 µg/mL, and positive control 200 µL α-amylase was taken with 200 µL of different concentration of acarbose 781.25, 390.62, 195.31, 97.65, 48.82, 24.41, 12.207, 6.10 µg/mL respectively. Similarly, for negative control 200 µL of α-amylase was added with the 200 µL of 2 % DMSO solvent. Then for the blank 200 µL, phosphate buffer was taken with 200 µL of DNS reagent (96 mM). And also, the positive control, negative control, blank, and extracts in test tubes were incubated at 30 ºC for 30 minutes. After incubation, 200 µL of 1 % starch was added to all the test tubes containing the positive control, negative control, blank, and extracts. Then, the solution was incubated at 30 ºC for 5 minutes for a second time. Further, all the solutions containing positive control, negative control, and extracts were treated with the 200 µL of DNS reagent respectively. However, for the blank 200 µL of distilled water was added and heated in a water bath for 15 minutes at 85 ° C. Finally, all the solutions of positive control, negative control, blank, and extracts were diluted with 3 mL of distilled water. The diluted solution was loaded in the 96 well plates in a triplicate manner, and absorbance was read at 540 nm by using a microplate reader. Percentage inhibition was calculated by the following equation.

$$\% \text{ inhibition} = \frac{\text{A control} - \text{A Test/Std}}{\text{Acontrol}} * 100$$

Where Acontrol = $A_{\text{negative control}} - A_{\text{blank}}$

$A_{\text{test}} = A_{\text{test}} - A_{\text{colour sample}} - A_{\text{blank}}$

Antimicrobial assay

The antimicrobial assay was performed by well diffusion technique by using a standard protocol (Holder & Boyce, 1994). Sterilization of all the equipment was done by autoclaving at 120 ºC for 15 minutes in autoclave. Initially, the overnight culture broth was transferred into another test tube containing media and matched the turbidity with a standard Mac-farland solution. Then after matching with standard, Carpet culture was done by using a sterilized cotton swab on Mueller Hinton agar media. The carpeting was done by swabbing all over the Petri dish by rotating at 120º. After carpeting completely, well boring was done by using cork bores of 30 µL. Finally, sample extract, positive control, a negative control was loaded in a respective well of 30 µL by using a micropipette in the sterilized zone and incubated for 24 hours at 37 ºC. After incubation, a clear zone of inhibition around the plant extract inhibitor was detected, and diameter was measured from four points of circumference of plate, by using a measuring scale.

RESULTS AND DISCUSSION

Percentage yield of sample extracts

The methanolic extract weight of *C. sinensis, B. asiatica,* and *P. arillata* were found to be 20.49 gm, 10.84 gm, and 12.57 gm, and the yield percentage was found to be 17.08 %, 9.03 %, and 10.48 %, respectively.

Phytochemical screening

Methanolic extract of *B. asiatica, C. sinensis*, and *P. arillata* roots are screened with suitable reagents and their result obtained are given in Table no. 2. Phytochemical screening indicates that they contain a phenolic compound, flavonoids, tannins, saponins, terpenoids, quinones, and carbohydrates in *C. sinensis*. *Buddleja asiatica* leaf contains phenolic compounds, flavonoids, terpenoids, sterols, saponins, reducing sugar, carbohydrates. Similarly, *P. arillata* contains alkaloids, reducing sugar, terpenoids, saponins, glycosides, carbohydrates, sterols. However, the preliminary analysis result of phytochemical screening for the sample may differ for the same plant due to environmental factors like temperature, pressure, minerals contained in soil, and sunlight. The other parameters during analysis like collection time of the plant, size of grinding, percolation method, laboratory setup, and chemical grades also cause variation in the analysis. Even though it is not reliable, it gives qualitative information about bioactive compounds contained in an extract.

Total phenolic content in plant extracts

Polyphenols are present in a wide variety of plant sources, which contain one or more acidic hydroxyl residues attached to an aromatic phenyl ring. Total phenolic content was obtained as milligram per gram of tested sample in gallic acid equivalent (GAE), by plotting the standard curve of gallic acid as shown in (Fig. 2A). While the regression equation of standard curve was Y = 0.03250*X + 0.01084, and R^2 value 0.9853. So, total phenolic contents in methanolic extract of *B. asiatica, C. sinensis,* and *P. arillata* were found to be 116.47 ± 6.62, 157.17 ± 18.79, and 44.27 ± 2.97 mg GAE per gram in dry weight of extracts (< 0.0001) respectively. On comparative analysis among the selected plants as shown in the bar diagram (Fig. 2B), *C. sinensis* contains a highest phenolic content than *B. asiatica* while lowest in a *P. arillata*.

Furthermore, in the previous study it is reported that in aqueous and ethanol extracts of black tea contain 82.86 ± 3.18 mg GAE/g and 29.32 ± 0.62 mg GAE/g (Oh *et al*., 2013). The total polyphenolic content in water extracts of chloroform, ethyl acetate, and n-butane fractions were found to be 9.8 ± 0.57, 787 ± 26.32, and 310.9 ± 7.25 mg/g by Cheng *et al*. (2015). Another research done by Liu *et al*. (2017) reported that polyphenol in Quingzhuan dark tea in different fractions of water, ethyl-acetate, n-Butanol fraction contains 18.25 ± 0.21, 62.72 ± 2.63, and 31.09 ± 0.73 mg GAE/g. These were lower to local orthodox tea *C. sinensis*. On the other hand, Tong *et al*. (2018) reported total polyphenols content of black tea and baked black teas were 362.37 ± 5.00 mg GAE/g and 347.33 ± 1.77 mg

GAE/g. As reported by Islam (2011) in the black tea extracts was found 293.04 ± 4.22 mg/mL GLE of phenolic content. Similarly, Sai *et al.* (2019) analyzed total polyphenol in methanolic extracts in leaves of *B. asiatica* was 127.48 ± 1.58 mg GAE/g higher compared to our obtained result. The total phenolic content of *P. arillata* in leaf and stem were investigated by Radhamani and Britto. (2016) found to be 39 and 25.1 µg/g. which is comparable with our finding (Radhamani & Britto, (2016).

Table 2. Results of phytochemical screening of methanolic extracts leaf of *B. asiatica, C. sinensis, and P. arillata* roots.

S.N	Phytochemicals	*B. asiatica root*	*C. sinensis leaf*	*P. arillata* leaf
1	Alkaloids	-	-	+
2	Flavonoids	+	+	-
3	Reducing sugars	+	-	+
4	Terpenoids	+	+	+
5	Saponins	+	+	+
6	Phenolic compounds	+	+	-
7	Tannins	-	+	-
8	Glycosides	+	-	+
9	Coumarins	-	-	+
10	Sterols	+	-	+
11	Quinones	-	+	-
12	Carbohydrates	+	+	+

Where, (+) = Present and (-) = Absent

Quantitative analysis of flavonoid contents in the selected plant was analyzed by constructing a standard curve of quercetin with regression equation Y = 0.05020*X + 0.04924, and R^2 value 0.9924 as in figure 2A. As a result, total flavonoid content in methanolic extracts of *B. asiatica, C. sinensis* and *P. arillata* were obtained 34.28 ± 2.63, 37.07 ± 2.24, and 5.99 ± 1.00 ($P_{-value} < 0.0001$) mg QE per gm of methanolic extract in dry weights respectively. Then, comparative analysis in the selected plant extracts on the bar chart in figure 2B. There was the largest portion of flavonoid in the extract of *C. sinensis* than content in *B. asiatica*, and *P. arillata,* however, *P. arillata* root extract contains the least.

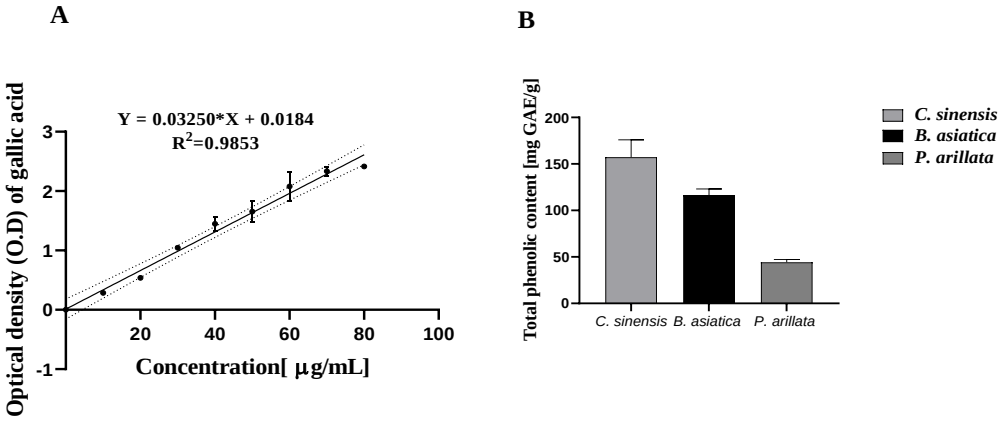

Figure 2. A) Standard curve of gallic acid; B) Bar-graph showing the total phenolic content (mg GAE/gram of dry extract)

Total flavonoid content in plant extract

Moreover, from the survey of literature as reported by Oh *et al.* (2013) total flavonoid content in black tea in water

and ethanol extracts was 14.89 ± 0.59 and 5.30 ± 0.03 mg QE/g. Similarly, Tong *et al.* (2018) reported 24.80 ± 60 mg of rutin equivalent/gram in Lipton black tea. Overall, the report suggests lower content of flavonoids than the result above. while, in the case reported by Sai *et al.* (2019) in the methanolic extracts of *B. asiatica* was 648.42 ± 2.88 mg of GAE/g.

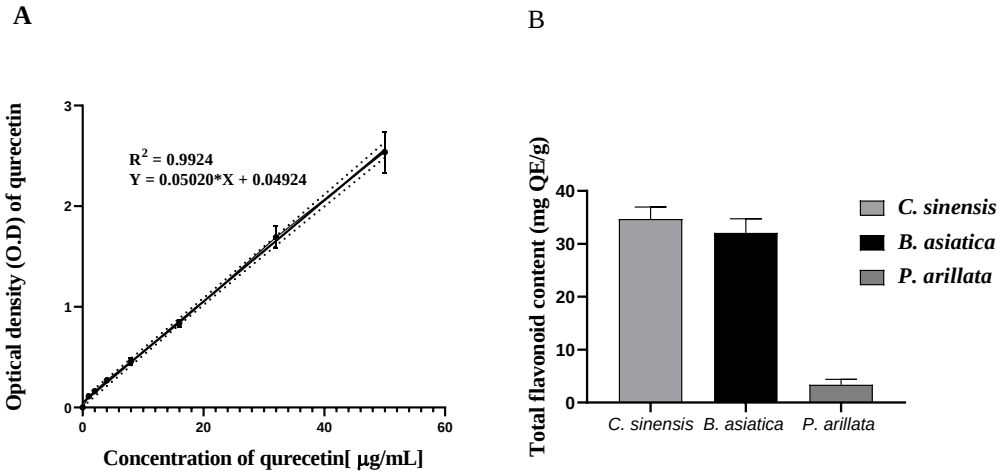

Figure 3. A) Standard curve of qurecetin B) Bar-graph showing the Total Flavonoid content (mg QE/gram of dry extract)

Antioxidant (DPPH) assay

During antioxidant analysis, quercetin was a positive control. The reading of percentage inhibition versus concentration as given in (Fig. 4A) was used to calculate fifty percentage inhibition of DPPH by using a nonlinear statistical method in a GraphPad prism 8.0.3. So, IC_{50} values of methanolic extracts of *B. asiatica, C. sinensis* and quercetin were found to be 26.86 ± 2.00, 19.15 ± 4.32, and 2.36 ± 0.13 µg/mL respectively. Therefore, the IC_{50} values of methanolic extracts *C. sinensis* (19.15 ± 4.32 µg/mL) and *B. asiatica* (26.86 ± 2.00 µg/mL) were lower than the Quercetin (2.36 ± 0.13 µg/mL) which means, that antioxidant potential activity of both the methanolic extracts of *C. sinensis* and *B. asiatica* were found to be lower than the standard quercetin. When comparing the methanolic extracts of *C. sinensis* and *B. asiatica*, the antioxidant potential of the methanolic extracts of *C. sinensis* was higher than that of *B. asiatica*. However, the IC_{50} value of *P. arillata* was not detected as shown in (Fig. 4B).

From the literature survey, Islam (2011) reported that aqueous Black tea shows IC_{50} values of 60.26 ± 1.74 mM, and Oh *et al.* (2013) analyzed in water fraction and ethanol fraction of black tea, and reported comparable their IC_{50} was 66.65 ± 1.55 and 28.91 ± 2.15 mg/g. While, in Lipton black tea, Tong *et al.* (2019) reported higher IC_{50} value of 0.077 ± 0.01 mg/mL. Cheng *et al.* (2015) also reported IC_{50} values of water extracts of Qingzhuan tea of chloroform, ethyl acetate, and n-butanol fractions, which were 244.6 ± 24.62, 6.6 ± 0.90, 17.3 ± 2.89 µg/mL respectively. These all IC_{50} are closer to the value of local tea *C. sinensis.* IC_{50} value of methanolic extracts of *B. asiatica* is reported in range from 3.04 -15 µg/mL while 26.86 ± 2.00 µg/mL in our study, which are quite comparable (Sai *et al* 2019, El-Domiaty *et al.* 2009, Sundararajan & Ilengesan. 2018). The result of this study also supports the potential antioxidant activity of the selected plants.

α-Amylase enzyme inhibition by Starch-iodine assay

To evaluate percentage inhibition starch as substrate, and acarbose as a positive control was taken. *C. sinensis* methanolic extracts show percentage inhibition of 61.431 ± 7.39, 41.405 ± 2.23, 25.977 ± 1.34, 16.640 ± 0.81, and 13.599 ± 2.25. Then methanolic extracts of *B. asiatica* shows percentage inhibition as 63.511 ± 5.60, 39.185 ± 4.46, 23.738 ± 3.69, 12.172 ± 0.27, 7.938 ± 0.60, and *P. arillata* shows the percentage inhibition as 34.670 ± 3.78, 23.393 ± 2.86, 21.369 ± 2.34, 16.865 ± 0.71, 9.514 ± 0.45. Similarly, Standard drug Acarbose shows percentage inhibition of 94.515 ± 3.29, 92.725 ± 1.78, 88.163 ± 4.09, 64.789 ± 4.84, 31.536 ± 1.34, 11.622 ± 1.17, 2.686 ± 1.09, 1.175 ± 0.40 respectively. So, the comparative graph of different plants and standard acarbose is plotted, and represented in graphical forms in figures 5A, 5B, and 5C. In addition, fifty Percentage Inhibitory Concentration (IC_{50}) of methanolic extracts of *C. sinensis*, *B. asiatica*, *P. arillata,* and acarbose were calculated by using nonlinear statistical method in a GraphPad prism 8.0.3. Therefore, the IC_{50} value of *C. sinensis, B. asiatica,* and acarbose were found to be 1737.65 ± 2.21 µg/mL, 1780.00 ± 3.06 µg/mL, and 55.74 ± 2.85 µg/mL respectively. As we know, the lower the IC_{50} the more efficient the extracts. Thus, the local tea *C. sinensis* extracts are more potent compared to the *B. asiatica* but both have less potential

compared to standard drug acarbose as shown in the bar diagram (Fig. 4D). However, the *P. arillata* IC_{50} value was not evaluated due to less percentage inhibition.

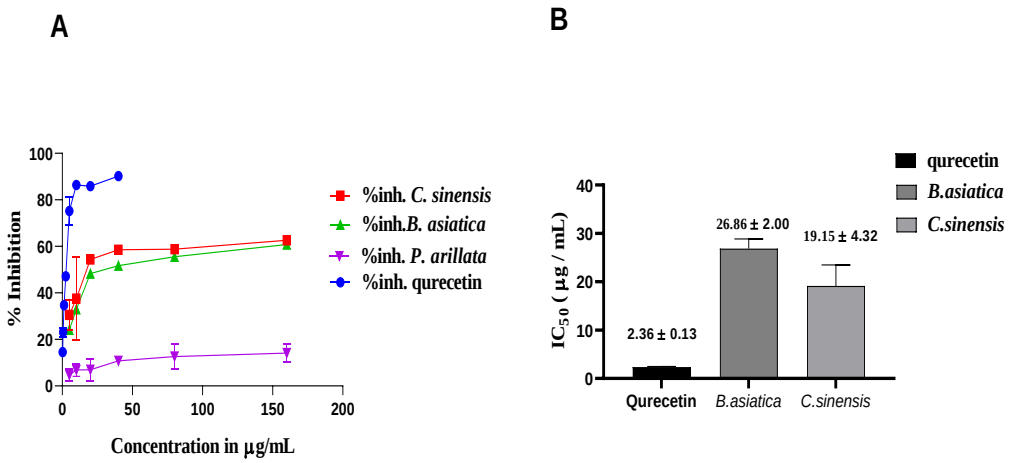

Figure 4. A) Plot of % inhibition of methanolic extracts *C. sinensis, B. asiatica, P. arillata,* and standard qurecetin vs concentration (µg/mL) B) Bar-graph showing Antioxidant inhibitory activity (IC_{50}) values for qurecetin and Methanolic plants extract.

α-Amylase inhibition by 3,5-dinitrosalicyclic acid (DNS) assay

Two α-amylase active extracts on starch-iodine assay were further fractionated and their α- amylase inhibition potential was further checked by 3,5-dinitrosalicyclic acid (DNS assay) method. The percentage inhibitions of local orthodox tea *C. sinensis* in a fraction of hexane (TH), dichloromethane (TD), ethyl acetate (TE), and in the *B. asiatica* fraction of hexane (KH), dichloromethane (KD), ethyl acetate (KE) was evaluated respectively. And the standard acarbose drug percentage inhibition was also calculated. Moreover, 50 % inhibitory concentration (IC_{50}) was evaluated, and represented in Table 3. 50 % inhibitory concentration IC_{50} values of fraction of *C. sinensis* fraction were TH (IC_{50} = 1296.13 ± 94.46 µg/mL), TD (IC_{50} = 675.97 ± 66.18 µg/mL), TE (IC_{50} = 338.52 ± 3.78 µg/mL) and *B. asiatica* fraction were KH (IC_{50} = 767.46 ± 18.44 µg/mL), KD (IC_{50} = 411.50 ± 79.07 µg/mL), KE (IC_{50} = 1051.62 ±12.05 µg/mL) in hexane, dichloromethane, and ethyl acetate respectively. The acarbose shows IC_{50} 70.89 ± 4.76 µg/ mL. Furthermore, a comparative study in bar diagram (Fig. 6), lower the IC_{50} values higher the inhibition potential content in the extracts. Therefore, the result shows that the ethyl acetate fraction of *C. sinensis* (TH) shows more inhibition potential compared to the dichloromethane (TD) and hexane fractions (TH). Similarly, the dichloromethane fraction (TD) shows more inhibition potential than the hexane fraction (TH). In the case of fractions in *B. asiatica* dichloromethane fraction (KD) shows greater inhibition potential than the ethyl acetate (KE) and hexane fraction (KH) and also, hexane fraction (KH) is more potent as compared to the ethyl acetate fraction (KE). However, all the fractions are less potent as compared to the standard acarbose (A-std).

The literature survey highlighted that for most extracts, tannins were responsible for α-amylase inhibition activity of plants. Tannin is reported as α-amylase inhibitor in black and green teas (Zhang &Kashket,1997). Phenolic compounds are also reported for inhibition of α-amylase in pears, cocoa, lentils, and many others (Queseda *et al.*,1995) and the same authors also reported that the α-amylase inhibitory activity of commercial tannic acids and condensed tannins. So, in the present study also, phenolic compounds and tannins that were detected on phytochemical screening can be credited for α-amylase inhibitory activity of study plants. Food-grade herbal is an attractive source for the isolation of natural α-amylase inhibitors. They may have potential for use in the treatment of diabetes mellitus and obesity by decreasing glucose release from starch without any side effect.

Antimicrobial assay

Antimicrobial activity of methanolic extracts of local orthodox tea *C. sinensis*, *B. asiatica, P. arillata* against *Staphylococcus aureus* and *Salmonella typhi* were studied, and their Zone of Inhibition (ZOI) was determined as shown in (Fig. 7A). Only two plants extract *C. sinensis* and *B. asiatica* have shown antimicrobial activity towards the *S. aureus*, and their zone of inhibition was found to be 17 mm and 12.5 mm respectively. However, *P. arillata* root does not show any antimicrobial sensitivity with *S. aureus,* and also these three plant extracts were unable to show antimicrobial activity toward *S. typhi.* Furthermore, a

comparative study was represented in bar- diagram 6B, and table no 4 clearly illustrates the zone of inhibition diameter of drug Neomycin (25 ± 0.82 mm) which is wider than both *C. sinensis* and *B. asiatica* extract. But the zone of inhibition diameter of *C. sinensis* extracts show greater than the *B. asiatica*. Thus, *C. sinensis,* and *B. asiatica* extract shows antimicrobial potential. From the previous study, black tea shows antimicrobial activity as reported by Armstrong *et al.* (2020) against *S. aureus*. In another study, commercial tea oil shows antimicrobial activity as reported by Brun *et al.* (2019). Similarly, Rasheed & Haider (1998) also reported that black tea can care dental carries against *S. mutans*.

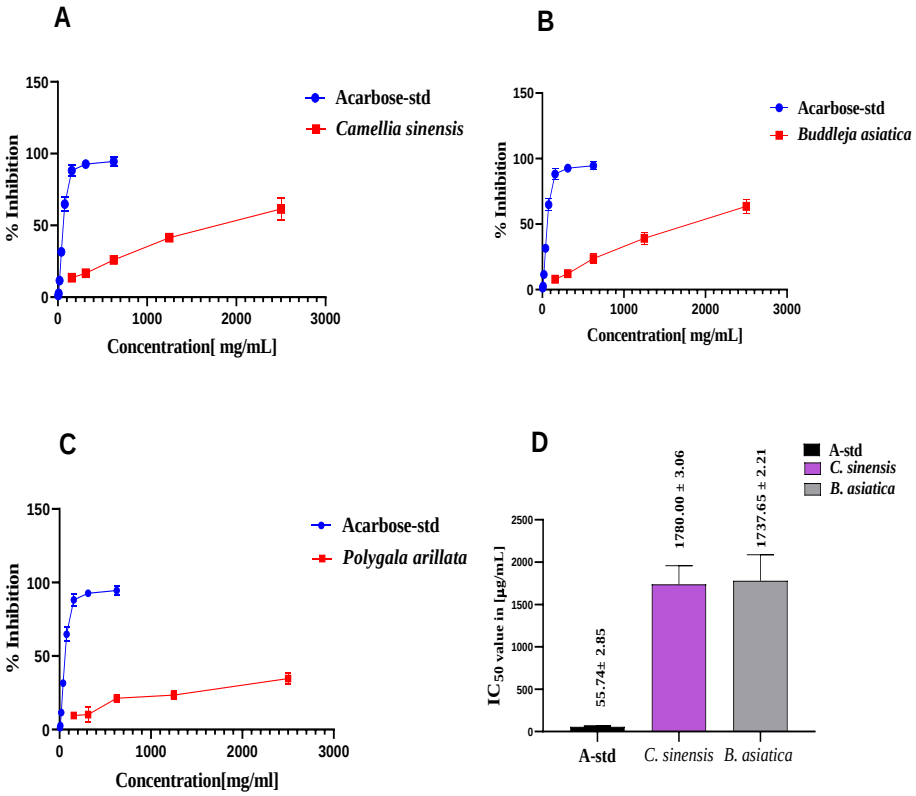

Figure 5. Graph showing Comparison of a different plant extract with standard Acarbose. A) Comparison of standard acarbose vs *C. sinensis*, B) Comparison of standard Acarbose vs *B. asiatica*, C) Comparison of standard acarbose vs *P. arillata* D) Bar-graph showing IC_{50} comparing plants extracts with standard acarbose.

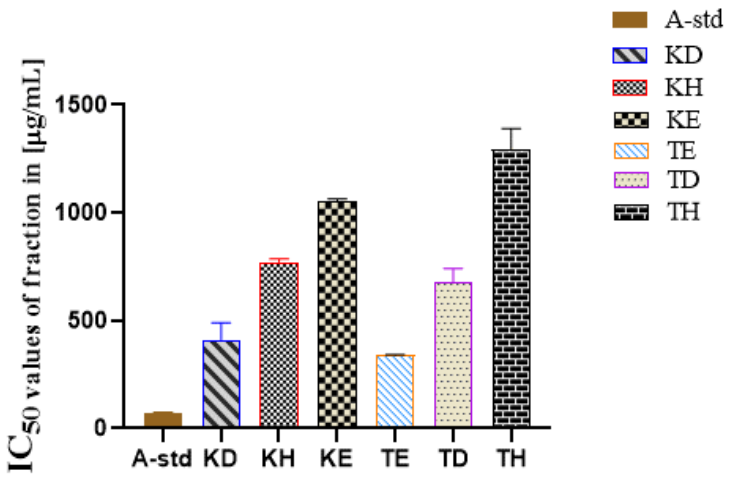

Figure 6. Bar-graph showing IC_{50} values of fractions *C. sinensis* (TE, TD, TH) and *B. asiatica* (KD, KH, KE) and Acarbose (A- std).

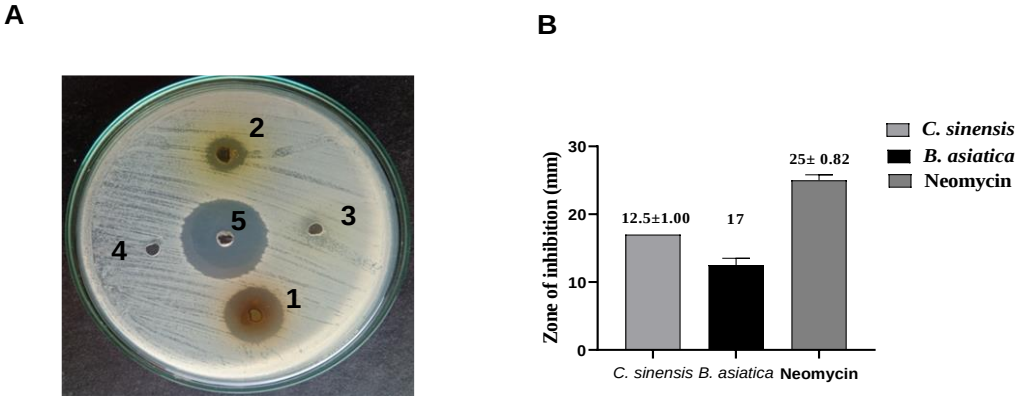

Figure 7. A) Antimicrobial screening result against *S. aureus* (gram-positive bacteria) (1) *C. sinensis,* (2) *B. asiatica,* (3) *P. arillata*, (4) Negative control (50%DMSO), (5) Positive control (Neomycin), B) Bar-graph showing Zone of inhibition (mm) against *S. aureus.*

CONCLUSIONS

It is concluded that *C. sinensis* (Panchthar District*,* 3000-6000 ft) is rich in bioactive phytochemicals and phytonutrients such as polyphenols, flavonoids, tannins, glucosides, saponins, and so on. In vitro antioxidant assay, and antimicrobial assay and α-amylase inhibition assay clearly indicated that among three study plants, *C. sinensis* had higher potential antioxidant, antimicrobial, and anti-diabetic activity. On the basis of this preliminary result, consumption of tea is also beneficial for human health. *P. arillata* was found deprived in phytochemicals content as well as antioxidant, antimicrobial and α- amylase inhibitory activity although it is used as traditional medicine. Further comprehensive investigations like fractionation, isolation and characterization of compounds in the extracts of these plants are suggested to elucidate their therapeutic potency at the molecular level.

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None

AUTHORS CONTRIBUTION STATEMENT

B. Subba analyzed the data, wrote the manuscript; K. Chemjong carried out the laboratory work.

CONFLICT OF INTEREST

The authors do not have any conflict of interest pertinent to this work.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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