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GC-MS Analysis and Evaluation of Bioactivities of Ethanolic Leaf Extract of *Araucaria heterophylla* (Salisb.) Franco

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Abstract

Araucaria heterophylla (Salisb.) Franco (Araucariaceae) is found all over the country mostly for ornamental purposes. It is widely used as an antiseptic, emollient and in the treatment of ulcers, rheumatism, toothache as well as respiratory infection. The present study was aimed to investigate GC-MS analysis, *in vitro* antioxidant activity and antimicrobial activity of ethanolic leaf extract of *A. heterophylla* (Salisb.) Franco (EAH). EAH was investigated by the GC-MS electron impact ionization (EI) method on a GC-2010 Plus gas chromatograph coupled to a GCMS-QP2010 Ultra mass spectrometer. *In vitro* antioxidant activity was evaluated by DPPH free radical scavenging assay along with the determination of total phenolic content (TPC) and total flavonoid content (TFC). Antimicrobial activity screening was performed by agar disc diffusion method against two Gram-positive (*Staphylococcus aureus* and *Bacillus megaterium*) and two Gram-negative bacteria (*Escherichia coli* and *Salmonella typhi***)** as well as a fungal strain (*Trichoderma harzianum*). Nine phytoconstituents were identified with the maximum concentration of 2,2,4-Trimethyl-3-pentanol. TPC of EAH was determined at 193.46 ± 4.93 mg GAE/g, whereas TFC was found at 560.16 ± 64.97 mg QE/g. Percentage (%) DPPH free radical scavenging by EAH was found in a dose-dependent manner. The IC_{50} value was calculated by regression analysis, and the value of ascorbic acid and EAH was found at 8.05 and 33.17 µg/ml, respectively. Antimicrobial activity was expressed in terms of the zone of inhibition which was found against *S. aureus*, *B. megaterium*, *E. Coli*, *S. typhi* and *T. harzianum* 29.87 ± 1.86, 20.60 ± 1.93, 37.63 ± 3.56, 20.43 ± 2.38, 13.20 ± 1.71 mm, respectively. The findings indicate this plant could be a potential source for drug development.

Key words: *Araucaria heterophylla*, GC-MS profiling, antioxidant, DPPH free radical scavenging, phenolic, flavonoids, antimicrobial activity.

Introduction

For the past sixty thousand years, people have been using plants as an alternative form of medicine (Yuan *et al.*, 2016). The WHO estimates that traditional medicine is used by about 80% of people globally (Farnsworth *et al.*, 1985). Approximately 25% of prescription drugs are derived from plants worldwide (Rates, 2001). Herbal medicine provides primary healthcare for almost 75% of our population (BFTI, 2016).

The Norfolk Island pine, or Christmas tree plant, is an Australian native known as *Araucaria heterophylla* (Salisb.) Franco (Araucariaceae) (Patil *et al.*, 2013; Verma *et al.*, 2014). It has been planted all across Bangladesh, mostly as an aesthetic. Its primary distribution regions include Venezuela, El Salvador, Ecuador, China, Belize and Honduras (Aslam *et al.*, 2013). Araucaria species are used as antiseptic, emollient and for treating several traditional illnesses, including rheumatism, ulcers, amenorrhea, toothaches and respiratory infections (Bussmann, 2008; Abd-ElGawad *et al.*, 2023). The essential oils from Araucaria plants have a high terpenoid concentration. For the GC-MS study of *A.*

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heterophylla (Salisb.) Franco's essential oil, few investigations have been conducted (Abd-ElGawad *et al.*, 2023; Elshamy *et al.*, 2020). Its resin oil has been reported to possess cytotoxic (Elkady and Ayoub, 2018), anti-ulcerogenic (Sattar et al., 2009), antibacterial (Verma *et al.*, 2014), antioxidant activity (Abd-ElGawad *et al.*, 2023) and anti-inflammatory activities (Elshamy *et al.*, 2020). The majority of the research that is currently accessible has been carried out on this plant's essential resin oil. The biological and GC-MS profiling of the alcoholic extract has not yet been investigated. So, the aim of the present study was to perform GC-MS analysis, *in vitro* antioxidant activity evaluation with TPC and TFC measurement, and antimicrobial screening of ethanolic leaf extract of *A. heterophylla* (Salisb.) Franco.

Materials and Methods

Solvents and reagents: For this experiment, analytical grade reagents and solvents were utilized. DPPH (Sigma-Aldrich, Germany), Folin & Ciocalteus Phenol (FCP) Reagent (SRL, India), aluminium chloride, ascorbic acid (RL, India), quercetin hydrate (Aldrich, India), gallic acid (RL, India), sodium carbonate anhydrous, potassium acetate, (Merk, India), absolute ethanol (99.9%) (Changshu Hongsheng Fine Chemical, China) were used.

Plant collection and preparation: A. heterophylla (Salisb.) Franco leaves were collected from Narayanganj, Bangladesh in December 2022. The Bangladesh National Herbarium (BNH) identified and verified the plant. For further reference, a voucher specimen (DACB-87368) was submitted to BNH. The collected leaves were dedusted and washed with demineralized water, shade dried with proper air flow for 15 days. The dried leaves were ground into a coarse powder using a high-speed grinder. Till they were needed again, ground leaves were kept in an airtight polybag.

Extraction of plant material: Approximately 1.5 L of ethanol was used to soak 615 g of coarsely powdered *A. heterophylla* (Salisb.) Franco leaves for 13 days, with periodic shaking and stirring. After cold extraction, the mixture was vacuum-filtered using Whatman No. 1 filter paper. A rotary evaporator was used to evaporate the filtrate at low pressure and temperature (below 50 ^{o}C). After that it was kept for air drying, which resulted in viscous crude extract. Percentage of yield was calculated as following:

$$\%\ yield = \frac{mass\ of practical\ crude\ extract}{mass\ of\ initial\ powder} \times 100\%$$

GC-MS analysis: EAH was analyzed using the Shimadzu GC-2010 Plus gas chromatograph and Shimadzu GCMS-QP2010 Ultra mass spectrometer via the GC-MS electron impact ionization method. An HS-5MS fused capillary column of 5% phenyl-95% methylpolysiloxane (film: 0.25 m, length: 30 m, diameter: 0.25 mm) was fitted with the gas chromatograph. The following was the programming for GC's parameters: temperature at inlet: 250^{o}C; temperature in oven: started at 40^{o}C for one minute, then increased to 200^{o}C for ten minutes (at a rate of 10^{o}C/min); carrier gas (Helium, at continuous pressure of 9.7 kPa), flow rate: 0.54 ml/min; a split ratio of 50 was used to administer the samples. Chloroform was used to dissolve the sample. For the chromatographic analysis, the total retention duration was 37 minutes. The following were the MS settings set: interface temperature: 450^{o}C ; ion source temperature: 200^{o}C; acquisition mode: scan mode; mass range: 50–1000 m/z.

Antioxidant activity evaluation

Determination of total phenolic content: Total phenolic content (TPC) of EAH was determined using the Folin-Ciocalteu reagent (FCR) following the method of *Velioglu et al.* (1998). FCR, a mixture of phosphomolybdate and phosphotungstate, facilitates a colorimetric assay for phenolic antioxidants (Singleton *et al.* 1999). The assay measures the ability of the sample to prevent FCR oxidation (*Vinson et al.*, 2005). 1 ml plant extract (100 µg/ml) or standard solution (6.25-100 µg/ml) was mixed with 5 ml of 10-fold diluted FCR and 4 ml of 7.5% sodium carbonate. After incubation (standard: 30 min; extract: 1 hr) at room temperature,

absorbance at 765 nm was recorded using a UV-Visible spectrophotometer against the blank. The TPC was calculated in gallic acid equivalents (GAE) per gram of dry extract by using the calibration curve.

Determination of total flavonoid content: Total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method (Wang and Jiao, 2000). One ml plant extract (100 µg/ml) or standard solution (6.25-200 µg/ml) was mixed with 3 ml ethanol, 200 µl potassium acetate (1M), 10 ml aluminum chloride solution (10% w/v), and 5.6 ml distilled water. The mixture was incubated for 30 minutes at room temperature. Absorbance at 415 nm was measured using a UV-Visible spectrophotometer against the blank. TFC in EAH was calculated in quercetin equivalents (QE) per gram of dry extract using the calibration curve.

DPPH free radical scavenging assay: DPPH free radical scavenging assay was conducted following the method of Braca *et al.* (2001), with modifications by Xiao *et al.* (2020). One milliliter of plant extract or standard solution (6.25-200 µg/ml) was mixed with 2 ml of 0.004% DPPH solution. A control was prepared using 2 ml of 0.004% DPPH and 1 ml ethanol, without ascorbic acid or extract. The mixtures were incubated in the dark at room temperature for 30 minutes, and absorbance was measured at 517 nm. The percentage of DPPH radical scavenging was calculated using the following formula:

$$\%\ Scavenging\ Activity = \frac{Absorbanc\ of\ the\ control - Absorbance\ of\ the\ test\ sample}{Absorbance\ of\ the\ control} \times 100$$

The IC_{50} value was determined using logarithmic regression analysis of inhibition percentages versus concentrations.

Antimicrobial screening: The antimicrobial assay was performed using the disc diffusion technique (Barry, 1980; Bauer *et al.*, 1966; Hussain *et al.*, 2008). The antibacterial activity of EAH (300 µg/disc) was evaluated against four pathogenic bacterial strains (*Staphylococcus aureus* (cars-2), *Bacillus megaterium* (BTCC-18), *Salmonella typhi* (K-323130), and one fungal strain (*Trichoderma harzianum* (carsm-2)). The diameter of the zone of inhibition was measured after the fungus and bacteria were cultured for 48 hours at 25°C and 24 hrs at 37°C, respectively.

Result and Discussion

Percentage (%) of yield of crude extract: A. heterophylla (Salisb.) Franco leaf powder was extracted using ethanol using a cold extraction method. A yield percentage of 3.41% was determined (Table 1).

Table 1. Crude extract yield value as a percentage

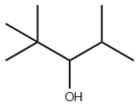
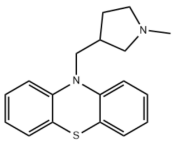
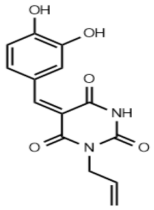
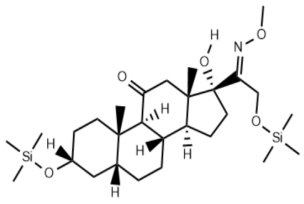
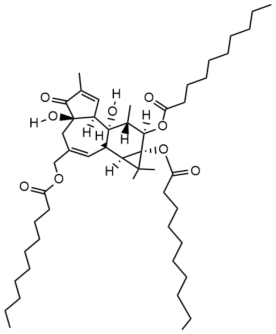
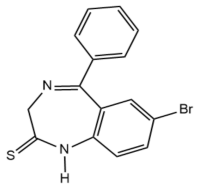
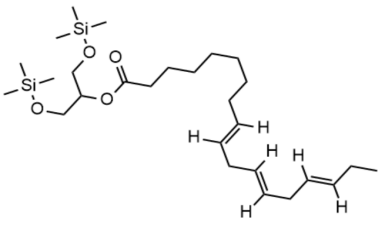
Plant used	Powder weight (g)	Extract obtained (g)	Yield (%)
A. heterophylla	615	21	3.41

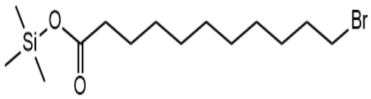
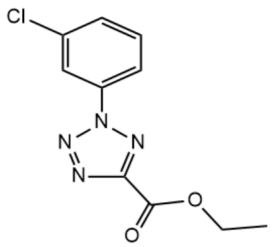
GC-MS analysis: GC-MS analysis of extract provides valuable information about phytoconstituents present in the sample. Using NIST database of the mass spectrum, the GC-MS analysis was interpreted. Nine (09) components from EAH were identified and quantified by the GC-MS analysis (Table 3).

Antioxidant activity evaluation

Determination of total phenolic content (TPC): The FCR was used to determine the TPC of the EAH, which was then represented as GAE/g plant extract. TPC was calculated using the standard curve of gallic acid ($y = 0.0155x + 0.0868$, $R^2 = 0.9982$) (figure 1).

Table 2. Chemical structures of constituents of EAH identified by GC–MS analysis

Sl. No.	Name of constituent	Molecular weight	Molecular formula	Chemical structure
1.	2,2,4-Trimethyl-3-pentanol	130	$C_8H_{18}O$	
2.	Methdilazine	296	$C_{18}H_{20}N_2S$	
3.	(5E)-1-Allyl-5-(3,4-dihydroxybenzylidene)-2,4,6(1H,3H,5H)-pyrimidinetrione	288	$C_{14}H_{12}N_2O_5$	
4.	Pregnane-11,20-dione, 17-hydroxy-3,21-bis[(trimethylsilyl)oxy]-, 20-(O-methyloxime), (3.alpha.,5.beta.)	537	$C_{28}H_{51}NO_5Si_2$	
5.	Decanoic acid, 1,1a,1b,4,4a,5,7a,7b,8,9-decahydro-4a,7b-dihydroxy-1,1,6,8- tetramethyl-5-oxo-3-[[(1-oxodecyl)oxy]methyl]-9aH-cyclopropa[3,4]benz[1,2-e]azulene-9,9a-diyl ester, [1aR-(1a.alpha.,1b.beta.,4a.beta.,7a.alpha.,7b.alpha.,8.alpha.,9.beta.,9a.alpha.)]-	826	$C_{50}H_{82}O_9$	
6.	7-Bromo-2,3-dihydro-5-phenyl-1H-1,4-benzodiazepin-2-thione	330	$C_{15}H_{11}BrN_2S$	
7.	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl] ethyl ester, (Z,Z,Z)- $$ 2-[(Trimethylsilyl)oxy]-1-([(trimethylsilyl)oxy]methyl) ethyl (9E,12E,15E)-9,12,15-octadecatrienoate	496	$C_{27}H_{52}O_4Si_2$	

8.	Undecanoic acid, 11-bromo-, trimethylsilyl ester	336	$C_{14}H_{29}BrO_2Si$	
9.	2H-Tetrazole-5-carboxylic acid, 2-(3-chlorophenyl)-, ethyl ester	252	$C_{10}H_9ClN_4O_2$	

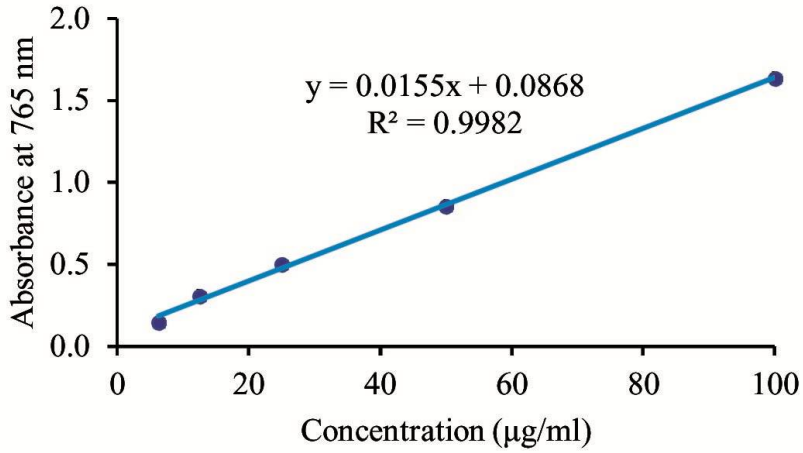


Figure 1. Calibration curve of gallic acid for determination of TPC.

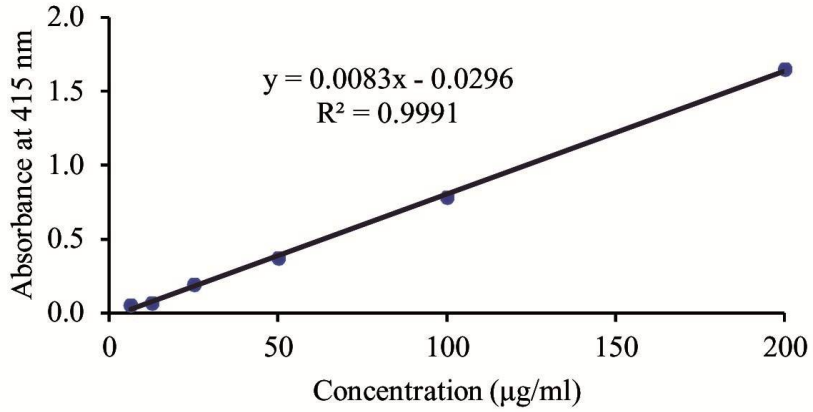


Figure 2. Calibration curve of Quercetin for determination of TFC.

Polyphenols' antioxidant characteristics result from their capability of hydrogen or electron donation (Dhakar *et al*., 2019). The present study's findings showed that the EAH equivalent to GAE had a high phenolic content (193.46 ± 4.93 mg/g). The substantial phenolic quantity suggests that the EAH has good antioxidant properties

Determination of total flavonoid content (TFC): The TFC of EAH was determined using the colorimetric technique with aluminum chloride. Utilizing the quercetin standard curve (y = 0.0083x - 0.0296, R^2 = 0.9991), the TFC were computed and represented as QE/g of plant extract (Figure 2).

Flavonoids are recognized for their antioxidant characteristics. The amount of flavonoids and the ability of plant extracts to serve as antioxidants have been discovered to be positively correlated. The present study's EAH shows a higher flavonoid quantity (560.16 ± 64.97 mg/g) which is in agreement with Manalo *et al*, (2020). According to the findings, EAH exhibit greater antioxidant properties in relation to their overall flavonoid concentration.

DPPH free radical scavenging assay: EAH contains high amount of polyphenols (193.46 ± 4.93 mg/g) and flavonoid (560.16 ± 64.97 mg/g). Polyphenols and flavonoid possess antioxidant activity. (Škrovánková *et al*., 2020; Frei & Higdon, 2003). The results showed that EAH had higher antioxidant capacities relative to their total concentration of flavonoids and polyphenols.

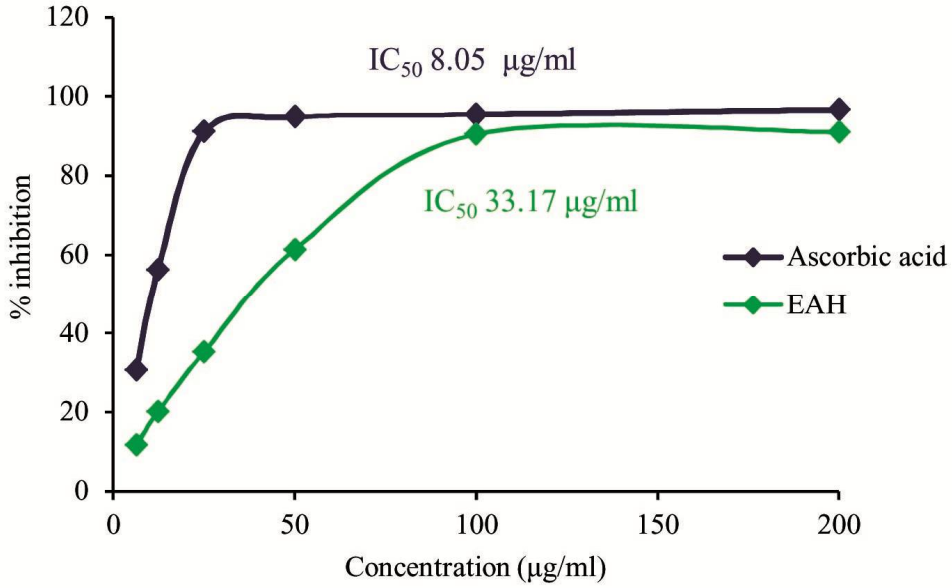


Figure 3. DPPH free radical scavenging assay

IC_{50} value of the EAH was 33.17 µg/ml, which is good antioxidant activity compared to standard ascorbic acid, 8.05 µg/ml (figure 3). High TPC and TFC are correlated with antioxidant activity.

Antimicrobial activity screening

Antibacterial activity: Two Gram-positive and two Gram-negative bacteria were tested for antibacterial activity using EAH at a concentration of 300 µg/disc. EAH showed potential antibacterial activity against all test bacterial (Table 3).

Table 3. Antibacterial potential of EAH

Test organism	Zone of inhibition (mm)
S. aureus	29.87 ± 1.86
B. megaterium	20.60 ± 1.93
E. coli	37.63 ± 3.56
S. typhi	20.43 ± 2.38

Antifungal activity: EAH at 300 µg/disc was screened for antifungal activity against *Trichoderma harzianum*. It showed good level of antifungal activity (13.20 ± 1.71 mm zone of inhibition).

Maximum inhibition was found against *E. coli*, whereas minimum against *S. typhi*. GC-MS analysis confirmed the presence of methdilazine having antibacterial activity (Chakrabarty *et al.*, 1993; Lagadinou *et al.,* 2020) in EAH. Flavonoids, which are widely recognized as functioning as antibacterial agents against a variety of pathogenic microorganisms, are abundant in EAH (Xie *et al.,* 2015; Farhadi *et al.,* 2019). Methdilazine with a high concentration of flavonoids may be the cause of the antibacterial activity that was discovered. Human pathogenic Gram-positive bacteria known as *S. aureus* are highly prevalent and are implicated in a wide range of infectious illnesses, such as pneumonia, bacteremia, osteomyelitis, endocarditis, and infections of the skin and soft tissues. Its medication resistance has been steadily increasing (Guo *et al.,* 2020). Meningitis and bacteremia may also have *B. megaterium*, another Gram-positive bacterium, as an etiologic component (Bocchi *et al.,* 2020). According to Allocati *et al.* (2013), the most common gram-negative bacteria, *E. coli*, is the primary cause of septicemia, enteritis, UTIs, and other severe disorders such neonatal meningitis. One of the main risks to the healthcare sector is the rising resistance of *E. coli* (Rodrigo, 2020). One of the most common bacterial diseases in the world is caused by *S. typhi*. It is becoming more resistant to many kinds of β-lactam antibiotics (Mina *et al.*, 2023). The observed antibacterial activity is in agreement with the study performed by Goud *et al*. (2017). The findings suggest that *A. heterophylla* (Salisb.) Franco may have the potentiality for acting as a source for developing newer antibiotic. In patients receiving peritoneal dialysis and hematological malignancies, *T. harzianum* induces invasive fungal infections (Sal *et al.,* 2022). The high concentration of flavonoids and polyphenols may be the cause of the observed antifungal action (Cushnie *et al.,* 2005; Orhan *et al.,* 2010; Daglia, 2012). The findings suggest that *A. heterophylla* (Salisb.) Franco might be potential source for developing newer antifungal drug.

Conclusion

Management of oxidative stress related diseses like diabetes, cancer and neurodegenerative disorder has becmome more challenging. Antimicriobial resistance is a global threat to the health sector. This research study was designed to evaluate antioxidant potenitial and antimicrobial acvtitiy of EAH for the hope of finding the source of new drugs. GC-MS analysis revealed nine constituents present in the EAH. Polyphenols have the potentilaity to fight against cardiovascular diseases, diabetes, cancer and neurodegenerative disorders. TPC and TFC of the EAH might be beneficial for managing the aforesaid disorders. Findings of antibacterial and antifungal disc diffusion assay might contribute to fighting the rapidly rising antimicrobial resistance. However, further research studies are required on these perspectives.

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References

Abd-ElGawad, A. M., Saleh, I., El-Razek, M. H. A., Elkarim, A. S. A., El-Amier, Y. A., Mohamed, T. A., El Gendy, A. E.-N. G., Afifi, S. M., Esatbeyoglu, T. and Elshamy, A. I. 2023. Chemical profiling of significant antioxidant and phytotoxic microwave-extracted essential Oil from *Araucaria heterophylla* Resin. *Separations* **10**, 141.

Allocati, N., Masulli, M., Alexeyev, M. F., and Di Ilio, C. 2013. *Escherichia coli* in Europe: an overview. *Int. J. Environ. Res. Public Health* **10**, 6235-6254.

Aslam, M. S., Choudhary, B. A., Uzair, M. and Ijaz, A. S. 2013. Phytochemical and ethno-pharmacological review of the genus *Araucaria* – review. *Trop. J. Pharm. Res.*, **12**, 651-659.

Bangladesh Foreign Trade Institute (BFTI). 2016. A Study on Sector-based Need Assessment of Business Promotion Council-Herbal Products and Medicinal Plants. http://www.bfti.org.bd/pdf/Herbal%20products%20and%20medicinal%20plants%20BPC%20research.pdf

Barry, A. L. 1980. In: *Antibiotics in Laboratory Medicine* (Lorian, V., Ed.), Willams and Wilkins, Baltimore, Procedures for Testing Antimicrobial Agents in Agar Media pp. 1-23.

Bauer, A. W., Kirby, W. M., Sherris, J. C. and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol* **45**, 493-496.

Bocchi, M. B., Cianni, L., Perna, A., Vitiello, R., Greco, T., Maccauro, G., and Perisano, C. 2020. A rare case of *Bacillus megaterium* soft tissues infection. *ActaBiomed*, **91**, e2020013.

Braca, A., Tommasi, N. D., Bari, L. D., Pizza, C., Politi, M. and Morelli, I. 2001. Antioxidant principles from *Bauhinia tarapotensis*. *J. Nat. Prod.* **64**, 892-895.

Bussmann, R.W. 2008 Antibacterial activity of northern-peruvian medicinal plants, *Arnaldoa* **15**, 127-148.

Chakrabarty, A. N., Bhattacharya, C. P. and DASTIDAR, S. G. 1993. Antimycobacterial activity of methdilazine (Md), an antimicrobic phenothiazine. *Apmis*, **101**, 449-454.

Cushnie, T. T. and Lamb, A. J. 2005. Antimicrobial activity of flavonoids. *Int. J. Antimicrob. Agents* **26**, 343-356.

Daglia, M. 2012. Polyphenols as antimicrobial agents. *COBIOT*, **23**, 174-181.

Dhakar, A., Chorotiya, P., Meena, M., Singh, C., Purvia, R.P. and Adlakha, M.K., 2019. Pharmacological properties and phytochemical of. *Limonia acidissima*: a review. *World J. Pharm. Res.* **8**, 637-645.

Elkady, W. M. and Ayoub, I. M. 2018. Chemical profiling and antiproliferative effect of essential oils of two *Araucaria* species cultivated in Egypt. *Ind. Crops Prod.* **118**, 188-195.

Elshamy, A. I., Ammar, N. M., Hassan, H. A., Al-Rowaily, S. L. Ragab, T. I., Gendy, A. E. G. and Abd-ElGawad, A. M. 2020. Essential oil and its nanoemulsion of *Araucaria heterophylla* resin: Chemical characterization, anti-inflammatory and antipyretic activities. *Ind. Crops Prod.* **148**, p.112272.

Farhadi, F., Khameneh, B., Iranshahi, M. and Iranshahy, M. 2019. Antibacterial activity of flavonoids and their structure–activity relationship: an update review. *Phytother. Res.*, **33**, 13-40.

Farnsworth, N. R., Akerele, O., Bingel, A. S., Soejarto, D. D. and Guo, Z. 1985. Medicinal plants in therapy. *Bull. World Health Organ.* **63**, 965-981.

Frei, B. and Higdon, J. V. 2003. Antioxidant activity of tea polyphenols *in vivo*: evidence from animal studies. *J. Nutr.* **133**, 3275S-3284S.

Goud, D. R., Reddy, G. R., Shivakoti, C., Ranjani, P. H., Vanitha, K. and Ramesh, A. 2017. Evaluation of antimicrobial activity of alcoholic extract of *Araucaria heterophylla* leaves. *World J. Pharm. Res.* **6**, 1287-1297.

Guo, Y., Song, G., Sun, M., Wang, J. and Wang, Y. 2020. Prevalence and therapies of antibiotic-resistance in *Staphylococcus aureus*. *Front. Cell. Infect. Microbiol.* **10**, 107.

Hussain, M. M., Rahman, M. S., Jabbar, A. and Rashid, M. A. 2008. Phytochemical and biological investigations of *Albizzia lebbeck* Benth. *Bol. Latinoam. Caribe Plantas Med. Aromát.* **7,** 273-278.

Lagadinou, M., Onisor, M. O., Rigas, A., Musetescu, D. V., Gkentzi, D., Assimakopoulos, S. F. and Marangos, M. 2020. Antimicrobial properties on non-antibiotic drugs in the era of increased bacterial resistance. *Antibiotics* **9**, 107.

Manalo, C. A. M., Arguelles, J. A. P., Bendaña, E. M., Comia, A. F. M., Ribao, M. R. B. and Dumaoal, O. S. R. 2020. *In vitro* antioxidant and DNA damage inhibition activity of ethanolic extract of *Araucaria heterophylla* (Salisb.) Franco (Norfolk Island pine). *The Steth* **14**, 31-45

Mina, S. A., Hasan, M. Z., Hossain, A. K. M. Z., Barua, A., Mirjada, M. R. and Chowdhury, A. M. M. A. 2023. The prevalence of multi-drug resistant *salmonella typhi* isolated from blood sample. *Microbiol. Insights* **16**, p.11786361221150760.

Orhan, D. D., Özçelik, B., Özgen, S. and Ergun, F. 2010. Antibacterial, antifungal and antiviral activities of some flavonoids. *Microbiol. Res.***165**, 496-504.

Patil, A. R., Darekar, A. B. and Saudagar, R. B. 2013. *Araucaria heterophylla*: the review. *World J. Pharm. Pharm. Sci.* **3**, 221-232.

Rates, S. M. 2001. Plants as source of drugs. *Toxicon:* **39**, 603-613.

Rodrigo, L. (Ed.). 2020. *E. Coli Infections:* Importance of Early Diagnosis and Efficient Treatment. *BoD–Books on Demand.*

Sal, E., Stemler, J., Salmanton-García, J., Falces-Romero, I., Kredics, L., Meyer, E., Würstl, B., Lass-Flörl, C., Racil, Z., Klimko, N., Cesaro, S., Kindo, A. J., Wisplinghoff, H., Koehler, P., Cornely, O. A. and Seidel, D. 2022. Invasive Trichoderma spp. infections: clinical presentation and outcome of cases from the literature and the FungiScope® registry. *J. Antimicrob. Chemother.*, **77**, 28500-2858.

Sattar, E. A., Monem, A. R. A., Ezzat, S. M., El-Halawany, A. M. and Mouneir, S. M. 2009. Chemical and Biological Investigation of *Araucaria heterophylla* Salisb. Resin. *Z. Naturforsch. C.*, **64**, 819-823.

Singleton, V. L., Orthofer, R. and Lamuela-Raventós, R. M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymol*, **299**, 152-178.

Škrovánková, S., Válková, D. and Mlček, J. 2020. Polyphenols and antioxidant activity in pseudocereals and their products. *Potr. S. J. F. Sci.*, **14**, 365-370.

Velioglu, Y. S., Mazza, G., Gao, L. and Oomah, B. D. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agric. Food Chem*, **46**, 4113-4117.

Verma, R. S., Padalia, R.C., Goswami, P., Verma, S. K., Chauhan, M. and Darokar, M. P. 2014. *Ind. Crops Prod.*, **61**, 410-416.

Vinson, J. A., Zubik, L., Bose, P., Samman, N. and Proch, J. 2005. Dried fruits: excellent in vitro and in vivo antioxidants. *J. Am. Coll. Nutr*. **24**, 44-50.

Wang, S.Y. and Jiao, H. 2000. Scavenging Capacity of Berry Crops on Superoxide Radicals, Hydrogen Peroxide, Hydroxyl Radicals, and Singlet Oxygen. *J. Agric. Food Chem.*, **48**, 5677-5684.

Xiao, F., Xu, T., Lu, B. and Liu, R. 2020. Guidelines for antioxidant assays for food components. *Food front.*, **1,** 60-69.

Xie, Y., Yang, W., Tang, F., Chen, X. and Ren, L. 2015. Antibacterial activities of flavonoids: structure-activity relationship and mechanism. *Curr. Med. Chem.*, **22**, 132-149.

Yuan, H., Ma, Q., Ye, L. and Piao, G. 2016. The Traditional Medicine and Modern Medicine from Natural Products. *Molecules*, **21**, 559.