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RESEARCH ARTICLE

Qualitative Analysis of the Antimicrobial, Phytochemical and GC-MS profile of the Stem ethanolic extract from *Anodendron borneense* (King & Gamble)

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ABSTRACT

Medicinal plants have long been recognized as a source of bioactive agents, and they continue to be a valuable resource for developing new drug prospects. In Mindanao, many indigenous plants are still used in treating multiple health issues. One of them is *Anodendron borneense* (King & Gamble) D.J. Middleton, locally known as "Lunas tag-uli" or "himag". Even with numerous ethnomedicinal uses of this species, its pharmacological properties still need evaluation. This study's primary objective was to understand some of the basis of the ethnomedicinal claims by assessing the extract's antioxidant, antibacterial, and antifungal properties, including a qualitative identification of bioactive compounds. The results showed growth inhibition against the positive bacteria *Staphylococcus aureus* and two fungal organisms - *Candida albicans* and *Aspergillus niger*. Phytochemical screening revealed abundant flavonoids and saponins and a moderate level of steroids but showed weak antioxidant activity. Twenty-five (25) bioactive compounds were reported in many studies to have varied pharmacological activities and importance. The research findings may highlight the significance of the ethnomedicinal value of *A. borneense* and as a potential source of compounds with medicinal importance.

KEYWORDS:
Antimicrobial activity, Antioxidant activity, GC-MS analysis, Lunas tag-uli, Indigenous plant,

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INTRODUCTION

Medicinal plants have long been recognized as a source of bioactive agents, and they continue to be a valuable resource for developing new drug prospects (1). Since time immemorial, these naturally occurring compounds have aided people in treating various diseases and illnesses (2). Today, many commonly available medicines are extracted from plants and their derivatives—galantamine from *Galanthus nivalis* (3) and sesquiterpene lactone endoperoxide from Artemisinin (4). Plant-derived products' predominantly unexplored structural diversity (5,6) has become its most compelling attribute, imposing enormous medical research and application potential. Furthermore, In the last decades, there has been a decline in the number of new drugs entering the market (7,8), and prices of drugs have been steadily increasing (9–11). These resulted in

renewed and increased interest in plants as a source of natural products for complementary and alternative treatments (12).

In Mindanao, a wide array of indigenous plants are still used in treating various diseases, which are commonly administered by tribe leaders, healers, or even by the elderly of the community (13). *Anodendron borneense* (King & Gamble) D.J. Middleton, locally known as "Lunas tag-uli" by the Manobo tribe of Agusan del Sur or "Himag" by the Bisaya communities of Mindanao, is one example of plants still utilized by the indigenous people and some locals of Mindanao (14). This plant treats various diseases, including cancer, diarrhea, stomach trouble, ulcer, toothache, arthritis, rheumatism, pregnancy, body ache, weakness, fatigue, cramp, spasm, relapse, and poisoning (15). The stem of the plant is used by making tinctures or decoctions and taken orally for colon and prostate cancer, cyst, tumor, diabetes, hypertension, pulmonary tuberculosis, diarrhea, stomach trouble, ulcer, toothache, swollen gums, arthritis, rheumatism, impotence, sterility, postpartum care and recovery, body ache, weakness, fatigue, cramp, spasm, relapse, gas pain, flatulence, sprain, and poisoning. Many local peoples also use the stem soaked with coconut or a local efficascent oil in the treatment for scabies, warts, impetigo, typhoid fever, boils, skin eruptions, skin rashes, and itchiness; arthritis, rheumatism, swellings, muscle pain, backache, body ache, weakness and fatigue, cramp and spasm, relapse gas pain and flatulence, allergy, burns, cuts and wounds, sprain, animal and insect bites, including contacts with plants and animal parts (15).

Studies conducted on two species of the same genus, i.e., *Anodendron parviflorum* and *Anodendron nervosum* showed triterpene ester and prenylbenzoic acid derivatives. These two compounds were known to have varied pharmacological functions and importance (16,17). Since A, borneense was argued to have many medicinal uses, there is a need for the plant to be assessed for its pharmacological properties. We, therefore, performed in vitro antioxidant, antibacterial, and antifungal properties evaluation on the extracts from the plant, including qualitative evaluation of bioactive compounds using phytochemical and Gas Chromatography-Mass Spectrometry analysis.

MATERIALS AND METHODS

Identification and Collection of plant material

A. borneense (King & Gamble) D.J. Middleton was identified by the local people of Agusan del Sur. The plants were then identified, authenticated, and certified by a botany professor. The stem bark was collected and stored in an airtight container and stored at room temperature until further use.

Preparation of samples

Five hundred (500) grams of the air-dried stem bark was powdered and soaked in two (2) liters of ethanol for a week, filtered using a Whatman no. 1 filter paper. The filtrate was further concentrated using a rotary-evaporating machine to a temperature of about 45 °C before further analysis.

Antioxidant Activity

The antioxidant activity was assayed using synthetic-free radical compound 1, 2-diphenyl-2-picrylhydrazyl (DPPH). Briefly, 0.1 mM solution of DPPH in ethanol was prepared and added to 3 ml of the ethanolic extract at different concentrations (10, 20, 30, 50, 100, 200, and 300 ppm). The mixture was shaken vigorously and allowed to stand at room temperature for 30 minutes, absorbance measured at 517 nm using a spectrophotometer (UV-VIS Shimadzu), and Ascorbic acid as the reference standard compound to calculate the IC50.

Phytochemical Screening

Qualitative evaluation of alkaloids, saponins, flavonoids, tannins, cyanogenic glycosides, anthraquinones, and steroids was recorded according to a 3-point scale (no signs, + weak, ++ moderate and +++ strong) scoring (Table 1) based on the Handbook on Philippine Medicinal Plants (18).

Antimicrobial Assay

Agar disk diffusion assay was used to determine the antibacterial and antifungal activities of *A. borneense* stem ethanolic extract following the methodology described (19). The following bacteria were used for the assay: gram-negative; *Salmonella typhimurium* UPCC 1368 and *Klebsiella pneumoniae* UPCC 1360; gram-positive bacteria; *Staphylococcus aureus* UPCC 1143 and *Bacillus subtilis* UPCC 1295. Two species of fungi were used for the assay: *Candida albicans* UPCC 2168 and *Aspergillus niger* UPCC 4219.

Gas chromatography-mass spectrometry

The *A. borneense* stem ethanolic extract was subjected to gas chromatography-mass spectrometry analysis to identify the substances present. The extract was analyzed using SHIMADZU GCMS-QP2010 Ultra. The compounds were detected by comparing the analyte's mass spectrum at a specific retention time to a reference standard from the National Institute of Standards and Technology's (NIST) library. The GC-MS experiment took 45 min. in total to complete. A similarity index of 80% or higher was deemed significant. The experiment was conducted at the Analytical Services Laboratory of the Chemistry Department, Ateneo de Davao University, Davao City, Philippines.

RESULTS AND DISCUSSION

Phytochemicals Screening

The phytochemical analysis of *A. borneense* stem ethanolic extract showed flavonoids, saponins, and steroids (Table 2). Flavonoids, one of the secondary metabolites known to be beneficial to human health (20), were found to be highly abundant. This group of compounds is reported to have protective associations with various diseases and help prevent premenopausal breast cancer, stomach, and lung cancer (21). Its abundant presence in the extract justifies the ethnomedicinal use of *A. borneense* to treat cancers and tumors. Additionally, flavonoids were also reported to be a potential source of antioxidants (22,23) and anti-inflammatory agents (24,25) and have also shown antibacterial and antifungal properties (26,27), which may further contribute to its effectiveness for its accorded ethnomedicinal use. Saponins and steroids were also found in the extract and were also known to have biological activities incredibly beneficial to human health (28–30). Although the ethanolic extract contains a high level of flavonoids, other potent phytochemicals were not detected in the sample. These include alkaloids and saponins, which were also valued for their biological activities (29–33). Plants rich in anthraquinones and cyanogenic glycosides (e.g., *Chromolaena odorata* (L.) R.M.King & H.Rob. and *Senna alata* (L.) Roxb) were described as having wound healing properties (34) and antitumor effect (35). The absence of alkaloids, anthraquinones, cyanogenic glycosides, and tannins may explain the decreased antioxidant capacity of the extract.

Antioxidant Content

The assay results show the IC50 values for the stem ethanolic extract and ascorbic acid were 187.2 ppm and 1.74 ppm, respectively, using DPPH as a scavenging assay method. The potency of a plant's antioxidant is classified according to the spectrum of its IC50 value (31). The IC50 value of less than 50 ppm is considered a powerful antioxidant, while more than 150 ppm IC50 value is deemed weak (31). Thus, the stem ethanolic extract of *A. borneense* with IC50 of 187.2 is regarded weak than ascorbic acid, which is one hundred and eight percent (108%) more potent. The weak antioxidant potency *of A. borneense* can be associated with the absence of most secondary metabolites (Table 2).

Antimicrobial Assay

The agar disk diffusion assay reveals that the plant has selected antibacterial and antifungal properties (Table 3). The extract showed growth inhibition to gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*, although the growth inhibition is nine (9) times lower than that of the standard antibiotic, chloramphenicol. No inhibitory activities were observed against gram-negative bacteria, *Staphylococcus aureus*, and *Klebsiella pneumoniae* (Figure 1, Table 3). The extract showed more significant inhibitory effects against fungi, *Candida albicans*, and *Aspergillus niger* with an antimicrobial index of 0.4 mm and 0.2 mm. These results may explain the plant's folk medicinal use, including treatment for various skin diseases, including scabies, warts, boils, skin eruptions, skin rashes, itchiness, allergy, and insect bites (14).

Gas chromatography-mass spectrometry (GC-MS) analysis

Qualitative assessment of the GC-MS chromatogram show twenty-five (25) peaks. The Similarity Index (SI) of the identified compounds ranges from 87-97, which is highly based on the reference standard found in the National Institute of Standards and Technology (NIST) library (Table 4). This twenty-five (25) identified compounds detected in *A. borneense* stem ethanolic extract (include seven (7) to have antifungal activity - Dodecane, 2- methyl-6-propyl-, Undecane, 5- methyl-, Hexadecane, Tridecane, 5-methyl-, Hexadecane, 2,6,10,14-tetramethyl, Hexadecanoic acid, ethyl ester, and Heptadecanoic acid, ethyl ester based on selected studies. The presence of these compounds may explain the inhibitory activities of *A. borneense* stem ethanolic extract against the two fungi species C. albicans and A. niger. The thirteen other compounds detected in the extract namely Dodecane, 2- methyl-6-propyl-, Hexadecane, Tridecane (CAS) n-Tridecane, Tridecane, 5-methyl-, Tetradecane, 5-methyl-, Hexadecane, 2,6,10,14-tetramethyl-, 1-Tetradecanol, Cyclopentane, 1-hexyl-3-methyl- (CAS), Dodecane, 2-cyclohexyl-, Hexadecanoic acid, ethyl ester, Heneicosane, Ethyl 9-hexadecenoate, and Heptadecanoic acid, ethyl ester were reported to have antimicrobial properties thus may explain the inhibitory effects observed to the gram-positive bacteria *S. aureus* as well as the two fungal fungi *C. albicans* and *A. niger*.(Figure 1, Table 4). One of the reported folkloric use of A. borneense is wound-healing (15). Although the extent of the plant's wound-healing potential has yet to be determined, indigenous peoples likely utilize *A. borneense* for infection control, which, if prevented, speeds up natural healing processes (36). Ethnomedicinally, *A. borneense* can treat many health conditions, including muscle pain, skin issues, and gastrointestinal problems (15). These ethnomedicinal claims may be explained by the detection of Propanedioic acid, diethyl ester, n-nonadecane, Hexadecanoic acid, ethyl ester, Heneicosane, Ethyl 9-hexadecenoate, and Octadecanoic acid; ethyl ester reported to be anti-inflammatory agents (37–41), and, Linoleic acid ethyl ester an antiarthritic compound (42). The detection of Ethyl Oleate said effective in respiratory problems treatment (43), might be responsible for the plant's pulmonary tuberculosis folk use. Linoleic acid ethyl ester is also reported to have an antieczemic and antiacne activity (42), thus crediting the plants' folk medicinal use for a broad set of skin problems. Indigenous peoples in the province also use the plant to lower high blood pressure or hypertension. The compound Ethyl 9-

hexadecenoate in the plant extract might be responsible since it was reported to be anemiagenic (40).

Compounds with known antitumor and cancer preventive activities were also identified in the extract. These were 1-Tridecene, Dodecane, 2-cyclohexyl-, Hexadecanoic acid, ethyl ester, and Ethyl 9- hexadecenoate. The presence of these compounds strengthens the plant's folk use as a treatment for a wide variety of cancers. Interestingly, Hexadecanoic acid, ethyl ester, Ethyl 9-hexadecenoate, and Linoleic acid ethyl ester found in the extract also reported hypocholesterolemic activities (40,42,44). These make *A. borneense* a potential alternative source of treatment for hypercholesterolemia and atherosclerosis (45,46). While the plant's hypocholesterolemic activities might have been perceived as antidiabetic based on its folkloric use, further research is needed to better understand these compounds' efficacy in treating the diseases described.

CONCLUSION

Anodendron borneense is an indigenous plant used by many community people in Mindanao, the Philippines, to treat many ailments; thus is considered a potential source of bioactive agents and a valuable resource for developing new drug prospects. Its potential was qualitatively assessed, especially the extract's antioxidant, antibacterial, and antifungal properties, including a qualitative identification of bioactive compounds and their biological properties. The results showed the antimicrobial potential of the extract. Both phytochemical and GC-MS screenings revealed abundant antioxidants and bioactive compounds reported in many studies to have varied pharmacological activities and importance. Therefore, the research findings may highlight the significance of the ethnomedicinal value of *A. borneense* as a potential source of compounds with medicinal importance.

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AUTHOR CONTRIBUTIONS

All authors contribute to the conception of the research design and implementation, analysis of data and interpretation of results, and publication writing.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

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Table 1: Detection of Secondary Metabolites.

Secondary metabolites	Methodology and confirmation of compounds
Alkaloids	Five (5) to ten (10) mL of 2M HCl and 0.5g of sodium chloride crystal were added to the extract, subjected to boiling bath for 5 mins, allowed to cool down, and filtered. 2M HCl was used to remove the debris from the filter paper. After washing the combined filtrate, 3-5 drops of Wagner's reagent were added. The development of brown precipitate with Wagner's reagent confirmed a positive finding..
	5-10 mL of 2M HCl and 0.5g of sodium chloride crystal were added, subjected to boiling bath for 5 mins, cooled and filtered and the residue washed with 2M HCl. The combined filtrate was washed, and Mayer's reagent was added where the formation of white precipitate indicates a positive result.
Saponins	Two (2) ml of extract were added from a test tube with an equal volume of water and shaken for about 30 seconds. The formation of a stable, persistent froth with 2 cm in height for 30 minutes was regarded as positive.
Flavonoids	The extract was defatted with hexane until it becomes clear. It was then dissolved with 10 ml of 80% alcohol and then filtered. The filtrate was divided into two equal parts with 0.5 ml of 12M HCl added to the 1st while the 2nd part served as the control and then subjected again to a hot water bath and observed for two (2) hours. Positive results were determined by the development of the red color in the solution.
Steroids	The extract was defatted with hexane. The extract was added with 3-5ml of ferric chloride reagent, filtered, divided into two equal parts. The first was added with one (1) ml of concentrated sulfuric acid. The brown (sometimes blue or green) formation at the aqueous extract's boundary region served as a positive result.
Tannins	20ml of boiling water was added to the extract, followed by the addition of 2-3 drops of 10% NaCl solution. The resulting solution was filtered, and the residue was washed with water. The combined filtrate was recovered, washed, and divided into three (3) equal parts. The first filtrate was added with 3-5 drops of 1% ferric chloride, 3-5 drops of Gelatin-Salt reagent in the second part, and the third part as the control. A positive result was indicated by the (1) formation of black or blue-black colored precipitate with ferric chloride and (2) white precipitate with Gelatin-sodium chloride test.
Cyanogenic Glycosides	One (1) ml of the plant extract was transferred into a 20 ml test tube and added with 4-5 drops of chloroform. A picrate paper was suspended above the solution while the test tube was placed in the hot water bath covered with a dropper in an inverted position. The immediate formation of red color on the surface of the picrate paper indicated a positive result.
Anthraquinones	The extract was defatted with hexane, and then 10ml of distilled water was added. The resulting solution was stirred continuously then filtered twice with 5ml of benzene. The extract was left to stand for a few minutes to complete the aqueous and benzene layer. The benzene layer was separated using a transfer pipet and was placed in a test tube containing one (1) ml of ammonia reagent. It was then shaken for few seconds. The reddish pink color in the aqueous layer of the solution indicated the presence of anthraquinones.

Table 2. Qualitative Phytochemical Analysis of *A. borneense* stem ethanolic extract.

alkaloids	Anthraquinones	Cyanogenic glycosides	Flavanoids	Saponins	Steroids	Tannins
-	-	-	+++*	+++	++	-

Remarks: - no signs, + weak, ++ moderate and +++ strong, * abundant

Table 3. Antibacterial and Antifungal Activity of *A. borneense* stem bark ethanolic extract showing inhibition zone (in mm) and antimicrobial index (AI).

Test Organism	Sample	Inhibition zone (ave. in mm.)	Antimicrobial Index (AI)
	Gram-Negative bacteria		
S. typhimurium UPCC 1368	*A. borneense* stem ethanolic extract	No inhibition	0
	Chloramphenicol disc	30	4
K. pneumoniae UPCC 1360	*A. borneense* stem ethanolic extract	No inhibition	0
	Chloramphenicol disc	38	5.3
	Gram-Positive bacteria		
S. aureus UPCC 114	*A. borneense* stem ethanolic extract	15	0.5
	Chloramphenicol disc	33	4.5
B. subtilis UPCC 1295	*A. borneense* stem ethanolic extract	Partial inhibition of growth of the test organism as shown by thinning of growth within the diameter	0

	Chloramphenicol disc	20	2.3
	Fungi		
C. albicans UPCC 2168	*A. borneense* stem ethanolic extract	14	0.4
	Canesten solutiond, 100 µL	32	2.2
A. niger UPCC 4219	*A. borneense* stem ethanolic extract	13	0.3
	Canesten solutiond, 100 µL	42	3.2

Table 4: Bioactive Compounds Identified in *A. borneense* stem ethanolic extract.

No.	Compounds	Molecular Formula	Mol. Weight	SI	Studies Undertaken
1	Propanoic acid (CAS)	$C_3H_6O_2$	74	88	Preservative [47].
2	1-Hexanol, 2-ethyl-	$C_8H_{18}O$	130	95	Reduce tremor amplitude [48].
3	Propanedioic acid, diethyl ester	$C_7H_{12}O_4$	160	92	Anti-inflammatory [37].
4	Undecane, 5- methyl-	$C_{12}H_{26}$	170	93	Antifungal activity enhancer [49].
5	Dodecane, 2- methyl-6-propyl-	$C_{16}H_{34}$	226	94	Antioxidant, antibacterial [50], antifungal, antimicrobial [51].
6	(+)-Isomethol	$C_{10}H_{20}0$	156	96	Anti-salmonella agents and antioxidant [52]
7	Hexadecane	$C_{16}H_{34}$	226	96	Antibacterial, antifungal, and antioxidant [51,53–56].
8	Tridecane (CAS) n-Tridecane	$C_{13}H_{28}$	184	97	Antibacterial, antioxidant, and antiproliferative against HeLa cells [57–59]
9	Tridecane, 5-methyl-	$C_{14}H_{30}$	198	96	Antifungal and antibacterial [49]
10	n-Nonadecane	$C_{19}H_{40}$	268	94	Anti-inflammatory [60]
11	1-Tridecene	$C_{13}H_{26}$	182	95	Antitumor [61]
12	Tetradecane, 5-methyl-	$C_{15}H_{32}$	212	93	Antifungal and antibacterial [55]
13	Hexadecane, 2,6,10,14-tetramethyl-	$C_{20}H_{42}$	282	94	Antifungal, antibacterial, antitumor, and cytotoxic effects [49].
14	1-Tetradecanol	$C_{14}H_{30}O$	214	92	Anti-microbial [62,63].
15	Cyclopentane, 1-hexyl-3-methyl- (CAS)	$C_{12}H_{24}$	168	89	Antibacterial [61], antimicrobial, antitumor [64].
16	Methanone, diphenyl- (CAS) Benzophenone	$C_{13}H_{10}O$	182	97	No report
17	Dodecane, 2-cyclohexyl-	$C_{18}H_{36}$	252	88	Anticandidal [64], anticancer, antioxidant, and antimicrobial activity [65]
18	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	95	Antibacterial, antioxidant, antifungal, antitumor, anticancer, anti-inflammatory, hypocholesterolemic [38,54,66–70].
19	Heneicosane	$C_{21}H_{44}$	296	96	Antibacterial, anti-inflammatory, antimicrobial, antioxidant [38,39,44,71]
20	Ethyl 9-hexadecenoate	$C_{18}H_{34}O_2$	282	87	Antibiotic, emulsifying agent [61], anti-inflammatory, antiandrogenic, cancer preventive, dermatitigenic, hypocholesterolemic, 5-alpha reductase inhibitor, anemiagenic, insectifuge [40]
21	n-Nonadecanol-1	$C_{19}H_{40}O$	284	96	Nematicide, pesticide [72]
22	Heptadecanoic acid, ethyl ester	$C_{19}H_{38}O_2$	298	93	Antifungal, antimicrobial, and antibacterial [49,73]
23	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	94	Hepatoprotective [74], anti-inflammatory agent, decrease sebum production [75], antioxidant [76], hypocholesterolemic, nematicide, antiarthritic, antiandrogenic, hypocholesterolemic, 5-alpha reductase inhibitor antihistaminic, anticoronary, insectifuge, antieczemic, antiacne [42]
24	Ethyl Oleate	$C_{20}H_{38}O_2$	310	88	Muscle weakness, pulmonary edema, anemia, respiratory failure, diarrhea, sleep disturbance, tetany [43]
25	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	92	Antioxidant, anti-inflammatory [41]
SI - Similarity Index					

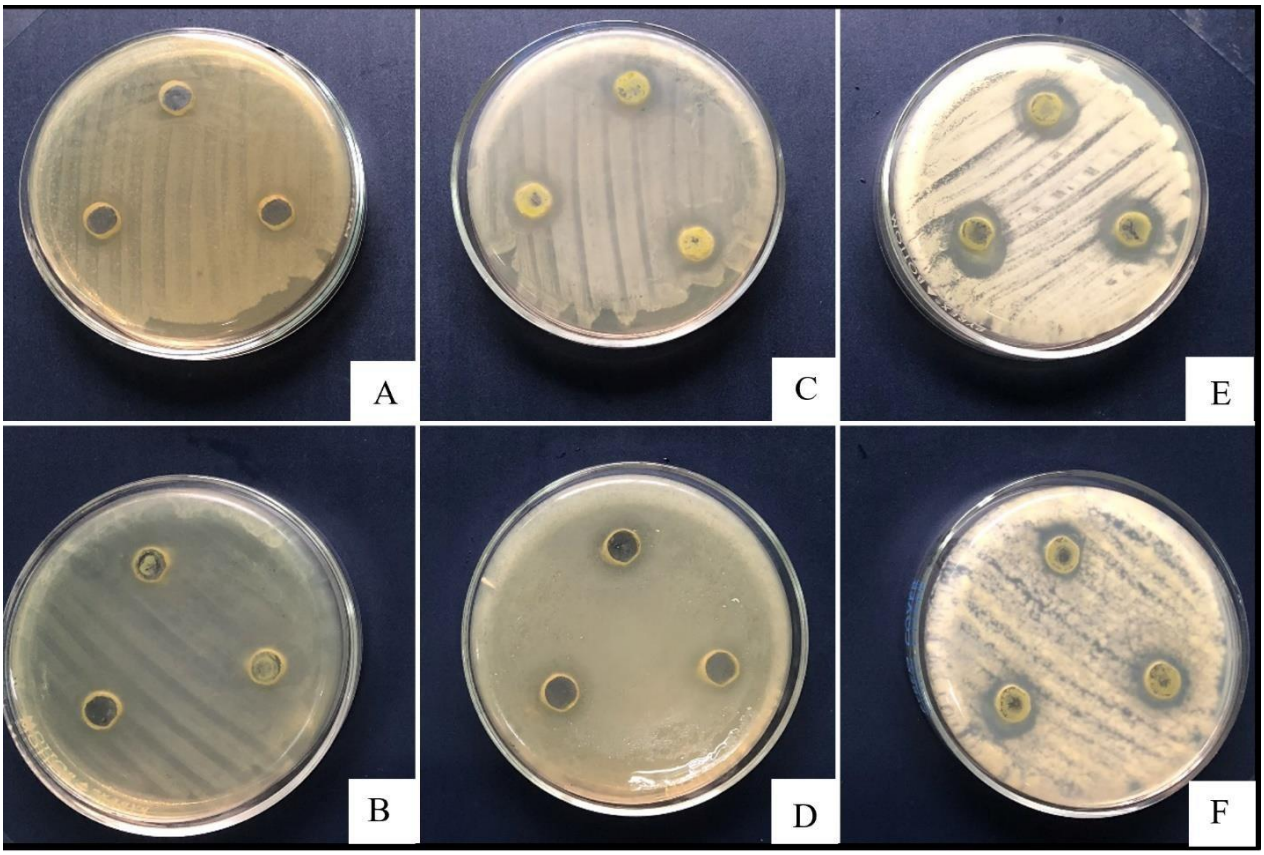

Fig.1: Inhibition zone of (A) *S. typhimurium* with AI= 0, (B) *K. pneumoniae* with AI= 0, (C) *S. aureus* with AI= 0.5 mm and (D) *B. subtilis* with AI= 0, (E) *C. albicans* with AI= 0.4 mm, and (F) *A. niger* with AI= 0.3 mm.

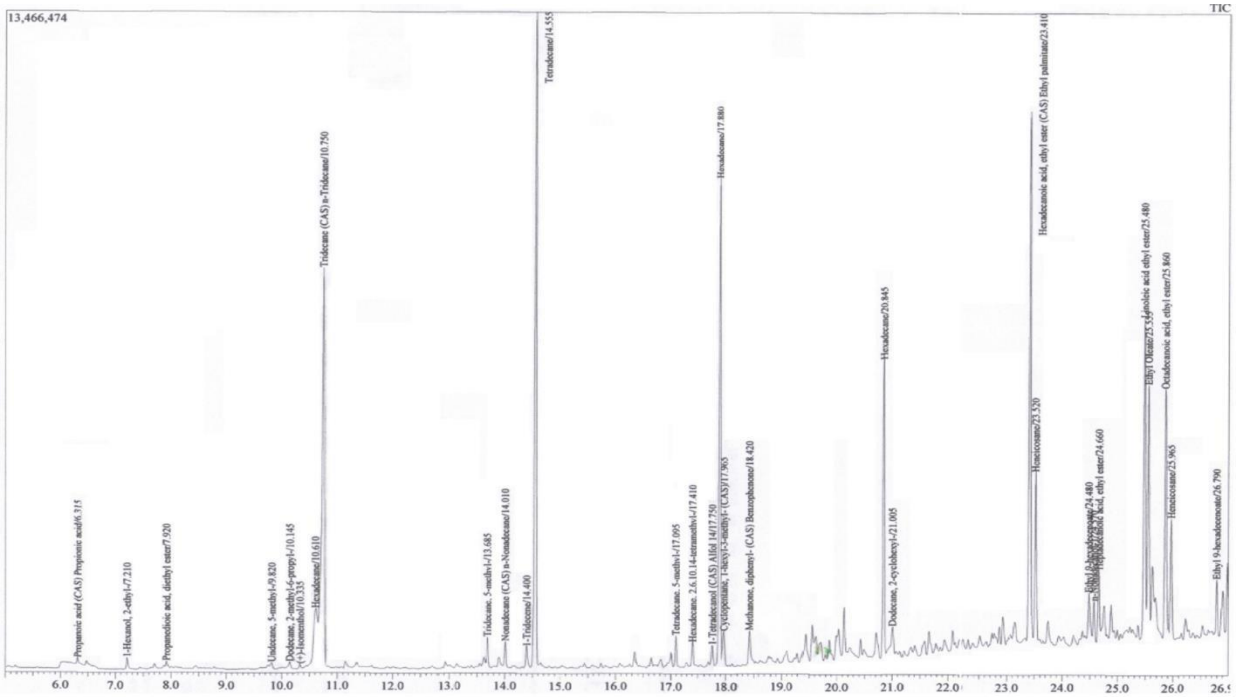

Fig.2: Gas chromatography-mass spectrometry chromatogram of *A. borneense* stem ethanolic extract.