Enhanced Knowledge in Sciences and Technology Vol. 2 No. 2 (2022) 043-051

EKST

Homepage: http://publisher.uthm.edu.my/periodicals/index.php/ekst
e-ISSN : 2773-6385

A Comparative Study On Phytochemical Constituents and Antimicrobial Activity of *Alternanthera Sissoo* Extracts

'Aina Khalida Khairul Anuar[1], Shakila Abdullah[2*]

[1]Department of Technology and Natural Resources,
Universiti Tun Hussein Onn Malaysia, Pagoh, 84600, MALAYSIA

[2]Faculty of Applied Science and Technology,
Universiti Tun Hussein Onn Malaysia, Pagoh, 84600, MALAYSIA

*Corresponding Author Designation

DOI: https://doi.org/10.30880/ekst. 2022.02.02.005
Received 03 January 2022; Accepted 08 June 2022; Available online 23 November 2022

Abstract: Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoulene (BHT) are widely used in this era. They are mostly can be found in packaging materials, cereals, sausage, chewing gum, potato chips and butter. Consuming synthetic antioxidant in high level for a long period of time may confer to some degree of carcinogenicity. An alternative is used to replace the synthetic to natural antioxidant such as plants that contains phytochemical and antimicrobial activity which that can give impact on human health and disease prevention therefore used as antimicrobial drugs in traditional medicine. This study aimed to identify the qualitative phytochemical components present in *Alternanthera sissoo* extracts under different processing forms and to evaluate the antimicrobial microbial activity of *Bacillus cereus* and *Escherichia coli* of *Alternanthera sissoo* extracts under different processing forms. The method used are qualitative phytochemical screening which revealed the presence of flavonoids, terpenoids, tannins and saponins. Antimicrobial activity performed disc diffusion and agar well diffusion method to measure the inhibition zones of Gram-positive and Gram-negative bacteria. The phytochemical screening shows that *A. sissoo* contains terpenoids in different processing forms. The ethanolic extract of *A. sissoo* showed antimicrobial activity against *B. cereus* in boiled sample (19 mm) followed by steam blanch sample (14 mm) and none against *E. coli*.

Keywords: *Alternanthera Sissoo*, Antimicrobial, *Bacillus Cereus*, *Escherichia Coli*, Flavonoids, Phytochemical, Saponins, Tannins, Terpenoids

1. Introduction

Antioxidants are compounds that have been employed in the food chain for the past half-decade to prevent or at least postpone the auto oxidation process [1]. Butylated hydroxyanisole (BHA) and butylated hydroxytoulene (BHT) are the most commonly utilised synthetic antioxidants in foods to

**Corresponding author: shakilaa@uthm.edu.my*

prevent food spoiling. However, some evidence suggests that BHA and BHT may have modest carcinogenic effects when used in large doses [2]. Long-term consumption of high doses of BHA and BHT has been shown to increase tumor formation in the body. Aside from that, large doses of BHT in food can induce toxicity, affecting liver, thyroid, and renal function, as well as lung function and blood coagulation. Consumers are concerned about the long-term safety of synthetic antioxidants and have a negative opinion of their use. As a result, there is a growing interest in finding alternative natural and safe sources of antioxidants, particularly from *Alternathera sissso*, a member of the *Amaranthaceae* family that includes a variety of phytochemicals that benefit consumers. Many studies have shown that medicinal plants contain flavonoids, alkaloids, terpenoids, tannin, and saponins. As many plant extracts have been shown to inhibit the growth of microorganisms, these extracts consist of chemicals and are usually considered to play a role in defense reactions of plants against infections by pathogenic microorganisms.

In popularity of natural antioxidants is growing based on the fact that few synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoulene (BHT) are widely used in food product. BHA and BHT are mostly can be found in packaging materials, cereals, sausage, chewing gum, potato cheeps and butter. However, it is suspected to be harmful to human health as they confer some degree of carcinogenicity when it is consumed at high level for a long period of time. Due to the carcinogenicity of BHA and BHT, an alternative is used to replace the synthetic antioxidant to natural antioxidant which can be found in plants as natural antioxidants has phytochemical and antimicrobial activity that can give impact on human health and disease prevention. Hence, *A. sissoo* is also possible to produce phytochemical and antioxidant but up till today, although some research on *A. sisso* has been documented, it is still limited to the survey of traditional utilization among local people. In this present study, ethanolic extracts of *A. sissoo* were screened to determine their phytochemical and potential antimicrobial activity using 4 techniques preparation which are fresh, sun-dried, boiled water and steam blanch. The antimicrobial activity was tested against gram-negative bacteria (*Escherichia coli*) and gram-positive bacteria (*Bacillus cereus*).

2. Materials and Methods

2.1 Materials

A. sissoo was purchased from local supermarket in area Pagoh. The *A. sissoo* was washed with tap water to remove the foreign matter, and kept at an open place for drying at room temperature. The sample was prepared with 4 techniques preparation method which are fresh, sun-dried, boiled water and steam blanch. The samples were crushed and extracted by solvent extraction for 3 days using 70% ethanol. The extract of *A. sissoo* obtained were filtered using Whatmann No. 1 filter paper and the supernatant was collected. The supernatant was pooled and evaporated at 40°C until the volume is reduced to result in thick green crude extract [3]. The extracts of *A. sissoo* were stored in air tight bottle for further use.

2.2 Identification of phytochemical

2.2.1 Fourier-transform infrared (FTIR) analysis

In FTIR analysis, ethanol extract of *A. sissoo* powder was employed. Functional group of phytochemicals were analyzed by using Fourier Transform Infrared Spectroscopy (Perkin Elmer 99365, USA) equipped with attenuated total reflectance (ATR). The sample was placed on top of the ATR crystal and the values of the peaks were recorded.

2.3 Qualitative phytochemical screening

The qualitative phytochemical screening was used to identify flavonoids, terpenoids, tannins, and saponins in the ethanolic extracts. Phytochemical screening of extracts was conducted using standard

procedure described by Johari & Khong (2019) [4]. The qualitative results were expressed as (+) for the presence and (-) for the absence of phytochemical.

2.3.1 Test for flavonoids

The 2 ml of extract, 1 ml of 2N sodium hydroxide (Qrec, Malaysia) were added. Flavonoid content was detected by the presence of yellow colour [5].

2.3.2 Test for terpenoids

The 0.5 ml of the extract was treated with 2 ml of chloroform (Spectrum, United States) and concentrated sulfuric acid (Spectrum, United States). The presence of terpenoids was revealed by the formation of a reddish-brown tint at the interface [5].

2.3.3 Test for tannins

The 1 ml of extract was mixed with 2 ml of 5% ferric chloride (Sigmaaldrich, United States). The presence of tannins was indicated by the formation of dark blue or greenish black [5].

2.3.4 Test for saponins

The 2 ml of extract, 2 ml of distilled water was added and shaken in a graduated cylinder for 15 minutes. It resulted in the creation of a 1 cm foam layer, indicating the presence of saponins [5].

2.4 Antimicrobial activity

The evaluation of antimicrobial activity was determined using agar disc diffusion and agar well diffusion methods towards Gram-positive bacteria and Gram-negative bacteria which are *Bacillus cereus* and *Escherichia coli* [6].

2.4.1 Disc diffusion

The antimicrobial activity was carried out using agar disc diffusion method. The inoculums were inoculated by swabbing the bacteria onto sterile Mueller-Hinton agar. The filter paper discs about 6 mm in diameter, containing plant extracts were placed on the agar surface. The petri dishes were incubated (MMM Incucell 222, German) at 37°C for 24-48 hours. The inhibition growth zones of *A. sissoo* extract was measured in mm after the incubation period [6].

2.4.2 Agar well diffusion

The bacteria were uniformly spread onto Mueller-Hinton agar. Mueller-Hinton agar was pouched by cork borer 6 mm in diameter and then the extracts were poured into agar well. Penicillin and distilled water used as positive and negative control, respectively. The plates were incubated (MMM Incucell 222, German) at 37°C for 24-48 hour. Antimicrobial activity of *A. sissoo* was detected by measuring the zone of inhibition appeared after the incubation period [6].

3. Results and Discussion

3.1 Qualitative phytochemical analysis of *A. sissoo*

The result for the qualitative phytochemical analysis of *A. sissoo* in different processing forms are given below in Table 1.

Table 1: Phytochemical analysis of ethanolic extracts of *A. sissoo* in different processing form

Sample	Phytochemical in *Alternanthera sissoo*			
	Flavonoids	Terpenoids	Saponins	Tannins
Fresh	-	+	+	-
Sun-dried	-	+	+	+
Boiled	-	+	-	-
Steam blanch	-	+	-	-

*Note: presence (+) absence (-)

Phytochemical of plant extracts have recently been a great significant in food industry. The presence of phytochemicals as natural additive has the tendency to replace synthetic antimicrobials with natural ones. Present study was carried out phytochemical screening on *A. sissoo* in different processing forms to determine its usefulness in traditional medicine. The fresh and sun-dried sample of *A. sissoo* showed absence of flavonoids but boiled and steam blanch sample showed presence of flavonoids. Terpenoid test of *A. sissoo* indicates the presence of terpenoid in all samples which are fresh, sun-dried, boiled and steam blanch. Phytochemical screening for saponins revealed the presence of saponins on fresh and sun-dried sample by showing the presence of foam layer. Finally, sun-dried sample of *A. sissoo* showed intense green color referring to the presence of tannins but none in fresh, boiled and steam blanch sample.

Flavonoid test indicated that the possible changes that can occur in the solvents during the extraction processes can have significant effects on the stability of the flavonoids and the efficiency of the treatments [7]. Boiling and steam blanch because of its chemical structure degrade during the process. Hence, the absence of flavonoid can be detected. The presence of terpenoids detected in different processing form is due to the chemical structure of terpenoid as it is the largest among saponins, tannins and flavonoids which cannot be simply degrade during the boiling and steam blanch process. The study identified that saponins were extremely sensitive to high temperature treatment. It was speculated that saponins might increase the loss in the blanching water or be degrade at the high blanching temperature [8]. While a study suggested that tannins are relatively stable at room temperature in slightly acidic soils but both higher temperature and neutral to basic pH may facilitate chemical degradation [9]. Hence, the absence of tannins in fresh, boiled and steam blanch sample of *A. sissoo.* Stabilizing the content, structure, and activity of tannin is difficult. Tannin content is decreased in storage time of materials. The results of the tannin activities are mainly at the laboratory level. At the same time, modern method of tannin extraction needs high investment, good manpower, and great market. There are some challenges in tannin extraction and its application into the life.

3.2 Identification of phytochemical

3.2.1 Fourier-transformed Infrared spectroscopy

The Fourier Transformed Infrared (FT-IR) spectroscopy was used to analyze the functional group present in the chemical structure. The FT-IR spectrum was used to identify the functional groups of the bioactive components present in the extract based on the peak values in the region of IR radiation [10]. FT-IR analysis was performed from wavenumber 450 to 4400 cm^{-1} using a Frontier (Perkin Elmer) FT-IR spectrometer. The results of FT-IR analysis showed the presence of N-H, O-H, C=C, C-H and C-O functional group.

Figure 1 showed the FTIR spectra for fresh sample, Figure 2 showed FTIR spectra for sun-dried sample, figure 3 showed FTIR spectra for boiled sample and figure 4 showed FTIR spectra for steam blanched sample. They possess a major broad band at 3333.587 cm^{-1}, 3327.209 cm^{-1}, 3320.655 cm^{-1}, and 3336.255 cm^{-1}. The presence of phenolic compounds in terpenoids is further assured by the weak-medium absorption band observed at 1649.956 cm^{-1}, 1653.454 cm^{-1}, 1649.172 cm^{-1}, and 1653.682 cm^{-1} which shows the C=C stretch in alkene contains in the structure of terpenoids. A previous study stated that a peak at 1654 cm^{-1} is due to chlorophyll and protein content [11]. Other than that, the absorption band observed at 1043.908 cm^{-1}, 1043.882 cm^{-1}, 1043.870 cm^{-1}, and 1043.932 cm^{-1}can be pointed to C-O found in primary alcohols. The presence of weak absorption at 2976.151 cm^{-1}, 2976.319 cm^{-1}, 2976.707 cm^{-1}, and 2976.400 cm^{-1} found in ethanol solution used in the extraction of *A. sissoo* can be attributed to C-H stretching vibration. These results demonstrated the presence of hydroxyl group, alkenes, carbonyl and alkane compounds.

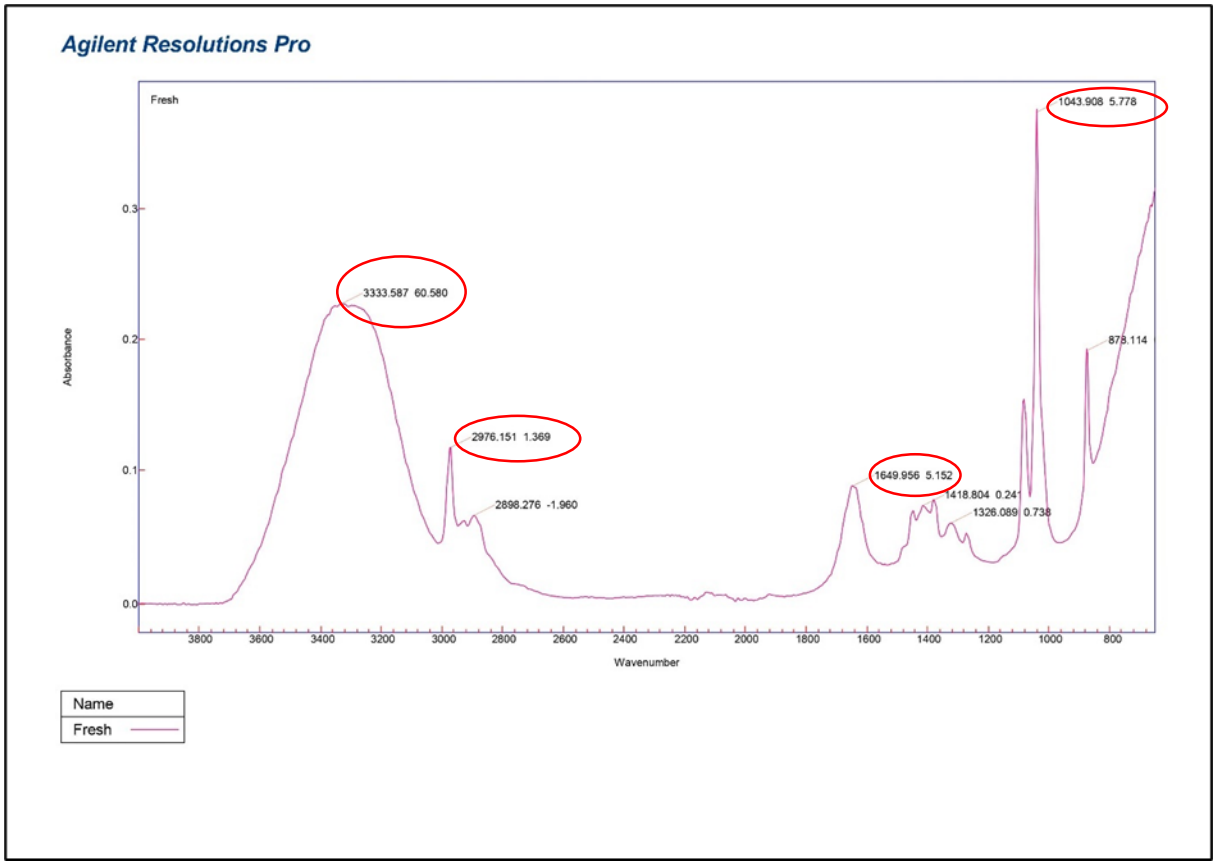

Figure 1: FT-IR spectra of fresh *A. sissoo* ethanolic extract

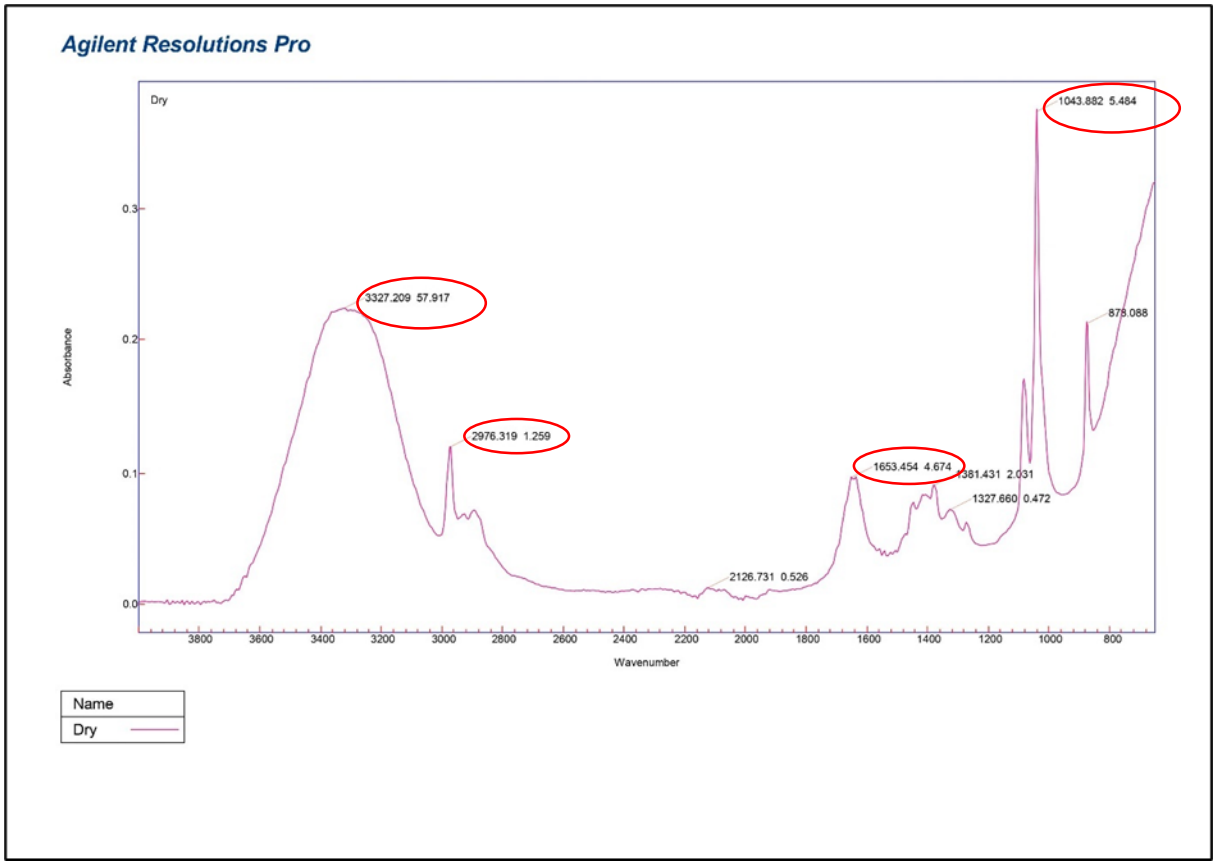

Figure 2: FT-IR spectra of sun-dried *A. sissoo* ethanolic extract

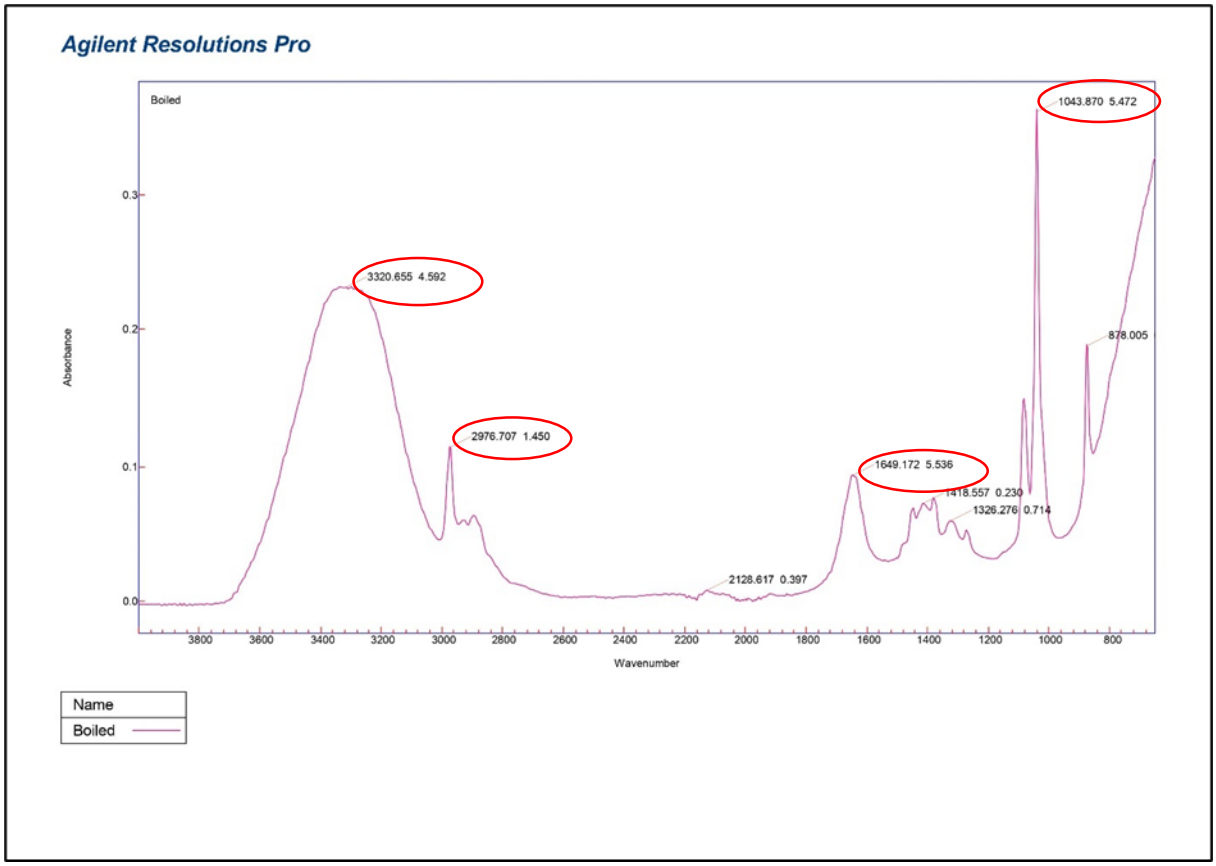

Figure 3: FT-IR spectra of boiled *A. sissoo* ethanolic extract

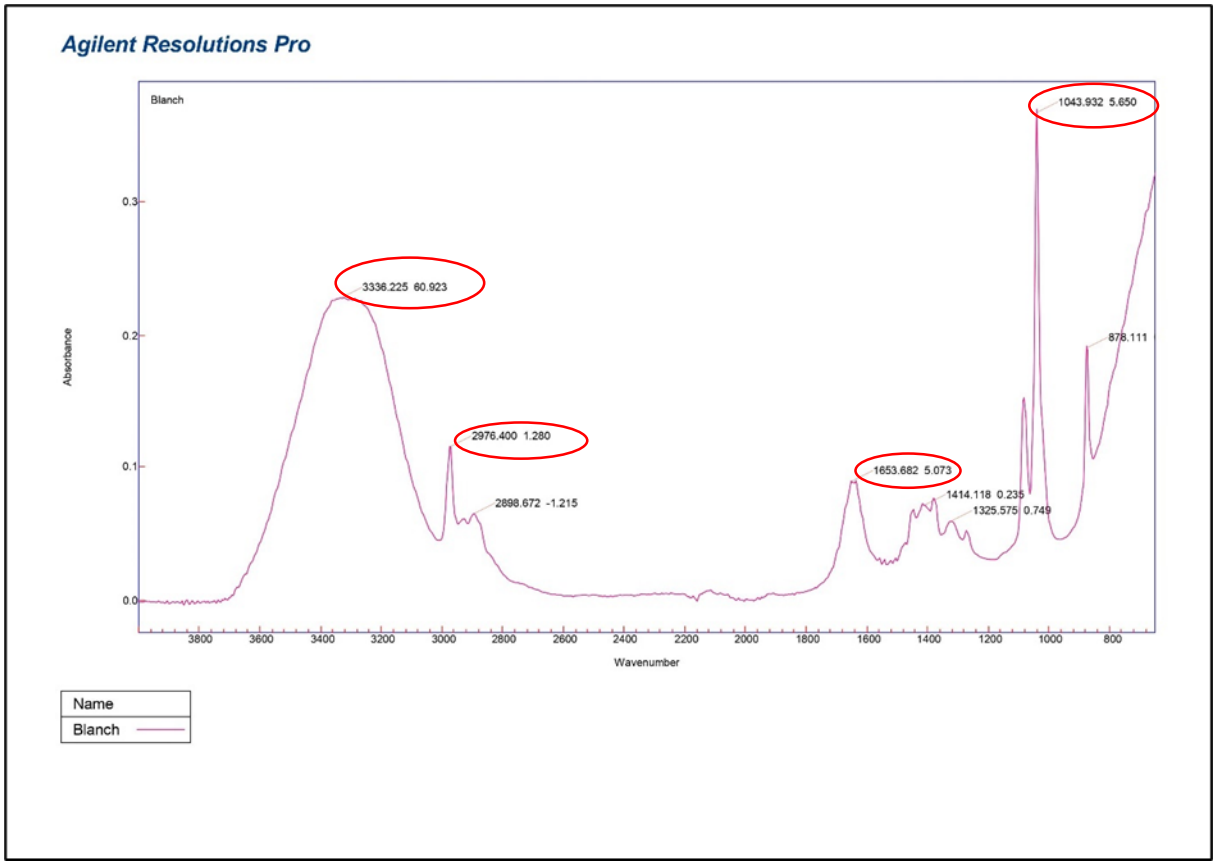

Figure 4: FT-IR spectra of steam blanch *A. sissoo* ethanolic extract

Based on the phytochemical compounds found in the FT-IR spectra in fresh, dry, boiled and steam blanch of *A. sissoo* extracts, it found that all the processing forms of *A. sissoo* has almost same FT-IR spectra as they give positive result in the presence of terpenoids. The healing properties of medicinal plants are usually linked with the presence of phytochemicals and these differ compound in one plant has the difference in pharmacological effects. Therefore, the presence of various phytochemicals in *A. sissoo* suggests that this plant possesses therapeutic importance. Thus, the preliminary screening test was useful in the detection of bioactive principles and subsequently may lead to drug discovery and development. Besides, flavonoids, tannins, saponins, and terpenoids which were extracted from this plant may be responsible for its pharmacological activities as well as a wound-healing agent for pharmaceutical and cosmeceutical use. As those phytochemicals seem to give a very positive impact to the consumers, the *A. sissoo* has the potential to be used for bio-medicinal applications, as phenolic compounds, tannins, flavonoids, chlorophyll and carotenoids are known to have antioxidant activities. These findings provide an opportunity for the development of natural products from *A. sissoo* in drug discovery.

3.3 Antimicrobial activity

From the result of the disc diffusion and agar well diffusion screening in Table 2, it summarizes that *B. cereus* (ATCC 14579) is shown to clearly possess antimicrobial properties against *E. coli* (ATCC 25922). The ethanolic extract of *A. sissoo* in boiled and steam blanch sample showed antimicrobial activity in *B. cereus* while dry and fresh sample of *A. sissoo* did not show any antimicrobial activity. The maximum antimicrobial activity was shown by boiled sample of *A. sissoo* and followed by steam blanch sample of *A. sissoo* with 19 mm and 14 mm inhibition zones respectively. Fresh and dried sample of *A. sissoo* in both gram-negative and gram-positive bacteria shows no inhibition zones as they might be contaminated during the processing of the extracts.

As *B. cereus* seems to give appreciable microbial activity against *E. coli*, this may indicate that the plant extract acts specifically against the gram-positive cell wall because they have a much thicker peptidoglycan layer than gram-negative bacteria [12]. The significant antibacterial activity of the *A. sissoo* extracts was comparable to the positive control, penicillin (10 µg/ml) and negative control distilled water. The antimicrobial potential of extracts obtained from *A. sissoo* could be attributed to presence of phytochemical listed above. Flavonoids are known to enhance the antimicrobial activity by creating complexes which will disrupt the function of cell membrane of microorganisms [13]. Antimicrobial property of tannins presence due to its ability to link amino acids in protein, inactivating adhesions, enzymes and transport proteins of cell membrane of microorganisms [14]. Saponins are known to have cytotoxic effects with surfactant properties on cell membrane, which assist in destruction of invading microorganism [15]. The results of the present study showed that oxygenated functional groups in terpenes compounds exhibited better antimicrobial activity than hydrocarbons.

Table 2: Antimicrobial screening test of *A. sissoo* extracts against *B. cereus* and *E. coli*

Sample	Inhibition zone in diameter (mm)			
	Gram-positive bacteria		Gram-negative bacteria	
	Bacillus cereus		*Escherichia coli*	
	Agar well	Disc diffusion	Agar well	Disc diffusion
Fresh	0	0	0	0
Dry	0	0	0	0
Boiled	8	19	0	0
Steam blanch	6	14	0	0

4. Conclusion

The leaves of *Alternanthera sissoo* in different processing forms contained different phytochemical including flavonoids, terpenoids, saponins and tannins. It can be concluded that high temperature during the processing degrades most of these phytochemicals in *A. sissoo* extracts. The results of FTIR analysis confirmed the presence of characteristics functional group in *A. sissoo* such as alcohol, phenols, alkanes, and alkanes which indicates the presence of terpenoids in different processing forms of *A. sissoo. A. sissoo* exhibited the highest antimicrobial activity in boiled sample followed by steam blanch sample against *B. cereus.* The presence of these bioactive compounds in *A. sissoo* revealed that it could be used as a potential source of natural antimicrobial components. However, further studies should be carried out to contribute to the improvement target phytochemical content in the *A. sissoo* extracts.

Acknowledgement

The authors would like to thank the Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia for its support and provide basic facilities to carry out this present research work.

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