Scientific Research Publishing

Advances in Bioscience and Biotechnology, 2017, 8, 149-162
http://www.scirp.org/journal/abb
ISSN Online: 2156-8502
ISSN Print: 2156-8456

Investigation of the Phytochemicals, Antioxidant, and Antimicrobial Activity of the *Andrographis paniculata* Leaf and Stem Extracts

Shakil Ahmed Polash[1], Tanushree Saha[2], Md. Sharif Hossain[1], Satya Ranjan Sarker[1*]

[1]Department of Biotechnology and Genetic Engineering, Faculty of Biological Sciences, Jahangirnagar University, Dhaka, Bangladesh
[2]Department of Textile Engineering, Dhaka University of Engineering and Technology, Gazipur, Bangladesh
Email: *satya.sarker@bgeju.edu.bd

How to cite this paper: Polash, S.A., Saha, T., Hossain, M.S. and Sarker, S.R. (2017) Investigation of the Phytochemicals, Antioxidant, and Antimicrobial Activity of the *Andrographis paniculata* Leaf and Stem Extracts. *Advances in Bioscience and Biotechnology*, **8**, 149-162.
https://doi.org/10.4236/abb.2017.85012

Received: April 17, 2017
Accepted: May 20, 2017
Published: May 23, 2017

Abstract

Andrographis paniculata (Kalmegh) has been considered as a medicinal shrub and used as a medicinal plant in the remote areas of Bangladesh. *A. paniculata* leaf and stem extracts were prepared using the polar (*i.e.*, water, and 70% ethanol) and nonpolar (*i.e.*, hexane) solvents. The phytochemical contents, total phenol contents (TPC), antioxidant activity, and antibacterial activity of all the extracts of *A. paniculata* leaf and stem were investigated. Both the gram-positive (*i.e.*, *Bacillus subtillis*) and gram-negative (*i.e.*, *E. coli*, and *Salmonella typhi*) strains of bacteria were used for the antibacterial activity assay of the sample extracts. The ethanolic stem extracts contained the maximum amount of TPC when compared to that of the leaf extracts. However, the aqueous stem extracts had the highest free radical scavenging activity *in vitro*. The extracts prepared from *A. paniculata* stem showed better antibacterial activity against all the strains of bacteria (*i.e.*, *E. coli*, *S. typhi*, and *B. subtillis*) when compared to that of the leaf extracts. More specifically, the aqueous stem extract showed superior antibacterial effect against *E. coli*, and *B. subtillis*, and the zones of inhibition were 21 mm, and 29 mm in diameter, respectively. On the other hand, the ethanolic stem extract showed the maximum antibacterial activity against *S. typhi* and the zone of inhibition was 8.15 mm. The minimum inhibitory concentration (MIC) value and IC_{50} value for all the *A. paniculata* extracts were ~0.05 μg/μL, and ~1 μg/μL, respectively.

Keywords

Andrographis paniculata, Total Phenol Content, Antioxidant, Antimicrobial, Zone of Inhibition

1. Introduction

Free radicals and other reactive oxygen species are being constantly produced in the human body and they are known to be responsible for various deadly diseases such as cancer, aging, atherosclerosis, immunodeficiency, and infections. On the other hand, synthetic drugs bring about various side effects such as gastrointestinal disturbances, hypoglycemia, and liver dysfunction [1]. Due to the toxicity and side effects of modern therapeutic drugs, people across the world are looking for the alternative medicines from natural sources and herbal plants are the most sought after [2].

Medicinal plants are an important source of valuable therapeutic agents, both in modern and in traditional medicine. As powders, extracts, decoctions or infusions, plants are being used in the traditional systems of medicine in many parts of the world, especially in rural communities, for the control, management, and/or treatment of a variety of human and animal ailments [3]. Among other medicinal plants, *Andrographis paniculata* is one of the most potential herbs for the treatment of various deadly diseases. Because *A. paniculata* extracts have antihypertensive effects and decreases plasma angiotensin converting enzyme (ACE) activity, and lipid peroxidation in kidneys [4]. They also have antimicrobial, antioxidant and anti-inflammatory activities, antihyperglycemic and hypoglycemic activities [5]. Moreover, *A. paniculata* extracts have hepatoprotective effects which help to cure damaged liver caused by agents with different hepatotoxic mechanisms [4].

However, when crude powder of *A. paniculata* was suspended in the water, it did not show any antibacterial activity against *Salmonella*, *Shigella*, *E. coli*, *Streptococci*, and *Staphylococcus aureus in vitro* even at a very high concentration (25 mg/mL) [5]. It has also been reported that the crude aqueous as well as the ethanolic extracts of *A. paniculata* do not show any antibacterial effect against gram-negative *E. coli* [6] [7]. Inconsistency in *in vitro* antibacterial effects could be due to several factors, the variation of the constituents in the material tested being the prime suspect [4]. The negative antibacterial results have been reported from Thailand by Leelarasamee *et al.* [7] [8] while the results reported from India [9] and Malaysia [6] have been positive. It is due to the place and timing of collection of the medicinal herbs, storage, and extraction conditions may affect the extracts constituents both qualitatively and quantitatively [4]. Herein, in this study we have collected *A. paniculata* herbs from the plain areas of Bangladesh during the rainy season (from July to September). The *A. paniculata* leaf and stem extracts were prepared using both the polar solvents (*i.e.*, water, and 70% ethanol), and nonpolar solvent (*i.e.*, hexane). The phytochemical contents, antioxidant, and antibacterial activity of all the extracts were investigated *in vitro*. To perform the antibacterial activity of the extracts, both the gram-positive (*i.e.*, *B. subtillis*), and gram-negative (*i.e.*, *E. coli*, and *S. typhi*) bacterial strains were used.

2. Materials and Methods

2.1. Chemicals and Reagents

Absolute ethanol, methanol, hexane, and Folin-Ciocalteu reagent were purchased from Merck, Germany. Gallic acid was purchased from Ashland Inc. USA. Ascorbic acid was bought from VEGA, China. 1-1-diphenyl-2-picryhydrazyl (DPPH) was collected from Sigma-Aldrich, USA. Agar powder was purchased from Titan Biotech Ltd., India. Peptone, yeast extract, sodium chloride and sodium carbonate were collected from UNI-CHEM, China.

Escherichia coli DH5α, *Bacillus subtilis* RBW, and *Salmonella typhi* were obtained from the department of Biotechnology and Genetic Engineering, Jahangirnagar University, Savar, Dhaka 1342, Bangladesh.

2.2. Plant Sample Collection

A. paniculata shrubs were collected from the surroundings of the department of Biotechnology & Genetic Engineering of Jahangirnagar University, Savar, Dhaka 1342, Bangladesh. Leaves and stems were first separated out and washed thoroughly with tap water followed by distilled water wash to remove any dirt, and dried naturally. The dried parts (*i.e.*, leaves, and stem) of *A. paniculata* were ground separately into fine powder.

2.3. Preparation of Extracts

The powder (5 gm) of both the leaves and stems were extracted separately with 100 mL of both the polar (*i.e.*, 70% ethanol, and distilled water) and nonpolar (*i.e.*, hexane) solvents for 72 hours at 37˚C with gentle shaking at 120 rpm and filtered using Whatman No. 1 filter paper. The solvents were then removed completely to get the dried extracts. Finally, 1% of the extract sample was prepared using 0.9% NaCl solution.

2.4. Determination of Total Phenol Content (TPC)

The total phenolic contents (TPCs) of the extracts prepared from both the polar (*i.e.*, water and ethanol) and nonpolar (*i.e.*, hexane) solvents were determined by Folin-Ciocalteu's reagent method [10] [11]. Briefly, different sample extracts (*i.e.*, 100 µL) were taken into separate test tubes containing 500 µL Folin-Ciocalteu's reagent (10% w/v). The concentration of phenolic contents in the samples were derived from a standard curve of Chlorogenic acid (*i.e.*, gallic acid) diluted with absolute methanol (0 - 100 µg/mL). Folin-Ciocalteu's reagent (500 µL, 10% v/v) was then added to all the test tubes containing different concentrations of gallic acid. Finally, 400 µL sodium carbonate solution (7.5% w/v) was added to all the test tubes containing the *A. paniculata* extracts as well as the gallic acid. The tubes were then incubated at room temperature for 60 minutes before measuring the absorbance at 765 nm using a UV-visible spectrophotometer (Optizen POP, Korea). The total phenol contents of the *A. paniculata* extracts prepared from both the polar and nonpolar solvent were expressed as µg CAE/mL.

2.5. Determination of Antioxidant Activity by DPPH Method

The antioxidant activity of *A. paniculata* extracts were determined according to the protocol reported by Manzocoo *et al.*, with slight modification [12] [13]. Briefly, sample extract (200 µL) or different concentrations of standard (*i.e.*, freshly prepared ascorbic acid) solution were taken into different test tubes. 50 µL HCL (1.0 M) was added to all the test tubes containing the sample extracts and ascorbic acid. The final volume of all the test tubes were made up to 500 µL with absolute methanol. Methanolic solution of DPPH (500 µL, 0.004%) was then added to all the test tubes and incubated in the dark and cold environment for 30 minutes to complete the reaction. The absorbance of the solution was measured at 517 nm.

The percentage (%) of free radical scavenging activity is calculated from (Ab – As)/Ab × 100.

Here, Ab is the absorbance of the blank, and As is the absorbance of the standard or extract sample. Ascorbic acid was used as a positive control. Percentage (%) of free radical scavenging activity was plotted against the concentration of the plant extracts and the value of IC_{50} (*i.e.*, the concentration of the plant extract required to inhibit the formation of free radicals by 50%) was calculated from the regression line obtained. Tests were carried out in triplicate and the average value was taken.

2.6. Determination of the Phytochemicals

The presence of phytochemicals in *A. paniculata* leaf and stem extracts prepared using different solvents were determined qualitatively. Standard procedures were followed to determine the presence of flavonoids (alkaline reagent test), tanins (ferric chloride test), saponins (foam test), phenols (ferric chloride test), and glycosides (KellarKillani's test) [14].

2.7. Preparation of Bacterial Starter Cultures and Test Plates

In a laminar air cabinet, Luria-Bertini (LB) broth was taken into three different test tubes and each test tube was inoculated with different microorganisms (*i.e.*, *E. coli*, *S. typhi*, and *B. subtilis*) and incubated overnight at 37˚C upon gentle shaking to prepare fresh bacterial cultures. The bacterial cultures (100 µL) were then transferred to different agar plates and spreaded uniformly with a sterile spreader to prepare test plates for antimicrobial test.

2.8. Preparation of Sample Discs with Test Samples

Sterile metrical filter paper discs (Oxoid, UK) were taken in a blank petri plate. Then the discs were soaked with different concentrations (*i.e.*, 5 µg/µL, and 10 µg/µL) of the test sample extracts and wait for several minutes to dry them properly.

2.9. Determination of Antibacterial Activity by Disc Diffusion Method

Antibacterial activity of the *A. paniculata* extracts were investigated by disc dif-

fusion method [15]. Briefly, LB agar medium was used to grow bacteria. Dried and sterile filter paper discs (6 mm diameter) containing *A. paniculata* extracts of known concentrations were placed on nutrient agar medium uniformly seeded with the test microorganisms. The plates were then incubated at 37˚C for 24 hours for optimum growth of the microorganisms. The extracts having antibacterial property prevent bacterial growth in the media surrounding the discs and yield a clear, transparent area defined as 'zone of inhibition'. The antimicrobial potency of the extract samples were measured by their activity to prevent the growth of the microorganisms surrounding the discs which gives clear zone of agar or inhibition. After incubation, the antimicrobial activities of the extract samples were determined by measuring the diameter of the zone of inhibition in millimeter with a transparent scale.

2.10. Determination of MIC (Minimum Inhibitory Concentration) and IC_{50} (Inhibitory Concentration 50) Value of the *A. paniculata* Extracts

The MIC and IC_{50} value of both the polar (*i.e.*, water, and ethanol) and nonpolar (*i.e.*, hexane) solvent extracts of *A. paniculata* leaf and stem were determined through serial dilution method. Briefly, different concentrations of the extracts (*i.e.*, 0, 0.05, 0.1, 0.2, 0.4, 0.8, 1.0, and 1.5 μg/μL) were added to the bacterial cultures and the final volume was made up to 1.0 mL before incubating over night at 37˚C upon gentle shaking. The absorbance of all the tubes was taken at 600 nm using a UV-visible Spectrophotometer (Optizen POP, Korea). The experiment was performed multiple times and the data expressed as a mean of several replications (n = 3) and standard deviations.

3. Results and Discussion

3.1. Estimation of Total Phenol Content (TPC)

The total phenol content (TPC) of the *A. paniculata* extracts was determined by Folin Ciocalteu method and the results were expressed in μg CAE/mL. The highest amount of TPC was found in the ethanolic extract of stem followed by its aqueous extract. On the other hand, the lowest amount of TPC was found in the hexane extract of both the leaf and stem (**Figure 1**). The TPC of the *A. paniculata* extracts showed the following order: 70% ethanolic extract > aqueous extract > hexane extract. The minimum amount of TPC in the hexane extract of leaf and stem is due to the use of nonpolar hexane as the solvent of extraction as because most of the phenolic compounds are soluble in polar solvents [16]. Therefore, the TPC was found more in the extracts obtained from the polar solvents (*i.e.*, water, and 70% ethanol) than that of the nonpolar solvents as shown in **Figure 1**.

3.2. Antioxidant Activity Assay

The free radical scavenging activity of *A. paniculata* extracts was carried out by DPPH assay. The highest free radical scavenging activity was found to be

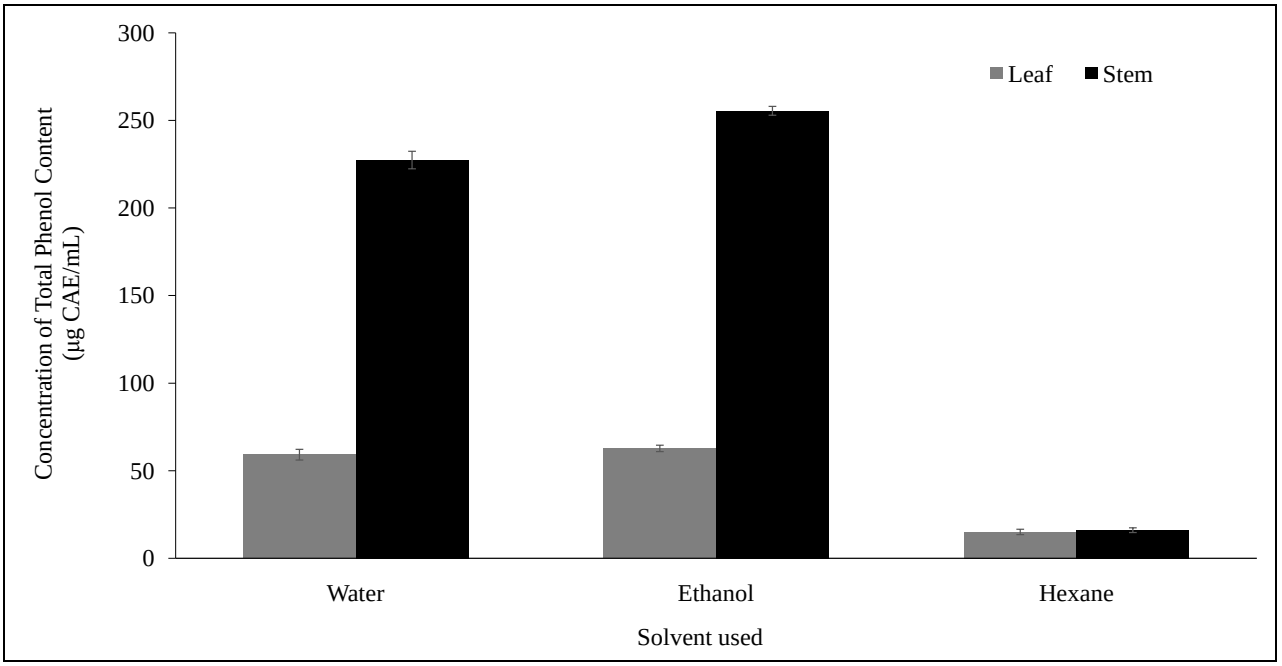


Figure 1. Total phenol content (TPC) of both the leaf and stem extracts of *A. paniculata*. Leaf and stem extracts were prepared using polar (*i.e.*, water, and 70% ethanol) and nonpolar (*i.e.*, hexane) solvents. Gray bar indicates the TPC of leaf extract and black bar indicates the TPC of stem extract. Data represent mean value ± SD (n = 3).

performed by the aqueous extract of stem followed by its ethanolic extract. On the other hand, the stem extract prepared by using hexane as the solvent showed the lowest antioxidant activity (**Figure 2**). The antioxidant activity of a plant extract depends on the presence of the compounds with free hydroxyl groups such as flavonoids [17]. The amount of flavonoids and phenols in the aqueous extracts of *A. paniculata* was found higher when compared to that of the ethanol and hexane extracts (**Table 1**), since water is the most polar solvent among the solvents used and flavonoids have free hydroxyl groups. Therefore, the highest antioxidant activity of the aqueous extract of stem was due to the presence of higher amount of free hydroxyl group containing flavonoids which can scavenge the free radicals more efficiently. Our data support the previously published reports that the antioxidant activity is present in the water soluble compounds [18] and high polarity solvents are the most efficient in the extraction of natural antioxidants [19] [20].

The IC_{50} value (*i.e.*, the amount of extracts required to scavenge the formation of free radicals by 50%) of *A. paniculata* extracts prepared from polar solvents were calculated from the liner regression curve (**Figure 3**). The lowest IC_{50} value was obtained from the aqueous extract of stem (*i.e.*, 4.42 µg/mL) followed by ethanolic stem extract (*i.e.*, 6.84 µg/mL). Hence, the aqueous extract of stem is the most efficient in scavenging the free radicals formed due to the oxidation of proteins, nucleic acids, and per oxidation of lipids.

3.3. Determination of the Phytochemicals

The qualitative phytochemical analysis data of both the polar and nonpolar

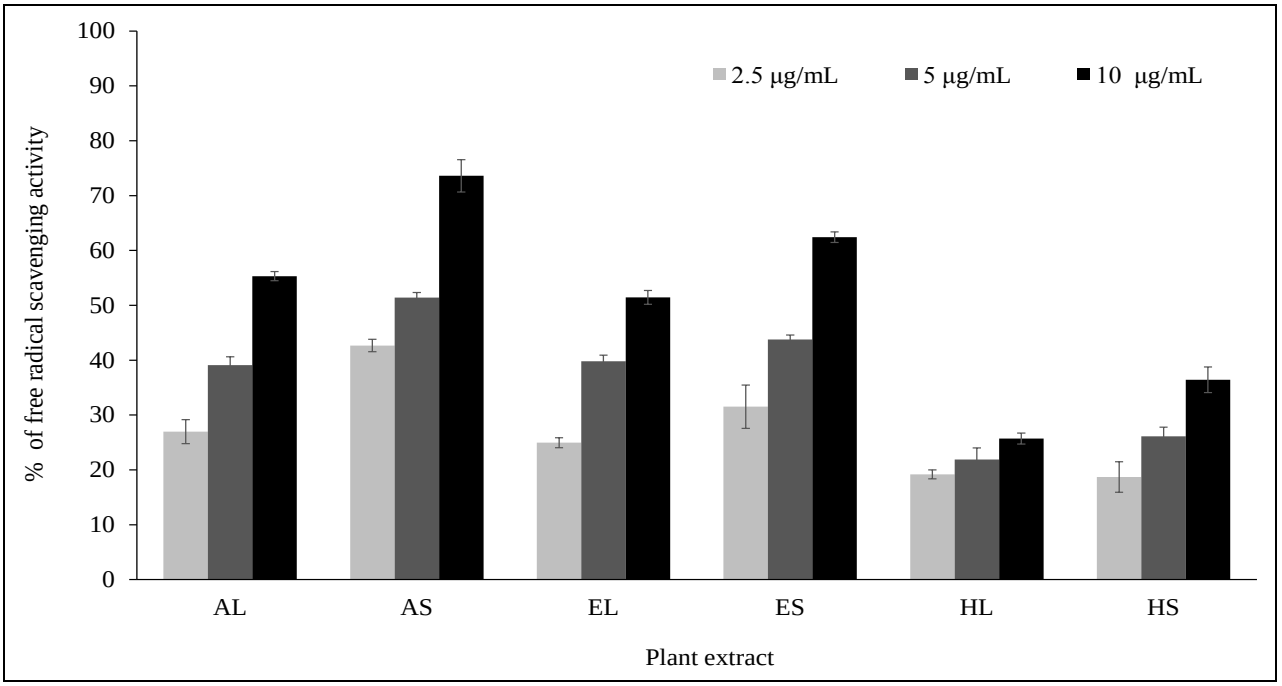


Figure 2. Free radical scavenging activity of both the leaf and stem extracts of *A. paniculata*. Leaf and stem extracts were prepared using polar solvents (*i.e.*, water, and 70% ethanol) and nonpolar (*i.e.*, hexane) solvent. Three different concentrations of the plant extracts (*i.e.*, 2.5 µg/mL, 5 µg/mL, and 10 µg/mL) were used and it showed that the free radical scavenging activity is proportional to the sample concentrations. Here, six different samples were taken: aqueous leaf extract (AL), aqueous stem extract (AS), ethanolic leaf extract (EL), ethanolic stem extract (ES), hexane leaf extract (HL), and hexane stem extract (HS). All the values are mean ± SD of three replications performed on three different times.

Table 1. Phytochemical tests of *A. paniculata* leaf and stem extracts prepared using water, ethanol, and hexane.

Test	Aqueous Leaf	Aqueous Stem	Ethanol Leaf	Ethanol Stem	Hexane Leaf	Hexane Stem
Tannin	+	++	+	+++		
Flavonoid	++	+++	++	+++		
Saponin	+	+++	+	+++		
Phenol	+++	++	++	+		
Glycoside	++	+++	++	+++	+	+

solvent extracts of *A. paniculata* leaf and stem showed that most of the phytochemicals are present in the aqueous and ethanolic extracts (Table 1). However, all the tested phytochemicals are absent in the hexane extracts except the glycosides which is present in a very little amount. Both the aqueous stem and ethanolic stem extracts contain the maximum amount (+++) of flavonoids followed by the aqueous and ethanolic leaf extracts. The flavonoid is known to be responsible for the antioxidant activity [21] [22] and, therefore, the aqueous stem extract showed the highest antioxidant activity (Figure 2). The aqueous stem and ethanolic stem extracts also contained the maximum amount (+++) of saponin and glycosides. The maximum amount (+++) of tannin and phenol were found in the ethanolic stem extracts, and aqueous leaf extracts, respectively.

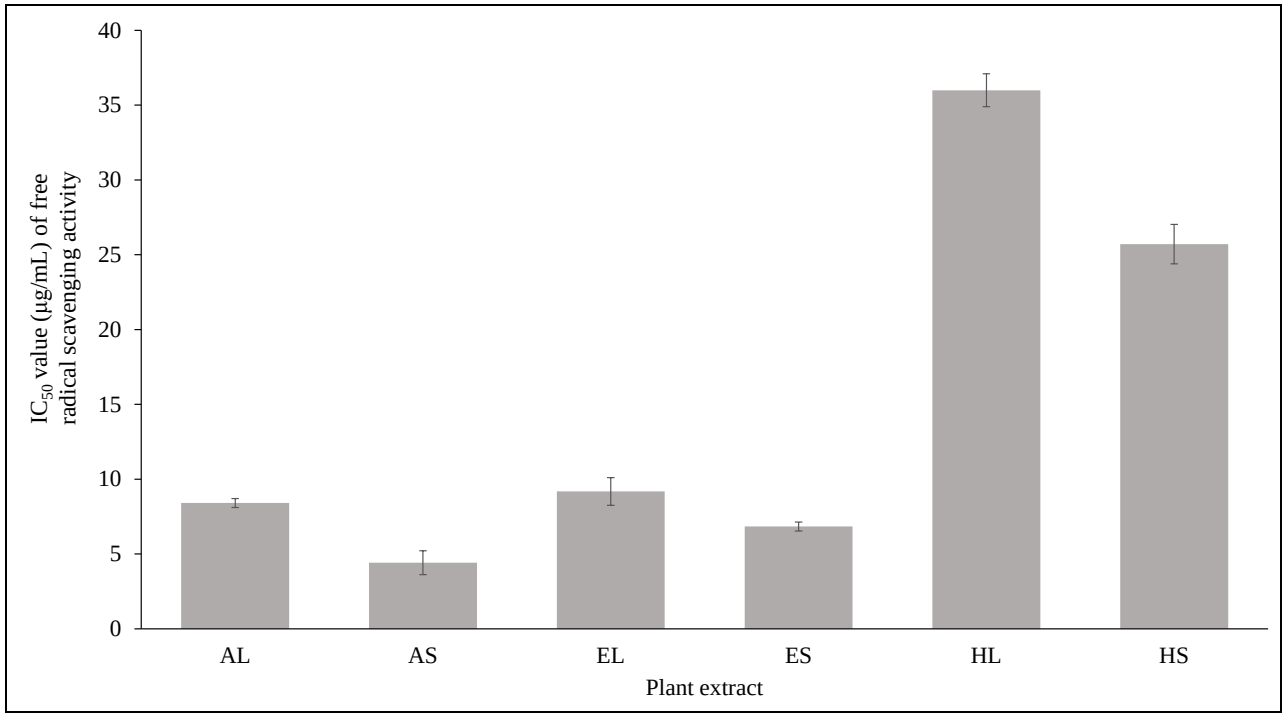


Figure 3. Comparison of IC_{50} value (µg/mL)) of *A. paniculata* leaf and stem extracts. After investigation of the free radical scavenging activity (%) of *A. paniculata* extracts *in vitro*, IC_{50} values were calculated.

3.4. Antimicrobial Activity

All the *A. paniculata* extracts were investigated for their antimicrobial activity assay against both the gram-positive (*i.e.*, *B. subtilis*) and gram-negative (*i.e.*, *E. coli*, and *S. typhi*) bacteria by simple agar diffusion method. Different concentration of the sample extracts (*i.e.*, 5 µg/µL, and 10 µg/µL) were used for the antimicrobial tests.

The extracts showed antimicrobial activity against all the microorganisms (Table 2) in both the concentrations.

Among all the *A. paniculata* extracts, the aqueous stem extracts showed the maximum antibacterial activity against gram-positive *B. subtillis* and diameter of the zone of inhibition was 29 mm followed by the ethanolic stem extract which was 23 mm in diameter (Table 2(a)). The aqueous stem extract also showed the maximum antibacterial activity against gram-negative *E. coli* and the zone of inhibition was 21 mm in diameter followed by the ethanolic stem extract which was 12 mm in diameter (Table 2(b)). However, the maximum antibacterial activity against *S. typhi* was performed by the ethanolic stem extract (8.15 mm) followed by the aqueous leaf, and stem extracts (Table 2(c)). The highest antibacterial activity of the aqueous stem extracts of *A. paniculata* is because of the presence of tannin, saponin, and glycosides which are known as potential antimicrobial agents. The minimum antibacterial activity was performed against the gram-positive and gram-negative strains of bacteria by the hexane extracts of both the leaf and stem. It was due to the presence of very little amount of glycosides (+) and the absence of excellent antimicrobial agents such as tannin, and saponin.

Table 2. Antibacterial activity of the *A. paniculata* leaf and stem extracts to (a) *B. subtilis* (b) *E. coli*, and (c) *S. typhi*. [Two different concentrations (5 μg/mL, and 10 μg/mL) of different extracts were used for the antibacterial assay. Values represent mean ± SD of three different replications].

(a)

Concentration (μg/μL)	Diameter of zone of inhibiton (mm)					
	Bacillus subtilis					
	Aqueous leaf (AL)	Aqueous stem (AS)	Ethanol leaf (EL)	Ethanol stem (ES)	Hexane leaf (HL)	Hexane stem (HS)
5	9.02 ± 0.04	18.12 ± 0.12	15 ± 0.12	12.05 ± 0.08	15.1 ± 0.15	7.97 ± 0.03
10	11.15 ± 0.20	29.12 ± 0.19	21.1 ± 0.12	23.12 ± 0.12	15.9 ± 0.12	10.01 ± 0.04

(b)

Concentration (μg/μL)	Diameter of zone of inhibiton (mm)					
	Eschericia coli					
	Aqueous leaf (AL)	Aqueous stem (AS)	Ethanol leaf (EL)	Ethanol stem (ES)	Hexane leaf (HL)	Hexane stem (HS)
5	8.02 ± 0.08	13.97 ± 0.10	10.12 ± 0.39	9.02 ± 0.04	7.95 ± 0.11	7.05 ± 0.08
10	9.25 ± 0.32	21.02 ± 0.04	10.95 ± 0.08	12 ± 0.12	8.77 ± 0.04	7.97 ± 0.10

(c)

Concentration (μg/μL)	Diameter of zone of inhibiton (mm)					
	Salmonella typhi					
	Aqueous leaf (AL)	Aqueous stem (AS)	Ethanol leaf (EL)	Ethanol stem (ES)	Hexane leaf (HL)	Hexane stem (HS)
5	6.42 ± 0.06	6.9 ± 0.08	6.45 ± 0.12	7.37 ± 0.08	6.4 ± 0.14	6.37 ± 0.05
10	7.27 ± 0.05	7.15 ± 0.04	6.77 ± 0.04	8.15 ± 0.12	6.85 ± 0.05	6.77 ± 0.09

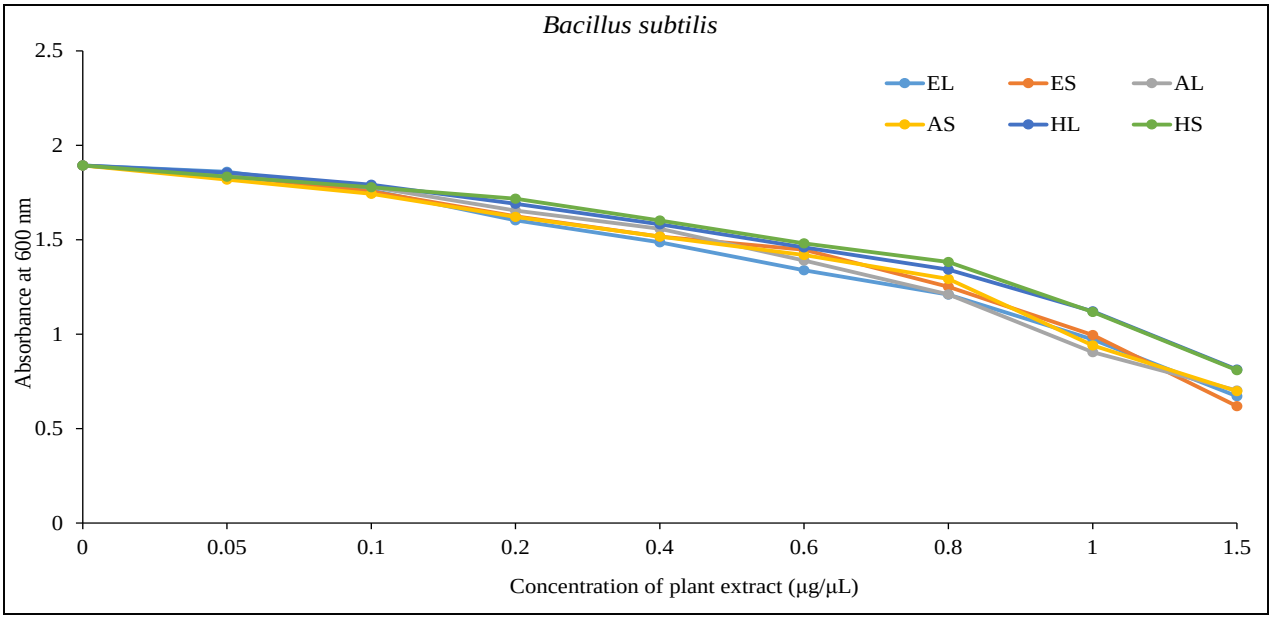
The antibacterial activity of the medicinal plant extracts depends on the presence of different concentrations of secondary metabolites such as tannin, saponin, and glycosides [23] [24] [25]. Herein, the highest amount (+++) of tannin was present in the ethanolic stem extract followed by the aqueous stem extract (++) (Table 1). The aqueous stem as well as the ethanolic stem extract contained the maximum amount (+++) of saponin and glycoside. The antimicrobial activity of hexane extracts was found due to the presence of glycosides.

Our data support the previously published reports that the gram positive bacteria are more sensitive to plant extracts when compared to that of the gram negative bacteria. Therefore, the *A. paniculata* extracts were tasted against a variety of gram-positive and gram-negative bacterial strains [26] [27]. The antibacterial activities of all the extracts at a same concentration against *B. subtillis* were stronger when compared to that of *E. coli* and *S. typhi.* Our results suggest that gram-negative bacteria are generally more resistance to *A. paniculata* extracts when compared to that of gram-positive bacteria. A possible explanation for these findings may lie in the significant differences in the outer layers of gram-negative and gram-positive bacteria. Gram negative bacteria possess an outer membrane and a unique periplasmic space which is not found in gram-positive bacteria and, therefore, they showed different antimicrobial activity

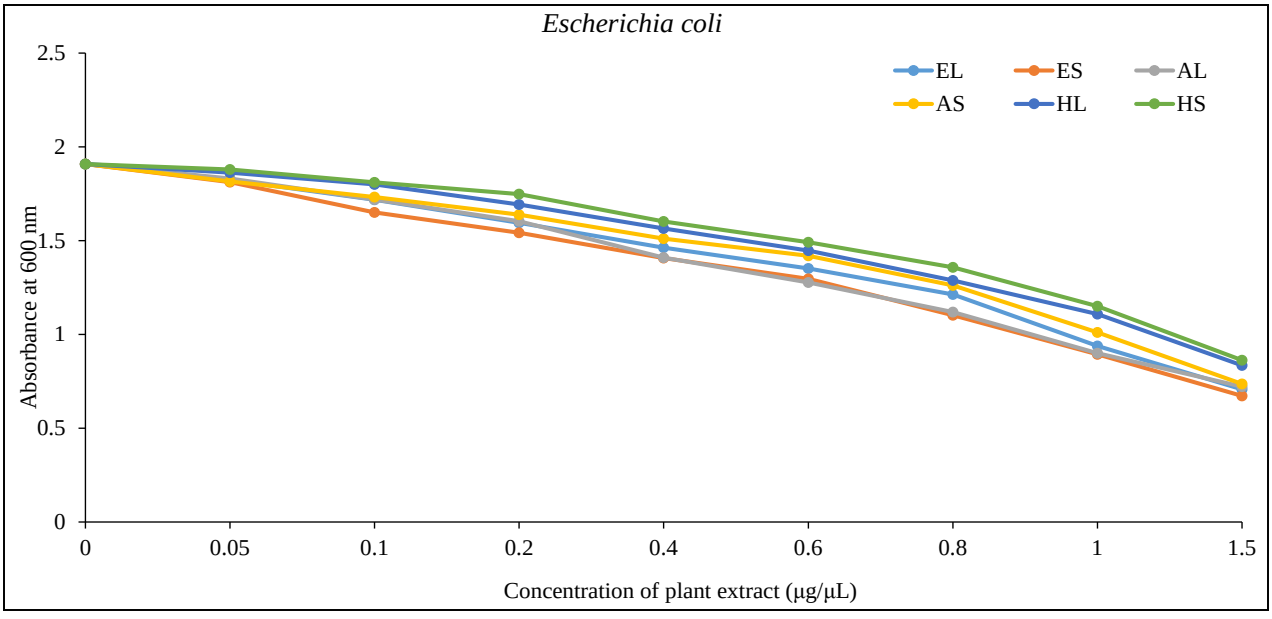
against the tested sample extracts [28]. Stem extracts have higher antimicrobial activity than that of the leaf extracts. Thus, *A. paniculata* stem extracts prepared from the polar solvents (*i.e.*, water, and ethanol)possess excellent antimicrobial property that can be used as a potential source of antibiotics.

3.5. Determination of MIC (Minimum Inhibitory Concentration) and IC_{50} (Inhibitory Concentration 50) Value

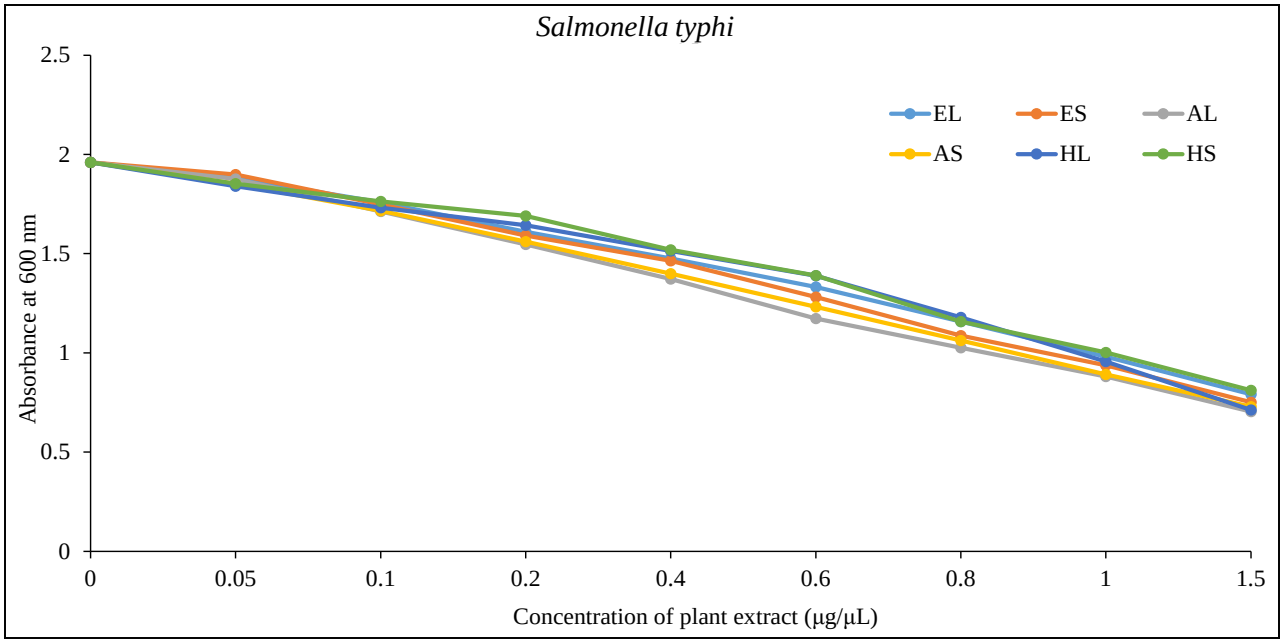
The absorbance of overnight grown bacterial culture was measured at different wavelengths ranging from 560 to 620 nm to determine the λ_{max} for the bacterial culture and observed that λ_{max} was 600. The minimum inhibitory concentration (MIC) value for all the extracts was ~0.05 µg/mL (**Figure 4**). Furthermore, the



(a)



(b)



(c)

Figure 4. Absorbance of overnight grown bacterial culture in the presence of different amounts of *A. paniculata* leaf and stem extracts. Six sample were taken under investigation: aqueous leaf (AL), aqueous stem (AS), ethanol leaf (EL), ethanol stem (ES), hexane leaf (HL), and hexane stem (HS). **Figures 4(a)-4(c)** depict the growth inhibitory response for *Bacillus subtilis*, *Escherichia coli*, *and Salmonella typhi*, respectively. In all the cases, the bacterial culture showed the maximum absorbance at 600 nm in the absence of any extracts. However, the absorbance value decreased with the increased amounts of sample extracts. All the values are mean ± SD of three determinations.

inhibitory concentration 50 (IC_{50}) value for all the extracts was ~1 µg/mL. The IC_{50} value explains the bacteriostatic as well as the bactericidal activity of the *A. paniculata* extracts that is required to inhibit the bacterial growth and multiplication by 50%. It is well known that the absorbance of bacterial culture increases with the increased concentration of bacteria. Since the extracts have both the bacteriostatic and bactericidal activity, the absorbance of bacterial culture reduced as the concentration of the extracts were increased. The IC_{50} values of HS and HL extracts were more among all other extracts and, therefore, it requires more amount of the extract to inhibit the growth of bacteria by 50% (*i.e.*, bacteriostatic effect) or to kill the 50% of the existing bacteria (*i.e.*, bactericidal effect). It is due to the absence of most of the phenolics except glycosides in hexane extract of *A. paniculata* leaf and stem (**Table 1**).

In the disc diffusion method, we showed the antimicrobial activity of the extracts in terms of their zone of inhibition which explains the ability of the extracts to inhibit the growth of bacteria up to a certain distance around the disc of placement.

4. Conclusions

We have evaluated the phytochemical contents, antioxidant efficiency, and antimicrobial potentiality of *A. paniculata* extracts in terms of their zone of inhibition (*i.e.*, diameter in millimeter), MIC value, and IC_{50} value. *A. paniculata* leaf

and stem extracts were prepared using different solvents such as water and ethanol as the polar solvents and hexane as the nonpolar solvent. The ethanolic stem extract showed the highest total phenol content and the aqueous stem extract showed the maximum free radical scavenging activity.

The ethanolic, aqueous and hexane extracts of *A. paniculata* leaf and stem showed significant TPC, antioxidant, and antimicrobial activity which supports the traditional use of plants in various diseases. In this study, we found that Kalomegh (*A. paniculata*) stem extract has the highest TPC and free radical scavenging activity. Stem extract also showed the maximum zone of inhibition (29.12 ± 0.19 mm) against gram positive microbial strain (*i.e.*, *B. subtillis*). So the *A. paniculata* can be further screened against various disease causing pathogens and can be a potential source of chemical and biologically important drug candidates.

Acknowledgements

This research was supported by Jahangirnagar University Research Grants 2014-15, Savar, Dhaka 1342, Bangladesh, and University Grants Commission Research Grants 2015-16, Govt. of Bangladesh.

Conflict of Interest

The authors declare no conflict of interest whatsoever.

References

[1] Rang, H.P., Dale, M.M. and Ritte, J.M. (2003) Pharmacology. 4th Edition, Churchill Livingstone, Melbourne, 340-385.

[2] Bothon, F.T.D., Debiton, E., Avlessi, F., Forestier, C., Teulade, J. and Sohounhloue, D.K.C. (2013) *In Vitro* Biological Effects of Two Anti-Diabetic Medicinal Plants used in Benin as Folk Medicine. *BMC Complementary and Alternative Medicine*, **13**, 51. https://doi.org/10.1186/1472-6882-13-51

[3] Kamboj, A., Kumar, S. and Kumar, V. (2013) Evaluation of Antidiabetic Activity of Hydroalcoholic Extract of *Cestrum nocturnum* Leaves in Streptozotocin-Induced Diabetic Rats. *Advances in Pharmacological Sciences*, **2013**, Article ID: 150401.

[4] Akbar, S. (2011) *Andrographis paniculata*: A Review of Pharmacological Activities and Clinical Effects. *Alternative Medicine Review*, **16**, 66-77.

[5] Zhang, X.F. and Tan, B.K. (2000) Antidiabetic Property of Ethanolic Extract of *Andrographis paniculata* in Streptozotocin-Diabetic Rats. *Acta Pharmacologica Sinica*, **21**, 1157-1164.

[6] Leelarasamee, A., Trakulsomboon, S. and Sittisomwong, N. (1990) Undetectable Anti-Bacterial Activity of *Andrographis paniculata* (Burma) Wall. ex ness. *Journal of the Medical Association of Thailand*, **73**, 299-304.

[7] Zaidan, M.R., Noor Rain, A. and Badrul, A.R. (2005) *In Vitro* Screening of Five Local Medicinal Plants for Antibacterial Activity Using Disc Diffusion Method. *Tropical Biomedicine*, **22**, 165-170.

[8] Voravuthikunchai, S.P. and Limsuwan, S. (2006) Medicinal Plant Extracts as Anti-*Escherichia coli* O157:H7 Agents and Their Effects on Bacterial Cell Aggregation. *Journal of Food Protection*, **69**, 2336-2341.

https://doi.org/10.4315/0362-028X-69.10.2336

[9] Singha, P.K., Roy, S. and Dey, S. (2003) Antimicrobial Activity of *Andrographis paniculata*. *Fitoterapia*, **74**, 692-694.

[10] Singh, R.P., Murthy, K.N.C. and Jayaprakasha, G.K. (2002) Studies on the Antioxidant Activity of Pomegranate (*Punica granatum*) Peel and Seed Extracts Using *in Vitro* Models. *Journal of Agriculture and Food Chemistry*, **50**, 81-86. https://doi.org/10.1021/jf010865b

[11] Terpinc, P., Čeh, B., Ulrih, N.P. and Abramovič, H. (2012) Studies of the Correlation between Antioxidant Properties and the Total Phenolic Content of Different Oil Cake Extracts. *Industrial Crop Products*, **39**, 210-217.

[12] Manzocco, L., Anese, M. and Nicoli, M.C. (1998) Antioxidant Properties of Tea Extracts as Affected by Processing. *LWT-Food Science and Technology*, **31**, 694-698. https://doi.org/10.1006/fstl.1998.0491

[13] Braca, A., Tommasi, N.D., Bari, L.D., Pizza, C., Politi, M. and Morelli, I. (2001) Antioxidant Principles from *Bauhinia terapotensis*. *Journal of Natural Product*, **64**, 892-895. https://doi.org/10.1021/np0100845

[14] Nagaraja, Y.P. and Chandrashekhar, B. (2014) Phytochemical, Antibacterial and Antioxidant Activity of *Andrographis paniculata* Nees. *International Journal of Science and Research*, **3**, 846-850.

[15] Bauer, A.W., Kirby, W.M., Sherris, J.C. and Truck, M. (1996) Antibiotic Susceptibility Testing by a Standardized Single Disk Method. *American Journal of Clinical Pathology*, **45**, 493-496.

[16] Doa, Q.D., Angkawijayaa, A.E., Tran-Nguyena, P.L., Huynhb, L.H., Soetaredjoc, . E., Ismadjic, S. and Ju, Y. (2014) Effect of Extraction Solvent on Total Phenol Content, Total Flavonoid Content, and Antioxidant Activity of *Limnophila aromatica*. *Journal of Food and Drug Analysis*, **22**, 296-302.

[17] Mensor, L.L., Menezes, F.S., Leitão, G.G., Reis, A.S., dos Santos, T.C., Coube, C.S. and Leitão, S.G. (2001) Screening of Brazilian Plant Extracts for Antioxidant Activity by the Use of DPPH Free Radical Method. *Phytotherapy Research*, **15**, 27-30. https://doi.org/10.1002/ptr.687

[18] Nurlaily, A., Noor Baitee, A.R. and Musalmah, M. (2012) Comparative Antioxidant and Anti-Inflammatory Activity of Different Extracts of *Centella asiatica* (L.) Urban and Its Active Compounds, Asiaticoside and Madecassoside. *Medicine & Health*, **7**, 62-72.

[19] Duh, P.D., Yeh, D.B. and Yen, G.C. (1992) Extraction and Identification of an Antioxidative Component from Peanut Hulls. *Journal of the American Oil Chemists' Society*, **69**, 814-818. https://doi.org/10.1007/BF02635922

[20] Tian, L.L. and White, P.J. (1994) Antioxidant Activity of Oat Extract in Soybean and Cottonseed Oils. *Journal of the American Oil Chemists' Society*, **71**, 1079-1086. https://doi.org/10.1007/BF02675900

[21] Chahmi, N., Anissi, J., Jennan, S., Farah, A., Sendide, K. and El Hassouni, M. (2015) Antioxidant Activities and Total Phenol Content of *Inulaviscosa* Extracts Selected from Three Regions of Morocco. *Asian Pasific Journal of Tropical Biomedecine*, **5**, 228-233.

[22] Kumar, S. and Pandey, A.K. (2013) Chemistry and Biological Activities of Flavonoids: An Overview. *The Scientific World Journal*, **2013**, Article ID: 162750.

[23] Soetan, K.O., Oyekunle, M.A., Aiyelaagbe, O.O. and Fafunso, M.A. (2006) Evaluation of the Antimicrobial Activity of Saponins Extract of *Sorghum bicolor* L. Moench. *African Journal of Biotechnology*, **5**, 2405-2407.

[24] Nwogu, L.A., Igwe, C.U. and Emejulu, A.A. (2008) Effects of *Landolphia owariensis* Leaf Extract in the Liver Functioning Profile Haemoglobin Concentrations of Albino rats. *African Journal of Biochemistry Research*, **2**, 240-244.

[25] Panathula, C.S., Mahadev, M.D. and Naidu, C.V. (2014) Phytochemical Investigation and *in Vitro* Antimicrobial Activity of *Centella asiatica* (L.) Urban. A Potent Antijaundice Medicinal Plant. *International Journal of Phytomedicine*, **6**, 195-200.

[26] Taguri, T., Tanaka, T. and Kouno, I. (2006) Antibacterial Spectrum of Plant Polyphenols and Extracts Depending upon Hydroxyphenyl Structure. *Biological and Pharmaceutical Bulletin*, **29**, 2226-2235. https://doi.org/10.1248/bpb.29.2226

[27] Padam, B.S., Tin, H.S., Chye, F.Y. and Abdullah, M.I. (2012) Antibacterial and Antioxidative Activities of the Various Solvent Extracts of Banana (*Musa paradisiaca* cv. Mysore) Inflorescences. *International Journal of Biological Science*, **12**, 62-73. https://doi.org/10.3923/jbs.2012.62.73

[28] Duffy, C.F. and Power, R.F. (2001) Antioxidant and Antimicrobial Properties of Some Chinese Plant Extracts. *International Journal of Antimicrobial Agents*, **17**, 527-529.