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Original article

Chemical composition of fixed oil and *in vitro* antimicrobial activity of *Andrographis paniculata* root

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ABSTRACT

The present study estimates the antimicrobial potential of root extract of *Andrographis paniculata* which are extensively used in traditional medicine against clinical pathogens. The *in vitro* antibacterial activities were done with ten pathogen bacterial strains. Among the three extracts, the methanol extract exhibited the highest zone of inhibition against *Salmonella typhimurium* (45 mm), while ethanol and ethyl acetate extract revealed strong *in vitro* antibacterial against *Shigella flagella* (42 mm) and *E. faecalis* (38 mm), respectively at 200 μg/ml concentration. Antifungal activities of ethanol and methanol extract were done against four reference fungal strains. The ethanol and methanol extract confirmed the highest antifungal activity against *Trichophyton mentagrophytes* (38 mm) and *Candida albicans* (38 mm), respectively. The constituents of the fixed oil of petroleum ether root extract were evaluated by Gas Chromatography-Mass Spectrophotometer. Eleven composites have recognized, methyl palmitate (56.732%) was the major constituent. The investigation come to an end that the phytochemical constituents of methanol and ethanol root extract have capacity to kill both drug resistant bacteria and fungal.

1. Introduction

Natural products are strong and potential sources of drugs as they are very safe and free from any toxicity (Deora, 2017). Synthetic drugs are often treated chemicals so sometimes our body has difficulty to recognize it and causes toxicity and unhealthy side effect by extreme uses of different synthetic drugs. Nowadays, from the medicinal herbs or partial chemical synthesis maximum drugs are developed (Sukanya et al., 2009). In ancient times medicinal plants are widely used around the whole world as a great source of antimicrobial agents as plants are rich in phytochemical constituents (Ravikumar et al., 2010; Pandy et al., 2012). *Andrographis paniculata* (Burm. F) Ness belongs to the Acanthaceae family which is one of the most valuable medicinal plant and commonly used for the design of natural medicine of various diseases like as diarrhea, eczema, dysentery, chickenpox, fever, common cold, pneumonia and hepatitis (Akbar, 2011; Jarukamjorn and Nemoto, 2008). *Andrographis paniculata* is widely used from the ancient periods in India, Bangladesh, Hongkong, Indonesia, Pakistan and Thailand (Kabir et al., 2014). In south Asian countries it is known as "Kings of bitters" or "Kalmegh". It has been used for various therapeutic values such as anti-cancer, anti-inflammatory, anti-HIV, anti-diabetic, anti-allergic, anti-oxidant, anti-microbial and reliefs in striking cholera, influenza, bronchitis, swellings, itches and gonorrhea (Richard et al., 2017; Harjotaruno et al., 2008; Sheeja et al., 2006).

Formerly different parts of *Andrographis paniculata* have been considered for the isolation of pharmacologically active compounds but no orderly study has been done for comparative study of *in vitro* anti-microbial activities of root against various pathogenic microorganisms and fatty acid existing in the root of this plant. For the discovery of pure compounds of natural herbs, extraction must be needed. Such as solvent extraction, distillation method, pressing and sublimation have been done on different conditions. Among all processes solvent extraction is very popular

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because of its different beneficial properties; needs minimum energy, worthy grade extract, control the outcome destruction and instability, the process is very ordinary and easy to separate and collection of the resultant and also can reuse the solvent. Ethyl Acetate, ethanol and methanol are highly recommended for phytochemical as they have great properties to the entrance. (Qing-Wen et al., 2018). In view of the enormous application of all the parts of *Andrographis paniculata* in Ayurveda and Siddha, *in vitro* microbiological study and fatty acid composition of root were analyzed by Gas Chromatography-Mass Spectrophotometer.

2. Materials and methods

2.1. Chemicals

This experiment employed only analytical or laboratory-grade chemicals and solvents. The solvents ethyl acetate (purity 99.5%), ethanol (purity 99.8%) and methanol (purity 99.8%) were procured from E. Merck (Germany).

2.2. Plant materials

Fresh *Andrographis paniculata* roots were obtained in April 2018 from Jahangirnagar University in Dhaka, Bangladesh, and identified by a taxonomist at the Bangladesh National Herbarium in Dhaka, where a voucher specimen (No. 45932) was submitted. The dried plant material was then ground into a fine powder and stored in an airtight container at ambient temperature for future uses. The moisture content of the powder sample was 2.8%.

2.3. Preparation of extracts

For five days at room temperature, 100 g of powdered sample was submerged in appropriate solvents with increasing polarity commencing with ethyl acetate, ethanol, and methanol in an airtight separating funnel with intermittent shaking and stirring. A rotary evaporator was used to concentrate the various extract under reduced pressure. Finally, the extracts were kept at 4℃ for subsequent analysis.

$$\% \text{ of yield} = (W1 \times 100)/W2$$

W1 = weight of the extract residue obtained after solvent removal

W2 = weight of the plant powder

2.4. Bioactivity screening

2.4.1. Microbial strains

The antibacterial properties of the plant sample were tested against four gram-positive bacteria, including *Bacillus cereus*, *Bacillus thuringiensis*, *Staphylococcus aureus*, and *Enterobacter faecalis*, with six gram-negative bacteria, including *Shigella flagella*, *Salmonella typhimurium*, *Klebsiella pneumonia*, *Escherichia coli*, *Pseudomonas arerogenosa*, and *Vibro parahaemolytices*. The antifungal test included *Aspergillus niger*, *Trichophyton mentagrophytes*, *Candida albicans*, and *Candida tropical*. The Institute of Food Science and Technology (IFST), BCSIR, Dhaka, Bangladesh, provided all microorganisms as pure cultures.

2.4.2. Antibacterial assay

The agar well diffusion method was used to investigate the possible antibacterial activity of root extracts (Valgas et al., 2007). The sterile petri plates were filled with 25 ml Muller Hinton agar, which was allowed to solidify. A sterile borer was used to punch 6 mm diameter wells in the medium before streaking the plates with bacterial culture. The bacterial cultures were inoculated after the agar had been set by distributing them in petri plates using sterilized cotton swabs. Then, directly onto the surface of Muller Hinton agar containing bacterial lawn, 0.1 ml of plant extract was put. The inoculation plates were kept at 37 °C overnight to allow for bacterial development, and the diameter of the zone of inhibition was measured in millimeters.

2.4.3. Antifungal assay

100 μl of the ethanol and methanol root extracts were put into the wells in the plates. 100 μl of 10% DMSO was used to make a negative control well. Plates were placed in a laminar flow for 30 min to allow for pre-diffusion of the extract, then incubated at room temperature for three days. After the incubation period, the inhibition zone was evaluated. All of the experiments were carried out in triplicate. Each sample was tested in three different ways.

2.5. Extraction of fatty acids and preparation of methyl ester (FAMEs)

The root of *Andrographis paniculata* was collected and cleaned individually under running tap water to remove dust, debris, and other soil particles. Then, the root was dried at room temperature and made powder by a Fritsch mortar grinder from Germany. The natural fatty acids were extracted separately from the plant's root powder (100 g) using petroleum ether (b.p 40 °C-60 °C) for 72 h in a Soxhlet device. In a rotary evaporator, the extracts were concentrated under reduced pressure. The extracts were filtered through Whatman No. 1 filter paper before being vacuum distilled to eliminate the solvent completely. In a refrigerator, the extracts were stored in a nitrogen atmosphere. The fatty acids in the extracts were first converted to fatty acid methyl esters (FAMEs), and then GC–MS was examined using the method described by Griffin (Griffin, 1960).

2.6. Gas Chromatograph-Mass spectrum analysis

On an Agilent 7890A system with a mass Spectrophotometer detector and split less injection system, GC–MS analysis of petroleum ether extract from the root was performed. An HP-5MS capillary column (30 m × 0.25 mm: film thickness: 25 m) was installed in the GC. The following was the temperature schedule: initial oven temperature was 70 °C, then increased at 10 °C/min to 150 °C for 5 min, then 12 °C/min to 200 °C for 15 min, and finally 12 °C/min to 220 °C for 15 min. Helium was employed as the carrier gas, with a flow rate of 0.6 ml/min and a pressure of 17.69 psi. The samples were dissolved in methanol, and a 1 μl aliquot was automatically injected. MS had been set to scan mode. It was electron ionization that caused the ionization. The mass range was chosen to be between 50 and 550 *m*/*z*. MS spectra of isolated components were identified for fatty acid composition using NIST libraries.

3. Results

3.1. Oil extraction yield

A. paniculata root showed different yield (%) for the different solvents; 6.4% for ethyl acetate, 8.9% for ethanol and 12.6% for methanol.

3.2. In vitro antibacterial activity assay

The *in vitro* antibacterial activity of various solvent extracts of *A. paniculata* root at different concentrations (10, 40, 80, 120, 160,

200 μg/ml) was qualitatively measured by zones of inhibition against employed bacteria. Consequences showed that all the crude extracts have potential against all the bacterial strains screened (Figs. 1–4). Methanol extract was more effective compared with the ethanol and ethyl acetate extract. Methanol extract was exposed the greatest amount of bacterial activity against the maximum number of pathogen bacteria. Maximum activity of methanolic extract was observed against *Salmonella typhimurium* (45 mm), while ethanol and ethyl acetate extract revealed strong *in vitro* antibacterial activity against *Shigella flagella* (42 mm) and *E. faecalis* (38 mm), respectively at 200 μg/ml concentration.

Furthermore, MIC and MBC values (Table 1) of the root extracts were studied and compared with the standard by the well diffusion method. Ethyl acetate extract and ethanol extract lowest MIC value was 2.5 μg/ml against *S. flagella* and *E. coli*, respectively, whereas methanol root extract stood 2.8 μg/ml against *P. arerogenosa*. *A. paniculata* root extract disclosed possibly antibacterial action opposed to the microorganism with MBC from 165 μg/ml to 195 μg/ml. The highest MBC value was 195 μg/ml for *Vibro parahaemolytices*. The lowest MBC value was 165 μg/ml for *Bacillus cereus* and *Salmonella typhimurium*. The MIC and MBC evaluation of vigorous solvent extract in contradiction of strains presenting auspicious outcomes of antibacterial perspective of the *A. paniculata*.

3.3. In vitro antifungal activity assay

Additionally, the results of fungal bioassays of root ethanol and methanol extract were also determined (Fig. 5 and Table 2). This assay exposed that the ethanol root extract showed the maximum amount of antifungal activity (38 mm) and also methanol root extract showed the same value against *Trichophton mentagrophytes* and *Candida albicans*, respectively. The lowest antifungal activity was disclosed (32 mm) by ethanol root extract against *Aspergillus niger*. This assay discovered that *A. paniculata* had a strong antifungal perspective too.

3.4. Investigation of fatty acid by GC–MS

The constituents of the fatty acids from the root of *Andrographis paniculata* from petroleum ether extract confirmed the presence of 11 compounds, comprising the vigorous type of fatty acids that have been represented in Table 3. The fatty acids composition of

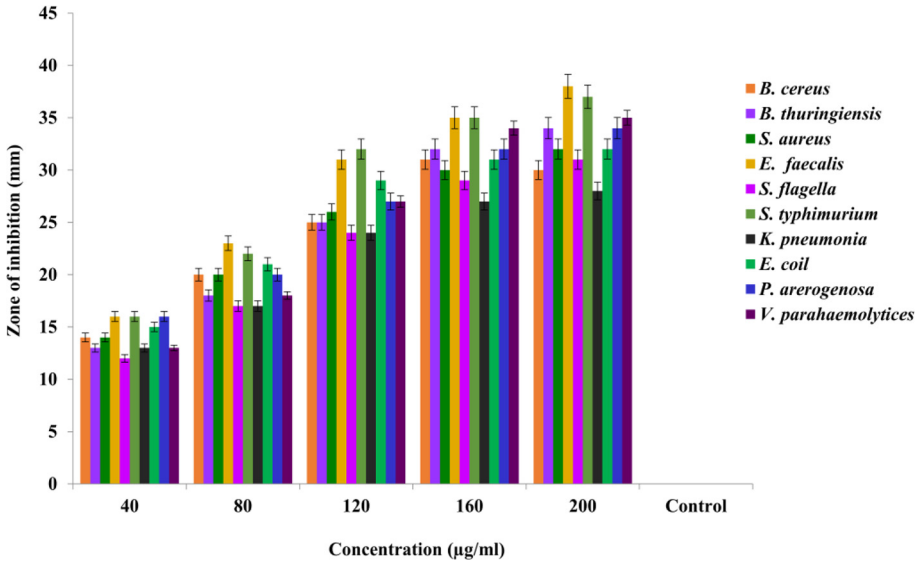

Fig. 1. Zones of inhibitions (mm) showing antibacterial activity of ethyl acetate extract of *Andrographis paniculata* root.

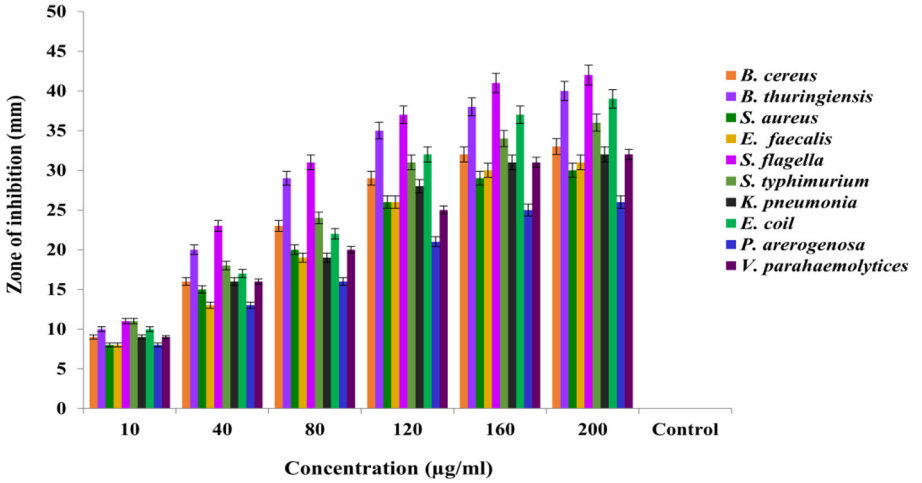

Fig. 2. Zones of inhibitions (mm) showing antibacterial activity of ethanol extract of *Andrographis paniculata* root.

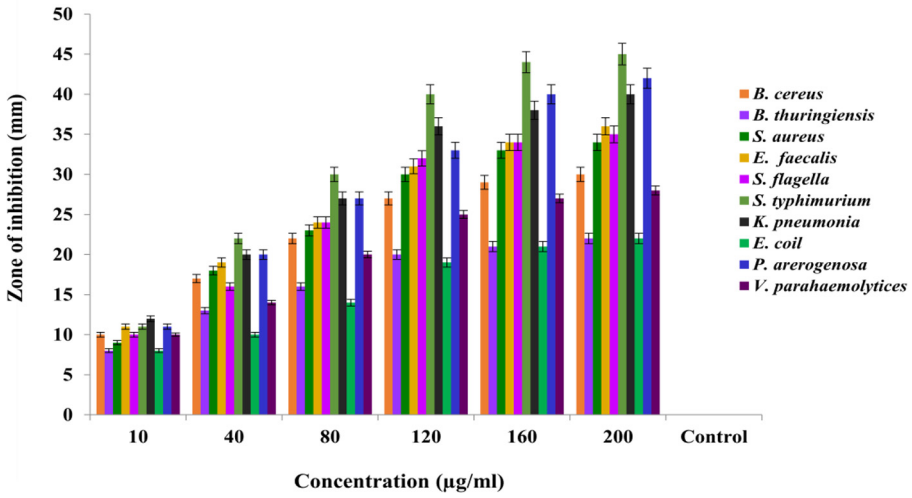

Fig. 3. Zones of inhibitions (mm) showing antibacterial activity of methanol extract of *Andrographis paniculata* root.

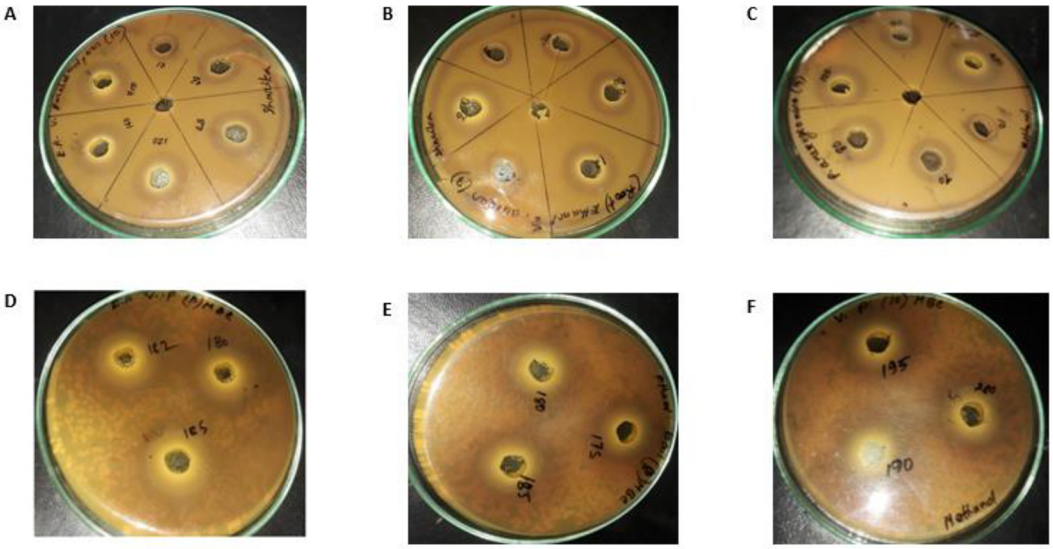

Fig 4. Antibacterial Zones of inhibitions of Root Extract (A) E.A extract with different con. against *V. parahaemolytices* (B) Ethanol extract with different con. against *S. aureus (C)* Methanol extract with different conc. against *P. arerogenosa* (D) MBC of E.A extract against *V. parahaemolytices* (E) MBC of Ethanol extract against *E. coil* (F) MBC of Methanol extract against *V. parahaemolytices*.

Table 1
Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of different extracts of *A. paniculata* root.

SI No	Name of Bacteria	Ethyl acetate extract		Ethanol extract		Methanol extract	
		MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)
1	*Bacillus cereus*	3	165	4	170	3.5	175
2	*Bacillus thuringiensis*	3	170	3.5	180	4	180
3	*Staphylococcus aureus*	3.5	185	3	175	3.5	185
4	*Enterobacter faecalis*	3	180	3	185	4.5	170
5	*Shigella flagella*	2.5	175	3	190	4	165
6	*Salmonella typhimurium*	3	165	3.5	194	3.5	185
7	*Klebsiella pneumonia*	3	170	3.5	187	3.6	175
8	*Escherichia coli*	3.5	175	2.5	180	3	170
9	*P. arerogenosa*	3.5	185	3	187	2.8	180
10	*Vibro parahaemolytices*	4	185	4.5	195	3.5	190

root defended the herb's acceptability in the ayurvedic remedies. They are associated with extended biological signing mechanisms. The highest component was methyl palmitate (56.732%) with a retention time of 11.59 min which is a saturated fatty acid. Most of the saturated fats have very important properties in heart disease. They also have been revealed to shield the glandular organ from liquor and drugs. Lack of them the immunity system of our body also reduced against predators or microorganisms. Unsaturated fatty acids are prime resources of power and strength from nutriment (Peichev et al., 2000).

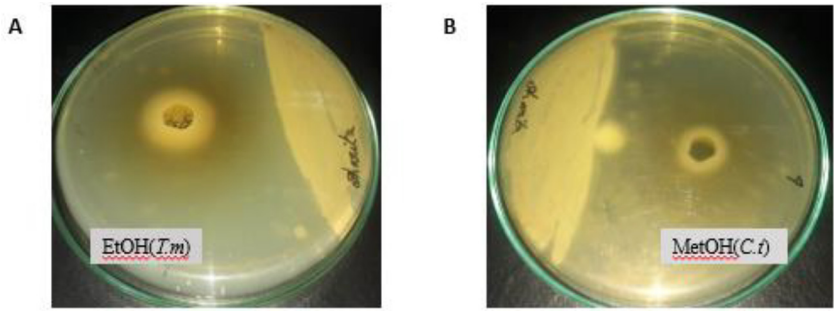

Fig 5. Antifungal ZIB (A) Ethanol Root Extract against *Trichophyton mentagrophytes* (B) Methanol extract against *Candida tropicals.*

Table 2
Antifungal activity of different extracts of *Andrographis paniculata* root.

SI No.	Fungal Strain	Zone of Inhibition (mm)	
		Ethanol (100 μg/ml)	Methanol (100 μg/ml)
1	*Aspergillus niger*	32 ± 0.2	34 ± 0.3
2	*Trichophton mentagrophytes*	38 ± 0.5	37 ± 0.7
3	*Candida albicans*	35 ± 0.7	38 ± 0.5
4	*Candida tropicals*	33 ± 0.3	36 ± 0.5

Table 3
GC–MS analysis of fatty acids from pet-ether extract of *Andrographis paniculata* root.

SI No.	Retention time (min)	Name of the compound	Types of fatty acid	Concentration (%)
1	5.62	Methyl Caproate/Methyl Hexanoate	Saturated	0.961
2	8.70	Methyl Laurate	Saturated	0.259
3	9.96	Methyl Myristate/ Methyl Tetradecanoate	Saturated	1.151
4	10.71	Methyl Pentadecanoate	Saturated	0.547
5	11.59	Methyl Palmitate	Saturated	56.732
6	12.56	Methyl Heptadecanoate	Saturated	0.897
7	13.65	Methyl Stearate	Saturated	9.978
8	13.82	Trans-9-Elaidic acid methyl ester	Unsaturated	5.968
9	13.96	Cis-9-Oleic acid Methyl ester	Unsaturated	8.875
10	14.57	Methyl Linoleate	Unsaturated	12.192
11	16.07	Methyl Eicosanoate	Unsaturated	2.442

4. Discussion

Globally transferrable diseases are the foremost reason for illness and temporariness. At this time, the enduring conflict contrary to bacteria and fungus wins through the confidence of growing, resistance. Nevertheless, development in health field outcomes in further sufferers being in serious and resistant repressed conditions, hence producing a continuous necessity for new antibiotics and antifungal drugs. Consequently, now is the great stage to find out novel microbicidal drugs (Mahesh and Satish, 2008). Natural medicines are a greater significant subject of interest in therapeutic industries for the preparation of important remedies (Sule et al., 2010). At extreme methanolic extract, concentration disclosed the highest bacterial inhibitory activity compared to other extracts. This result is a similar statement stated by several scientists (Parekh and Chand, 2010; Al-Bayati, 2008; Kaushik and Goyal, 2011, Shalini and Narayanan, 2015). There is very few work has been done on the antimicrobial activity of *A. paniculata* root, this study was done with ten microorganisms and interestingly the growth of all the bacteria has inhibited whereas researchers previously did by four bacteria (Shalini and Narayanan, 2015). The composition of fatty acid is subject to changing in topographical and environmental circumstances. Their hydrophobic behaviors, good collaboration with phospholipid bilayer characteristics might show the anti-microorganism properties. (Carvalho de Lima et al. 2016).

5. Conclusion

From the primordial periods, *A. paniculata* has been used in traditional treatments as a chemotherapeutical agent. It has the least side effects and worldwide field for numerous infections in the pharmacological markets with extract or isolated composites. The research was accomplished to open a new era in improving traditional medicine with modern microbiology. The encouraging outcome of this investigation may bring about the root of *A. paniculata* would be a prospective source of antibiotics. This *in vitro* study of *A. paniculata* is an absolutely new finding in Bangladesh. This is not to claim that the assays' results are conclusive; they may be the first stage in a considerable process that will ultimately lead the new researchers to the selection of more valuable substances in the plant.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Akbar, S., 2011. *Andrographis paniculata*: a review of pharmacological activities and clinical effects. Altern. Med. Rev. 16 (1), 66–77.

Al-Bayati, F.A., 2008. Synergistic antibacterial activity between *Thymus vulgaris* and *Pimpinella anisum* essential oils and methanol extracts. J. Ethnopharmacol. 116 (3), 403–406.

Carvalho de Lima, F.G., Pessoa da Silva, M.A., Freitas, B.T., Freitas, J.C.M., De Souza Teixeira, C., Bezerra, J.W.A., 2016. Evaluation of antimicrobial activity of the fixed oil of *Bowdichia Virgilioides* Kunth (fabaceae) seeds. Gaia Scientia 10 (4), 681–689.

Deora, G., 2017. *In vitro* assessment of antioxidant and antiproliferative efficacy of *A. paniculata*. Int. J. Pharma. Sci. Res. 5 (10), 4456–4466.

Griffin, R.C., 1960. ASTM, Technical Method of Analysis. McGraw-Hill Co. Inc Publication, New York and London.

Harjotaruno, S., Widyawaruyantil, A., Zaini, N.C., 2008. Apoptosis inducing effect of andrographolide on TD-47 human breast cancer cell line. Afr. J. Tradit. Complement. Altern. Med. 4 (3), 345–351.

Jarukamjorn, K., Nemoto, N., 2008. Pharmacological aspects of *Andrographispaniculata* on health and its major diterpenoid constituents andrographolide. J. Health Sci. 54 (4), 370–381.

Kabir, H., Hasan, N., Rahman, M.M., Rahman, M.A., Khan, J.A., Hoque, N.T., Bhuiyan, M.R., Mou, S.M., Jahan, R., Rahmatullah, M., 2014.A. survey of medicinal plants used by the Deb barma clan of the Tripura tribe of Moulvibazar district, Bangladesh. J. Ethnobiol. Ethnomed. 10 (1), 1–28.

Kaushik, P., Goyal, P., 2011. Evaluation of various crude extracts of *Zingiber officinale* rhizome for potential antibacterial activity: a study *in vitro*. Advance Microbiol. 1, 7–12.

Pandy, G., Madhuri, S., Mandloi, A.K., 2012. Medicinal plants useful in fish diseases. Plant Arch. 12 (1), 1–4.

Mahesh, B., Sathis, S., 2008. Antimicrobial activity of some important medicinal plants against plant and human pathogens. World J. Agric. Sci. 4, 839–843.

Parekh, J., Chand, S., 2010. Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. Afr. J. Biomed. Res. 10, 175–181.

Peichev, M., Naiyer, A.J., Pereira, D., Zhu, Z., Lane, W.J., Williams, M., Oz, M.C., Hicklin, D.J., Witte, L., Moore, M.A.S., Rafii, S., 2000. Expression of VEGFR-2 and AC133 by circulating human CD34+ cells identifies a population of functional endothelial precursors. Blood 95 (3), 952–958.

Qing-Wen, Z., Li-Gen, L., Wen-Cai, Y., 2018. Techniques for extraction and isolation of natural products: a comprehensive review. Chin. Med. 13, 20. https://doi.org/10.1186/s13020-018-0177-x.

Ravikumar, S., Palani Selven, G., Anitha Anandha Gracelin, N., 2010. Antimicrobial activity of medicinal plants along Kanyakumari cost, Tamil Nadu, India. Afr. J. Basic App. Sci. 2 (5–6), 153–157.

Richard, E.J., Murugan, S., Bethapudi, B., Illuri, R., Mundkinajeddu, D., Velusami, C.C., 2017. Is *Andrographis paniculata* extract and andrographoide anaphylactic? Toxicol. Reports 4, 431–437.

V, B.S., J, S.N., 2015. Antibacterial activity of *Andrographispaniculata*Nees against selective human pathogens. Afr. J. Microbiol. Res. 9 (16), 1122–1127.

Sheeja, K., Shihab, P.K., Kuttan, G., 2006. Antioxidant and anti-inflammatory activities of the plant *Andrographis paniculata* nees. Immunopharmacol. Immunotoxicol. 28 (1), 129–140.

Sukanya, S.L., Sudisha, J., Hariprasad, P., Niranjana, S.R., Prakash, H.S., Fathima, S.K., 2009. Antimicrobial activity of leaf extracts of Indian medicinal plants against clinical and phytopathogenic bacteria. Afr. J. Biotech. 8 (23), 6677–6682.

Sule, A., Ahmed, Q.U., Samah, O.A., Omar, M.N., 2010. Screening for antibacterial activity of *Andrographis paniculata* used in Malaysian folkloric medicine: a possible alternative for the treatment of skin infections. Ethnobotanical Leaflets 14, 445–456.

Valgas, C., Souza, S.M.d., Smânia, E.F.A., Smânia Jr., A., 2007. Screening methods to determine antibacterial activity of natural products. Braz. J. Microbiol. 38 (2), 369–380.