

# Anti-bacterial property and anti-nutritional contents of five wild fruits of Assam, India

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## ARTICLE INFO

Received on: 10/09/2018

Accepted on: 11/11/2018

Available online: 30/12/2018

### Key words:

Anti-bacterial activity, anti-nutritional content, MBC, MIC, wild edible fruits.

## ABSTRACT

**Background:** Wild fruits have traditionally been important food sources for rural population which are known to contain many biologically active compounds that can help in the prevention of several diseases.

**Objective:** To investigate the anti-bacterial property and anti-nutritional contents of *Grewia sapida* Roxb. ex DC., *Ottelia alismoides* (L.) Pers., *Aporosa dioica* (Roxb.) Muell.-Arg., *Antidesma bunius* (L.) Spreng., and *Eugenia operculata* Roxb. fruits obtained in Assam, India.

**Methods:** The methanolic extracts of five wild fruits were studied for anti-bacterial activities against two strains of gram-positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) and two strains of gram-negative bacteria (*Escherichia coli* and *Proteus vulgaris*) using the disc diffusion method. The anti-nutritional contents such as oxalate, phytate, tannins, saponins, and alkaloid were also assessed following the reported procedures.

**Results and Conclusion:** All the fruit extracts exhibited different degrees of anti-bacterial activities. *E. operculata*, *A. dioica*, and *O. alismoides* extracts were the most effective extracts, which showed strong anti-bacterial activities against the studied bacteria. The five wild fruits showed varied concentrations of anti-nutritional factors and very high levels of anti-nutritional compounds were not observed.

## INTRODUCTION

Wild edible fruits are considered as excellent sources of nutrients and various bioactive compounds; especially, the antioxidant and anti-bacterial compounds. These fruits have received worldwide increasing attention because of their important contributions to the health of rural communities. It is well known that most of the drugs in present days are of natural products and their derivatives. Antibacterials of plant origin have immense therapeutic potentials and are effective in the treatment of infectious diseases than synthetic antimicrobials which have adverse effects (Brandi *et al.*, 2006). Polyphenols from plants possess several benefits including antimicrobial properties against pathogenic and spoilage microbes and

variations in the chemical compositions of these compounds lead to remarkable differences in their antimicrobial activity (Savoia, 2012). Plant-derived antimicrobials have been recognized for centuries, but only scientifically confirmed in the last 30 years (Gyawali *et al.*, 2012). Natural antimicrobials play a significant role in food control by preventing microbial contamination or growth and by extending shelf life due to the removal of undesirable pathogens (Lanciotti *et al.*, 2004). In addition to the nutrients and bioactive compounds, anti-nutrients are also naturally occurring organic compounds found in plants which in the intake of excess play some adverse roles (Akindahunsi *et al.*, 2005). They reduce the maximum utilization of nutrients especially vitamins, minerals, and proteins, and thus prevent the optimal exploitation of the nutrients present in a food and decrease the nutritive value. Anti-nutrients are not always harmful even though they lack nutritive values. Anti-nutrients are beneficial for human health if consumed at appropriate amounts, and can prevent diseases including cancers and coronary diseases (Ugwu *et al.*, 2006).

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The objective of the present study is to investigate the anti-bacterial properties of five wild edible fruits, *viz.*, *Grewia sapida*, *Ottelia alismoides*, *Aporosa dioica*, *Antidesma bunius*, and *Eugenia operculata* against strains of gram-positive and gram-negative bacteria and determine the anti-nutritional contents of these wild fruits.

## MATERIALS AND METHODS

### Collection of sample

#### Wild fruits

Fresh fruits of *G. sapida*, *A. bunius*, and *E. operculata* were collected in April 2015 from Chirang district of Assam (India) whereas the fresh fruits of *A. dioica* and *O. alismoides* were obtained in April and October 2015, respectively, from Kokrajhar district of Assam (India). The plants were authenticated at Botanical Survey of India, Meghalaya (Ref. No. BSI/ERC/Tech./Plant Iden./2015/211, Dated 25-06-2015) and Department of Botany, Bodoland University, Assam (Collection No. 501 and 502).

#### Bacterial strains

Two strains of gram-positive bacteria (*Staphylococcus aureus* MTCC-7443; MTCC = microbial type culture collection and *Bacillus cereus* MTCC-430) and two strains of gram-negative bacteria (*Escherichia coli* MTCC-40 and *Proteus vulgaris* MTCC-7299) were obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India for the study of the anti-bacterial activity of fruit extracts.

### Extraction of wild fruits

The collected fruits were washed properly with distilled water followed by freeze drying for 72 hours and milling to a fine powder using a grinder, which were then kept in the air-tight container. For the anti-bacterial study, the powdered sample was extracted with methanol using 1:10 ratio (w/v) by stirring vigorously at a normal temperature for 72 hours. The mixture was filtered using Whatman No. 1 filter paper and evaporated using Buchi Rotavapor (R-215, Switzerland) at 40°C. The dried extract was kept in the plastic container at 4°C for analysis.

### Determination of anti-bacterial activity of fruit extract

The anti-bacterial activity of each fruit extract was determined using the disc diffusion method (Jorgensen *et al.*, 2007) with a slight modification. The methanol extract (100 mg) was dissolved in 2 ml of dimethyl sulfoxide and sterilized through a membrane filter (0.22 µm). It was then diluted to different concentration (10, 20, 30 mg/ml) and then 5 µl was loaded over 6-mm diameter of sterilized filter paper discs. After that 10–15 ml of Mueller–Hinton agar medium was poured into sterilized Petri-dishes and allowed to solidify for 15 minutes followed by addition of 100 µl of bacterial culture on top of the plate, spread with sterile L-loop and allowed to dry for few minutes. The sterile filter paper disc loaded with different concentrations of fruit extract was placed on the top of Mueller–Hinton agar plates and allowed to incubate for 24 hours. *Escherichia coli* and *S. aureus* were incubated at 37°C and *P. vulgaris* and *B. cereus* were incubated at 27°C. Filter

paper disc loaded with 30 µg/ml of amoxicillin was taken as a positive control. Filter paper disc soaked in sterile distilled water and DMSO was taken as a negative control. The inhibition zones were measured by vernier calliper and the diameter of inhibitions were expressed in millimetre (mm).

### Determination of minimum inhibitory concentration and minimum bactericidal concentration

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) in the methanol extracts of fruits were determined according to the reported protocol of Kitzberger *et al.* (2007).

### Determination of anti-nutritional contents

The powdered samples were investigated for anti-nutritional contents. Tannin content was evaluated as a catechin equivalent following vanillin–HCl method (Price *et al.*, 1978). Oxalate content was determined by the titration method described by Day and Underwood (1986). Saponin content was estimated following the gravimetric method described by Obadoni and Ochuko (2001). Phytate content was evaluated according to the procedure reported by Vaintraub and Lapteva (1988). The procedure described by Griffiths (2000) was followed for the determination of alkaloid contents.

### Statistical analysis

The experiments for anti-nutritional contents were performed for three replicates and the results were represented in terms of mean ± standard deviation. OriginPro 8.5 software (MA 01060, OriginLab Corporation, Northampton, MA, USA) was used for the statistical analysis and executed by the one-way analysis of variance *t*-test at  $p < 0.05$ .

## RESULTS AND DISCUSSION

### Anti-bacterial activity of fruits extract

Anti-bacterial activities in the methanol extracts of five wild edible fruits were studied by disc diffusion method at different concentrations (10, 20, and 30 mg/ml) against four bacterial strains, *viz.*, two gram-positive bacteria (*B. cereus* and *S. aureus*) and two gram-negative bacteria (*E. coli* and *P. vulgaris*). All the fruit extracts showed anti-bacterial activities against bacterial strains and the results in terms of zone of inhibition of bacterial growth are presented in Table 1. In this study, the fruit extracts showed a comparatively smaller zone of inhibitions in comparison to the standard antibiotics. The largest zone of inhibition (16 mm) has been recorded against *E. coli* and *P. vulgaris* at 30 mg/ml concentration in the methanolic extracts of *O. alismoides* and *G. sapida*, respectively. *Eugenia operculata* indicated inhibition zones between 7 mm (10 mg/ml) and 13 mm (30 mg/ml) and showed the minimum activity of 7 mm (10 mg/ml) against *B. cereus*. But in the other four fruits studied, no activity was observed at the lower concentration (10 mg/ml) against *B. cereus*. In all the tested bacteria, *G. sapida* fruits showed growth inhibition zones between 9 mm (20 mg/ml) and 16 mm (30 mg/ml) but no activity was observed at 10 mg/ml. Growth inhibition zones observed in *O. alismoides* fruit against *B. cereus* and *P. vulgaris* at 30 mg/ml were 10 and 9 mm, respectively. However, no activities were noted at 10 and

**Table 1.** Anti-bacterial activities of methanol extracts of fruits against four bacterial strains.

Sample	Zone of inhibition at different concentration (mm)			Amoxicillin (30 µg/ml)	MIC (mg/ml)	MBC (mg/ml)	Bacterial strains
	10 mg/ml	20 mg/ml	30 mg/ml				
<i>G. sapida</i>	0	9.5	11	23	15	<30	<i>B. cereus</i>
<i>A. bunius</i>	0	0	13	28	<30	<30	
<i>E. operculata</i>	7	9	13	23	7.5	15	
<i>A. dioica</i>	0	10	13	27	15	<30	
<i>O. alismoides</i>	0	0	10	26	<30	<30	
<i>G. sapida</i>	0	9	11	25	15	<30	<i>S. aureus</i>
<i>A. bunius</i>	0	9	14	26	15	<30	
<i>E. operculata</i>	9	10	14	28	7.5	15	
<i>A. dioica</i>	9.5	12	14	23	7.5	15	
<i>O. alismoides</i>	9	10	12	29	7.5	15	
<i>G. sapida</i>	0	12	14	30	15	15	<i>E. coli</i>
<i>A. bunius</i>	0	0	15	27	<30	<30	
<i>E. operculata</i>	0	0	12	28	<30	<30	
<i>A. dioica</i>	0	8	13	26	15	<30	
<i>O. alismoides</i>	8	12	16	29	7.5	15	
<i>G. sapida</i>	0	14	16	28	15	15	<i>P. vulgaris</i>
<i>A. bunius</i>	0	8	13	24	15	<30	
<i>E. operculata</i>	0	0	13	25	<30	<30	
<i>A. dioica</i>	0	12	14	28	15	15	
<i>O. alismoides</i>	0	0	9	22	<30	<30	

20 mg/ml of *O. alismoides* fruit against *B. cereus* and *P. vulgaris*. At the concentration of 10 mg/ml, no anti-bacterial activity was observed in *A. bunius* against all the tested bacterial strains. But, at the concentration of 20 mg/ml, *A. bunius* showed an inhibition zone of 8 mm against *P. vulgaris* and 9 mm against *S. aureus*, whereas no activity was observed against *B. cereus* and *E. coli* at the same concentration. Growth inhibition zones observed in *A. dioica* was 8 mm (20 mg/ml) against *E. coli* and 14 mm (30 mg/ml) against *S. aureus* and *P. vulgaris*. *Aporosa dioica* showed inhibitory zones of 9.5 mm at 10 mg/ml against *P. vulgaris* but no activity was observed at 10 mg/ml with other tested bacteria. Similarly, Saklani *et al.* (2012) studied the anti-bacterial activity of ethanolic extract of *Rubus ellipticus* fruit and reported  $15 \pm 1$  mm zone of inhibition against *E. coli* which is comparable to the results of the current study. The results reported for the inhibition zone of strains by Babahmad *et al.* (2018) ranged from  $11.20 \pm 1.5$  mm to  $20.02 \pm 0.9$  mm for gram-positive bacteria and from  $10.2 \pm 1.2$  mm to  $11.35 \pm 0.5$  mm for gram-negative bacteria which are comparable to the values of the present study. The results of present study are in agreement with the findings of Qadir *et al.* (2015) who reported that the inhibitory zone of seed extracts of *Anamirta cocculus* against *P. vulgaris*, *S. aureus*, *E. coli*, *K. pneumonia*, and *S. typhi* that ranged from  $5 \pm 1.10$  to  $20 \pm 0.31$  mm.

The results revealed that all plant extracts were potentially effective in suppressing the microbial growth of food poisoning bacteria with variable potency. *Eugenia operculata* was the most effective extract retarding the microbial growth of *S. aureus* and *B. cereus* at all concentrations (10, 20, and 30 mg/ml) while the extract of *O. alismoides* was effective against *S. aureus* and *E. coli*. *Aporosa dioica* fruit was found to be effective only against

*S. aureus*. Other fruit extracts showed variable anti-bacterial activity against food poisoning bacterial strains. *Grewia sapida* exhibited inhibitory effect against all bacterial strains (*B. cereus*, *S. aureus*, *E. coli*, and *P. vulgaris*) at two concentrations (20 and 30 mg/ml), whereas *A. bunius* was effective against two of them (*S. aureus* and *P. vulgaris*). Results of anti-bacterial activity of the five wild fruit extracts could suggest that *P. vulgaris* was the most resistant strain to fruit extracts followed by *E. coli* and *B. cereus*, whereas *S. aureus* was the most susceptible strains to the extracted fruits. Moreover, *E. operculata* and *O. alismoides* extracts were the most effective extracts and showed a strong anti-bacterial activity against food poisoning bacteria.

#### Determination of MIC and MBC

The anti-bacterial activities in methanol extracts of five wild edible fruits against four species of bacteria were assessed by evaluating MIC values. It is observed from Table 1 that all the fruit extracts showed varying degrees of anti-bacterial activity against all tested strains. The MIC and MBC values of microbial strains were in the range from 7.5 to <30 mg/ml and 15 mg/ml to <30 mg/ml, respectively, depending on the nature of the bacterial strains. *Eugenia operculata* showed the lowest MIC values (7.5 mg/ml) with respect to the two strains such as *B. cereus* and *S. aureus*. *Ottelia alismoides* also showed the lowest MIC values (7.5 mg/ml) against *E. coli* and *S. aureus* whereas *A. dioica* exhibited the lowest MIC value (7.5 mg/ml) only against *S. aureus*. On the other hand, *G. sapida* showed the lowest MIC value of 15 mg/ml against all the tested strains. MIC is defined as the lowest concentration of the plant extract that inhibits the microbial growth after 24 hours of incubation (Kitzberger *et al.*, 2007).

MBC is the minimum amount of plant extract to completely kill the respective microorganism after 24 hours of incubation on the freshly inoculated agar plates (Kitzberger *et al.*, 2007). The lower MIC and MBC values indicate higher anti-bacterial activities (Cowan, 1999). The MIC values of the fruit extracts studied were in accordance with the results reported by Tshikalange *et al.* (2017). The methanol extract of *Psidium sartorianum* reported by Pio-Leon *et al.* (2013) had MIC value of 2 mg/ml and MBC value of 4 mg/ml against *S. aureus* which are lower than the MIC and MBC values of the present study. The MIC values of wild berry fruit species studied by Radovanovic *et al.* (2013) against several bacterial species ranged from 15.6 to 500 µg/ml and MBC values ranged from 31.2 to 500 µg/ml which are higher in comparison to the values of the fruits studied herein. Dua *et al.* (2013) reported MIC value of cumin extract effective against *E. coli*, *S. aureus*, *P. aeruginosa*, and *B. pumilus* that ranged from 6.25 to 25 mg/ml and these values are comparable to the results of the present study. Akinpelu *et al.* (2015) also reported crude and butanolic extracts of *Persea americana* against *B. cereus* and both the extracts exhibited anti-bacterial activity at concentrations of 25 and 10 mg/ml with MBC values of 3.12 and 12.5 mg/ml, respectively. In this study, *G. sapida* fruit extract showed potential bactericidal activity against the tested pathogenic bacteria (*E. coli* and *P. vulgaris*) with MBC value of 15 mg/ml. Likewise, *E. operculata* also showed bactericidal activity against *B. cereus* and *S. aureus*. *Aporosa dioica* fruit also showed bactericidal activity against *S. aureus* and *P. vulgaris* with MBC value of 15 mg/ml while MBC value of *A. bunius* fruit reached to <30 mg/ml against all the four tested strains which was less sensitive. The MIC values of *Vitex doniana* studied by Lagnika *et al.* (2012) ranged from 0.039 to 2.5 mg/ml and these values are lower than the MIC values of the fruits studied herein. The MBC values obtained in the study conducted by Mahboubi *et al.* (2015) ranged from 0.78 to 50 mg/ml and the MBC values obtained in the present study are within this range.

#### Anti-nutritional contents of five wild fruits

The tannin contents of the fruits were determined from the standard equation ( $y = 0.0009x + 0.0979$ ,  $R^2 = 0.9821$ ) obtained from the standard graph shown in Figure 1. The phytate contents were also determined from the standard equation ( $y = 0.0003x + 0.0036$ ,  $R^2 = 0.9549$ ) obtained from the standard graph shown in Figure 2. The anti-nutritional contents of the five wild fruits are presented in Table 2. In this study among five wild

fruits, *E. operculata* and *A. bunius* showed low anti-nutritional factors, whereas *G. sapida*, *A. dioica*, and *O. alismoides* exhibited comparatively higher level anti-nutritional factors. The tannin contents in the tested samples ranged from  $0.18 \pm 0.03$  mg/g DW in *E. operculata* to  $1.03 \pm 0.01$  mg/g DW in *O. alismoides* which are lower compared with the results observed in the fruits (7.5 mg/g) reported by Kozioc and Marcia (2004) and comparable to the values reported by Rout and Basak (2014). The tannin contents of the current study are higher than the tannin contents of *Mordii whytii* ( $1.55 \pm 0.02$  mg/100 g) and *Spondias mombin* ( $2.41 \pm 0.02$  mg/100 g) reported by Adepoju (2009). Umaru *et al.* (2007) also studied tannin contents of some wild fruits of Nigeria and reported the highest tannin content in *Balanite aegyptiaca* ( $7.40\% \pm 0.14\%$ ) and the lowest in *Parkia biglobosa* ( $0.93\% \pm 0.11\%$ ) and *Phoenix dactylifera* ( $0.93 \pm 0.215$ ). Tannins are well known to possess anti-diarrhoeal, anthelmintic, and antimicrobial activities and also possess astringent properties that accelerate wound healing (Morton, 2001). The level of phytate in the fruits was found the highest in *A. dioica* ( $7.15 \pm 0.01$  mg/g) followed by *O. alismoides* ( $5.53 \pm 0.01$  mg/g), *E. operculata* ( $5.31 \pm 0.02$  mg/g), *G. sapida* ( $4.73 \pm 0.01$  mg/g), and *A. bunius* ( $3.79 \pm 0.02$  mg/g), and these results are comparable to that of 16 wild fruits reported by Rout and Basak (2014). Mahadkar *et al.* (2012) reported phytic acid content in wild fruits of *Gmelina arborea* (0.02 g/100 g) and *Oroxylum indicum* (0.04 g/100 g) which are lower in comparison to the results of the fruits studied herein. The phytate contents of five wild fruits (Table 2) are found higher than the levels of phytate in Thai fruits consumed by diabetic patients such as pineapple (0.90 mg/g), mango (0.86 mg/g), guava (0.8 mg/g), durian (0.51 mg/g), dragon (0.39 mg/g), and longan (0.37 mg/g) reported by Suree *et al.* (2004). In plant tissues, phytic acid is the major storage form of phosphorus. The daily average intake level of phytate for humans on vegetarian diets is 2,000–2,600 mg while it is around 150–1,400 mg on mixed diets for inhabitants of rural areas in developing countries (Reddy, 2002). Oxalate content was found the highest in *O. alismoides* ( $8.93 \pm 0.93$  mg/g) and the lowest in *A. bunius* ( $3.15 \pm 0.45$  mg/g) which is comparable to the oxalate contents of wild edible fruits of Odisha (India) reported by Rout and Basak (2014). The higher level of oxalate content was reported in *Ziziphus spichristi* (16.20%) by Umaru *et al.* (2007). Oxalate is a concern because it has undesirable effects on mineral availability. Diets rich in

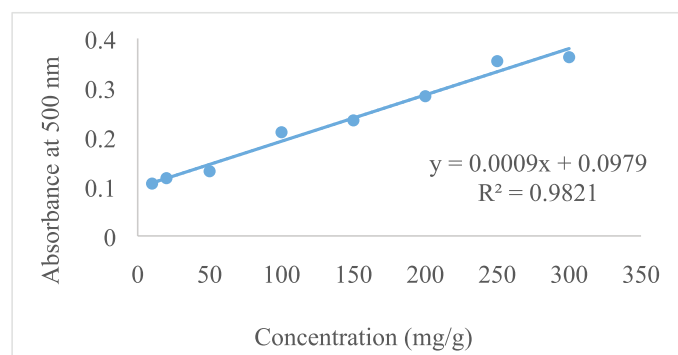


Figure 1. Standard graph of catechin for determination of tannin content.

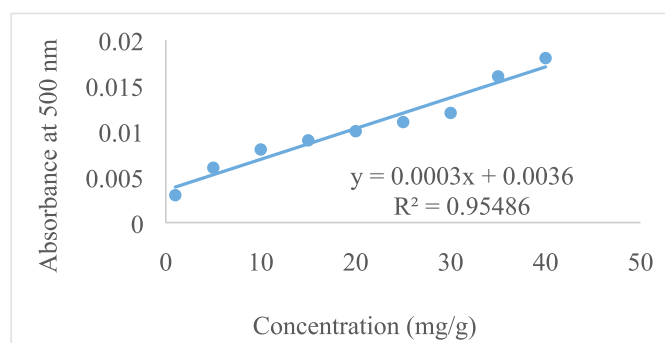


Figure 2. Standard graph of phytic acid for determination of phytate content.

**Table 2.** Anti-nutritional contents of wild fruits in mg/g dry weight (DW).

Plants	Oxalate	Tannin	Phytate	Saponin	Alkaloid
<i>G. sapida</i>	6.83 ± 0.34 <sup>a</sup>	0.35 ± 0.03 <sup>a</sup>	4.73 ± 0.01 <sup>a</sup>	0.09 ± 0.02 <sup>a</sup>	0.69 ± 0.07 <sup>a</sup>
<i>E. operculata</i>	4.15 ± 0.38 <sup>b</sup>	0.18 ± 0.03 <sup>a</sup>	5.31 ± 0.02 <sup>b</sup>	0.06 ± 0.03 <sup>a</sup>	0.56 ± 0.02 <sup>a</sup>
<i>A. dioica</i>	6.25 ± 0.96 <sup>a</sup>	0.91 ± 0.03 <sup>b</sup>	7.15 ± 0.01 <sup>c</sup>	0.13 ± 0.02 <sup>b</sup>	1.34 ± 0.02 <sup>b</sup>
<i>A. bunius</i>	3.15 ± 0.45 <sup>c</sup>	0.68 ± 0.02 <sup>a,b,c</sup>	3.79 ± 0.02 <sup>d</sup>	0.07 ± 0.02 <sup>a</sup>	0.31 ± 0.06 <sup>a</sup>
<i>O. alismoides</i>	8.93 ± 0.93 <sup>d</sup>	1.03 ± 0.01 <sup>b,c</sup>	5.53 ± 0.01 <sup>b</sup>	0.17 ± 0.03 <sup>b</sup>	1.68 ± 0.02 <sup>b</sup>

Values were expressed as the mean of three replicates ± standard deviation; the data with different letters in a column are significantly different from each other at  $p < 0.05$ .

oxalates can increase the risk of renal calcium absorption which is linked to the formation of kidney stones (Chai *et al.*, 2004). The level of saponins in the fruits ranged from  $0.06 \pm 0.03$  mg/g DW in *E. operculata* to  $0.17 \pm 0.03$  mg/g DW in *O. alismoides*. The saponin contents reported by Yadhu *et al.* (2011) in the seeds of black gram varied 9.2 to 21.4 mg/g which are much higher compared with the results of the present study. Gemah *et al.* (2007) also reported higher saponin content in African locust bean fruit (17.80 mg/100 g) compared with this study. Saponins are considered as harmful because of its toxicity and hemolytic activity (Campos-Vega *et al.*, 2010) and high levels of saponins more than 10% could cause gastroenteritis manifested by dysentery and diarrhoea (Agbaire, 2012). Saponins are also known to have antimicrobial property (Maatalah *et al.*, 2012). In present study, alkaloid contents varied from  $0.31 \pm 0.06$  mg/g in *A. bunius* to  $1.68 \pm 0.02$  mg/g in *O. alismoides* which are lower than the alkaloid contents of *Ficus asperifolia* ( $6.40 \pm 0.11$  g/100 g) and *Ficus sycomorus* ( $5.64 \pm 0.41$  g/100 g) reported by Nkafamiya *et al.* (2010). Sango *et al.* (2016) also reported the higher concentration of alkaloids in *Cleome gynandra* (3.44%) and *Solanum nigrum* (15.160%) which are much higher compared with the current study. However, Anhwange *et al.* (2015) reported lower levels of alkaloids in some indigenous fruits which ranged from 0.0097 mg/100 g in *P. americana* to 0.1075 mg/100 g in *Dialium guineense*. Alkaloids are a group of organic compounds with nitrogen obtained from plants which are toxic to many living organisms. They are known to possess anti-bacterial, anti-spasmodic, and analgesic properties (Yadav *et al.*, 2011). Alkaloids present in plants can prevent chordate and insects from eating it and they also cause infertility, neurological disorders, and gastro-intestinal upsets especially when consumed in excess (Olayemi, 2010).

## CONCLUSION

In this study, it was found that all the five wild fruit extracts were effective in suppressing the microbial growth of food poisoning bacteria with variable potency. *Ottelia alismoides* and *G. sapida* fruit extracts are exhibiting the highest inhibition zone of 16 mm at 30 mg/ml against *E. coli* and *P. vulgaris*, respectively. *Grewia sapida* exhibited inhibitory effect against all bacterial strains, viz., *B. cereus*, *S. aureus*, *E. coli*, and *P. vulgaris* at concentrations of 20 and 30 mg/ml. *Ottelia alismoides*, *A. dioica*, and *O. alismoides* fruits showed the lowest MIC value of 7.5 mg/ml against *S. aureus* with MBC value of 15 mg/ml indicating stronger anti-bacterial activities. In the current study, very high levels of anti-nutritional compounds were not observed in the fruits which may not be harmful for human consumption.

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#### How to cite this article:

Islary A, Sarmah J, Basumatary S. Antibacterial property and anti-nutritional contents of five wild fruits of Assam, India. *J App Pharm Sci*, 2018; 8(12): 170–175.