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Article

Identification and biological activity of some new compounds isolated from aerial parts of *Polygonum hydropiper*

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Abstract: New natural compounds, Polygonolic acid (1), Polygonumate (2) and Hydropiperoic acid (3) along with some known compounds were isolated from aerial part of medicinal plant *Polygonum hydropiper*. The compounds were isolated upon repeat column chromatography, HPTLC, RP-18 reverse phase column of dichloromethane fraction of the crude methyl extract. Their structures were identified by using spectroscopic technique. Structure of Hydropiperoic acid (3) was identified by single crystal X-ray diffraction study. Microbiological activities of these compounds against some of phytopathogenic fungi and bacteria have been investigated in this study.

Keywords: *Polygonum hydropiper*; polygonolic acid; polygonumate; hydropiperoic acid; antimicrobial activities

1. Introduction

Polygonum hydropiper Linn. (syn. *Persicaria hydropiper*) is an annual herb growing abundantly in wet places in tropical region. In Bangladesh, the plant found during the period in October-November and exists for about 4 to 5 months. It is known for centuries for its medicinal and insecticidal values (Hassan *et al.,* 2009; Rahman *et al.,* 2002; Haraguchi *et al.,* 1992). Polygonum hydropiper is used as anti-cancer and anti-rheumatic agent in folk medicine and used as potentials sources of therapeutic agents against cancer (Ayaz *et al.*, 2016a), plant extracts exhibited broad spectrum of activity against bacterial and fungal strains (Ayaz *et al.*, 2016b) also exhibit antiacetyl choline sterase and immune stimulation activities (Miyazaki Y, 2016). The herbs possess bitter, stimulant, tonic, diuretic, carminative, anthelmintic, emmenagogue, haemostatic and lithotripter properties (Sharma, 2003). Liquid extract of this plant is reported to be used as an oral contraceptive. The bruised leaves and seeds are used as vesicants and are substituted for mustard poultice. Their juice is used as a wash for skin affections (Krishnamurthi, 1969; Watt, 1962). It has been used as a hot-tasting spice in Japan, China, and Europe, and also used as a folk medicine for cancer and haemostatics (Haraguchi, 1992). The stems and leaves of this plant are used to treat snake-bite and used as diuretic and anthelmintic agent in Vietnam (Loi, 2000). Juice of leaves is used in headache, pain, toothache, liver enlargement, gastric ulcer, dysentery, loss of appetite and dismenorrhoea; roots are used as stimulant; juice is applied to wounds, skin diseases and painful carbuncles (Ghani, 2003). The bruised tender leaves are used for menstrual treatment of women (Akamatsu, 1970). The plant also possesses anti-carcinogenic activity (Hartwell, 1970). In antimalarial, antimicrobial, anti-inflammatory, PPAR and cytotoxic assays, some compounds isolated from this plant have demonstrated moderate inhibitory potentials (Xiao H *et al.*, 2017).

Bangladesh is a tropical country and the monsoon water blessed her to host innumerable number of green plants and herbs of different families which are very much important both from the economic and medicinal point of

view. These compounds are used for the treatment of various diseases as well as to kill the harmful insects. A huge amount of poisonous chemicals are used every year as insecticide to save the crops and to preserve the seeds. These chemicals pollute the environment, damage the life and health of animals and men, causing great ecological imbalance. These harmful insecticides may be replaced by plant-derived active principles which will be environment–friendly and economically profitable. In Bangladesh the plant is used as an insecticide. The farmer used the dry leaves with the seeds to store food grains; the leaves juice is used to spray on wheat and/or paddy field as insecticidal agent. The dried powder of the herb is spread on cloths to guard against moths. The greenish mucilaginous juice of the plant kills mosquito larvae (Krishnamurthi A, 1969). In agriculture, it is used as a poison for the insects. Powder of the dried *Polygonum hydropiper* plants (Figure 1) are used for preservation of tobacco (Akamatsu, 1970). Laboratory studies indicated that the larvae were deterred from feeding of *Polygonum hydropiper* treated wheat flour. Cessation of food intake increased with higher concentrations of extract. Phytochemical investigation of *Polygonum hydropiper* is essential from the point of economical, medicinal and biological importance.

Earlier investigation on the medicinal plant *Polygonum hydropiper*, Linn, reported some drimane-type sesqui- and nor sesquiterpenoids (Huq *et al.,* 2014; Sultana *et al.,* 2011; Haraguchi *et al.,* 1992; Fukuyama *et al.,* 1985, 1982), flavonoid (Peng *et al.,* 2003; Smolarz, 2002; Furuta *et al.,* 1986), and recently, cerebroside (Sultana *et al.,* 2015) type compounds have been isolated. It is also found that one report has been made on the phytochemical investigation of the Bangladeshi origin of this species. It is very much essential to study more extensively on this valuable plant of Bangladeshi origin. This concentrative study may contribute a lot to the treatment of a number of diseases. So, it will be very interesting to work on isolation, characterization and bio-assay studies on main constituents of *Polygonum hydropiper*.

Figure 1. *Polygonum hydropiper* plant.

2. Materials and Methods

Melting points were determined on BUCHI digital melting point apparatus (model- 535) and were uncorrected. Optical rotations were measured on JASCO polarimeter (model P-360), with a 10 cm cell and it was measured in methanol at given temperatures and concentrations. Ultraviolet (UV) spectra were recorded in methanol on HITACHI spectrophotometer (model U-3200) and absorption values (λ_{max}) are given in nm. Infrared (IR) spectra were measured as KBr discs on SIMADZU FTIR spectrophotometer (model 8900) and presented in cm^{-1}. All the chemicals and solvents used in the reactions were of AR grade and obtained from commercial sources (Merck, Germany). TLC chromatograms were viewed under ultraviolet light at 254 nm for fluorescence quenching spots, and at 366 nm for fluorescent spots. ^{1}H-NMR, ^{13}C-NMR spectra were recorded in deuterated solvents ($CDCl_3$) on *Bruker Avance* spectrometers equipped with 600 & 300 and 150 & 125 MHz, respectively. Residual proton of the solvent were used as an internal standard to measure the chemical shifts (δ) and these were measured in ppm relative to $CDCl_3$ (δ 7.25), and coupling constants *(J)* are given in Hz. Electron impact mass spectrometry (EI MS) was scanned on *Joel D-300* mass spectrometer. High resolution electron spin ionization mass (HR ESI MS) were measured on *Bruker (ULTRA FLEX III TOF/TOF)* mass spectroscopy. Structure of crystalline compound was unambiguously determined by single crystal X-ray diffraction techniques on *Bruker SMART Apex II* diffractometer at 293K. Cu-Kα radiations of 0.7Å with a graphite monochromator were used to collect the diffraction pattern. TLC was conducted on pre-coated silica-gel F_{254} aluminum sheets (0.25 mm thickness). The

compounds were visualized by heating the cards after spraying ceric sulfate reagent. All these analysis were performed in various laboratories of the H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Science (ICCBS), University of Karachi, Karachi-75270, Pakistan.

2.1. Phytochemical investigation on *Polygonum hydropiper,* L.

The plant *Polygonum hydropiper* (syn. *Persicaria hydropiper*) (*fm*. Polygonaceae) were collected from the compound of Chittagong University of Engineering and Technology (CUET), Rawjan, Chittagong, Bangladesh. The air dried plants was pulverized and macerated in methanol (60 Liters x3) at room temperature (27°-33°C) for 3 days. The extracts were concentrated to dryness with rotary vacuum evaporator. The methanolic extract was dissolved in methanol and fractionated with n-hexane by solvent-solvent extraction to remove gummy and sticky materials. Methanol was removed to dryness under vacuum evaporation. This crude methanol extract was suspended in distilled water and fractionated by solvent-solvent extraction with dichloromethane, ethyl acetate and n-butanol, subsequently. The dichloromethane fraction of the crude methyl extract was subjected to repeat column chromatography, HPTLC, RP-18 reverse phase column. By these techniques some new compounds along with some known compounds were isolated from this sub-fraction. The structures of the isolated compounds were identified with the help of extensive 1D & 2D NMR, MS spectroscopic and single crystal X-ray diffraction techniques.

2.2. Biological activity

The test organisms are phytopathogenic. For this reason, all steps of the work were done with high precaution and aseptic condition.

2.2.1. Antibacterial activities

Antibacterial activities of these compounds against selected bacteria were assessed by the Agar Well Diffusion Method. All these cultures were kept at 4°C prior to testing. They were sub-cultured in liquid nutrient broth and incubated at 37^{o} C for 18-24 hrs and then used for the screening. Two fold dilution of sample was prepared by using nutrient agar (19 mL agar + 1 mL sample). 10 µL of 10^{6} cells/mL suspension of each culture was inoculated in each tube containing two fold dilution of sample. Nutrient agar was poured on sterile plates and plates were incubated at 37ºC for 24 hours. Minimal inhibitory concentration was defined as the lowest concentration of sample that inhibited visible growth of microorganisms (Alves *et al*., 2000; Stepanović *et al*., 2003; Carron *et al*., 1987). The activity is expressed in terms of diameter of zone of inhibition in mm.

2.2.2. Antifungal activities

In this study Agar Tube Dilution Method was used to measure antifungal activity as it can screen a large number of Plants extracts for their antifungal activity. PDA was used as a growth medium for the test. DMSO was used as a solvent initially to prepare solution of the compounds. Such solutions were then mixed with the sterilized PDA to maintain desired concentration of the compounds and the mixture (20 cm^{3}) was poured in each Petri dish (Nath *et al*, 2017). Linear growth of the fungus was measured in mm after seven days of incubation at (35 ± 2) °C.

3. Results and Discussion

Dichloromethane (DCM) soluble part was applied to column chromatography over silica gel (30 cm x 8 cm) and eluted by ethyl acetate/hexane with increasing polarity by increasing amount of ethyl acetate. Each fraction is categories as sub-fraction A, B, C & D. Sub-fraction B was repeatedly subjected to reverse phase column chromatography on RP-18 silica gel (12x6 cm) with methanol/water (7:3) and followed by Sephadex LH 20 with methanol/DCM (2:1) got four sub-fractions named B1, B2, B3 and B4. Sub-fraction B1 was again subjected on silica gel flash column with elution system ethyl acetate/hexane and got the compounds 1 & 2 at different polarity. Small amount of sub-fraction B1 was kept for three months with solvent DCM, a beautiful crystal was obtained in the vial. The crystal was separated and washed with DCM/hexane (1:1) yielded a pure new nor-sesquiterpene compound 3 as white crystalline solid. The structure of this compound was detected by single crystal X-ray diffraction technique.

3.1. Identification of new compound

First time isolated new compounds from *Polygonum hydropiper* are Polygonolic acid (1), Polygonumate (2) and Hydropiperoic acid (3).

3.1.1. Polygonolic acid (1)

Compound 1 (Figure 2) was isolated as colorless amorphous solid (20.2 mg, 1.55x10^{-4}%) with $[\alpha]_D^{25}$ -10.7 (c 1.8, MeOH), R_f 0.35 (4% MeOH/DCM), m.p. 199.8°C. The molecular formula $C_{14}H_{22}O_3$ with four degrees of unsaturation was determined by EI MS at *m/z* 238 $[M]^+$, HRTOF-ESI-MS $[M+NH_4]^+$ at *m/z* 256.1923 (calcd 256.1913 for $C_{14}H_{22}O_3$+NH_4) and ^{13}C-NMR (BB), DEPT 135° and DEPT 90° experimental data (Table 1). The IR spectrum showed absorption band for hydroxyl group (3436 cm^{-1}) with *α,β*–unsaturated carbonyl group (1703 cm^{-1}) and olifinic bond (1651 cm^{-1}) functionalities. It also showed absorption band at 1388 cm^{-1} for *gem*-dimethyl group (Peng *et al.*, 2003). The UV spectrum (MeOH) exhibit terminal absorption at 193 nm indicating absence of any chromophore. ^{13}C-NMR (BB), DEPT 90º and DEPT 135º experiments showed 14 signals for carbon atoms, includes three methyl, four methylene, three methine and four quaternary carbons. The HSQC, HMBC, ^{1}H-^{1}H COSY and NOESY spectra were employed to assign spectroscopic data of compound **1**. One of the quaternary carbon exhibit a resonance in down field at δ_C 170.4 for an acid carbonyl carbon (C-11), other resonance in down field region for one double bond [δ_H/δ_C 7.08 (dd, $J_{6,7}$=5.3, 2.6 Hz)/144.1 (CH-7) and δ_C 132.6 (C-8)] in conjugation with carbonyl carbon, an isolated oxygenated methine [δ_H/δ_C 3.73 (s)/73.6 (CH-9)] and a methine [δ_H/δ_C 1.64 (dd, J5,6-11.7, 5.3 Hz)/41.3 (CH-5)] in up-field. Four methylene shows resonance in up-field at δ_H/δ_C 1.18, 1.85 (m)/34.9 (CH_2-1), 1.52, 1.50 (m)/19.4 (CH_2-2), 1.25, 1.45 (m)/43.5 (CH_2-3) and δ_H/δ_C 2.34 (dt, J=20.1, $J_{5,6}$=5.3 Hz), 2.11 (ddd, J=20.1, 11.7, $J_{6,7}$=2.6 Hz)/26.0 (CH_2-6)]. Three tertiary methyl groups were observed in up-field region at δ_H/δ_C 0.92(s)/33.1 (CH_3-12), 0.95(s)/22.1 (CH_3-13), 0.78(s)/18.9 (CH_3-14). Key HMBC and ^{1}H-^{1}H COSY correlations are shown in Figure 3. The cross peak in the NOESY spectrum showed between CH-9 (δ_H 3.73) and biogenetically *β*-oriented methyl protons CH_3-14 (δ_H 0.78) supported *α*-orientation of hydroxyl group at C-9 position (Figure 4). 14 carbons in the molecule and NMR chemical shift data were characteristic of a drimane-type nor-sesquiterpene skeleton and the structure of compound 1 was elucidated as Polygonolic acid, IUPAC nomenclature is [(1*S*,4a*S*,8a*R*)-1-hydroxy-5, 5,8a-trymethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalene-2-carboxylic acid].

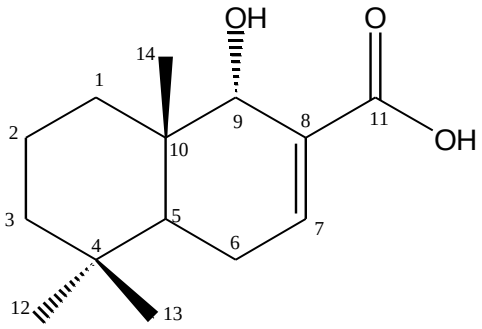

Figure 2. Polygonolic acid (1).

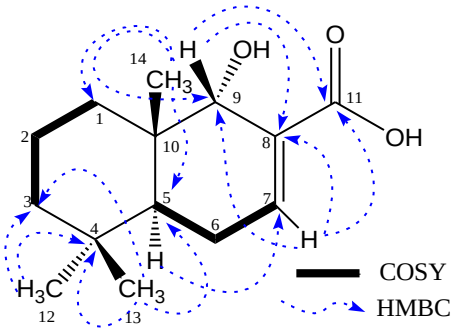

Figure 3. Key HMBC and COSY correlation of 1.

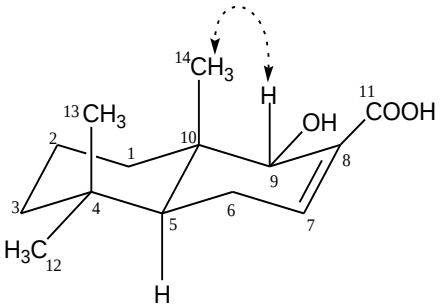

Figure 4. Key NOESY correlation of 1.

Table 1. ^{1}H and ^{13}C-NMR chemical shift and multiplicity for compound 1.

C No.	δ_C (‡)	Mult	δ_H (m, *J* in Hz) (†)	HMBC (‡)
1	34.9	CH_2	1.18, 1.85 (m)	C- 2,3,10,14
2	19.4	CH_2	1.52, 1.70 (m)	C- 1,3,4,10
3	43.5	CH_2	1.25, 1.45 (m)	C- 4,12,13,10,2,1
4	33.5	C	-	-
5	41.3	CH	1.64 (dd,11.7, 5.3 Hz)	C- 6,7,12,13,10
6	26.0	CH_2	2.34 (dt, 20.1, 5.3 Hz), 2.11(ddd, 20.1, 11.7, 2.6 Hz)	C- 7,8,5,10
7	144.1	CH	7.08 (dd, 5.3, 2.6 Hz)	C- 8,9,11,5
8	132.6	C	-	-
9	73.6	CH	3.73 (s)	C- 8,11,1,10,5
10	38.4	C	-	-
11	170.4	C	-	-
12	33.1	CH_3	0.92 (s)	C- 4,3,5
13	22.1	CH_3	0.95 (s)	C- 4,3,5
14	18.9	CH_3	0.78 (s)	C- 9,5,10,1

‡ 125 MHz and (†) 300 MHz, solvent: $CDCl_3$

3.1.2. Polygonumate (2)

Compound 2 (Figure 5) was isolated as colorless amorphous solid (5.2 mg, 4.0x10^{-5}%) with $[\alpha]_D^{25}$ +17° (c 0.11, MeOH), R_f 0.51 (4% MeOH/DCM). Sixteen resonance found in ^{13}C-NMR broad band spectra and DEPT 135° and DEPT 90° experimental data shows the molecular formula $C_{16}H_{22}O_4$ with six degrees of unsaturation was confirmed by EI MS at *m/z* 278 $[M]^+$, HRTOF-ESI-MS with pseudo-molecular ion peak $[M+NH_4]^+$ at *m/z* 296.1869 (calcd 296.1862 for $C_{16}H_{22}O_4$+NH_4). The UV spectroscopy showed absorption at 194 and 212 nm. The IR spectrum showed absorption for *α,β*-unsaturated carbonyl group (1701 cm^{-1}), olifinic bond (1655 cm^{-1}) and *gem*-dimethyl group (1385 cm^{-1}) (Peng *et al*., 2003). To assign spectroscopic data of compound 2, the spectra of HSQC, HMBC, ^{1}H-^{1}H COSY and NOESY correlations were employed. ^{13}C-NMR showed resonances for sixteen carbons and DEPT 90° and DEPT 135° resolved three methyl, six methylene, one methine and six quaternary carbons (Table 2). Two quaternary carbon showed resonance in down field for two ester carbonyl carbons [δ_C 177.4 (C-14) and 172.2 (C-11)], one double bond [δ_C 159.5 (C-8) and 134.5 (C-9)] in conjugation with carbonyl carbon and other two quaternary carbon were assign at δ_C 43.5 (C-4) and 35.2 (C-10). The only methine carbon exhibit a resonance in up-field region at δ_H/δ_C 1.38 (dd, $J_{5,6}$=18.6, 1.8 Hz)/53.3 (CH-5). One methylene attach with an olifinic carbon and an oxygen exhibit resonance in down field at δ_H/δ_C 4.53 (d, $J_{12a,12b}$=16.8 Hz), 4.61 (d, $J_{12a,12b}$=16.8 Hz)/70.6 (CH_2-12), Five other methylene showed resonances in up-field at δ_H/δ_C 2.24 (overlap) 1.08 (d, J=4.2 Hz)/37.9 (CH_2-3); 2.56 (d, J=13.2 Hz), 1.12 (d, J=4.2 Hz)/34.7 (CH_2-1); 2.35 (d, J=5.4 Hz), 2.25 (brs)/25.7 (CH_2-7); 2.18 (d, J=6.0 Hz), 1.80 (d, J=1.8 Hz)/20.0 (CH_2-6) and 1.55, 1.49 (overlap)/18.8 (CH_2-2). Herein, some multiplicities are not clear. Two tertiary methyl groups were observed in up-field region at δ_H/δ_C 1.22(s)/28.6 (CH_3-13) and 0.95(s)/17.4 (CH_3-15). Rest tertiary methyl group give resonance as methoxy gtoup at δ_H/δ_C 3.64(s)/51.4 (CH_3-16). Key HMBC and ^{1}H-^{1}H COSY correlations are shown in Figure 6. The NEOSY spectrum showed cross peak between the protons of δ_H 3.64 (-OCH_3-16) and biogenetically *β*–oriented methyl protons at δ_H 0.95 (CH_3-15), which revealed the *β*-orientation of the carbonyl ester unit (Figure 7).

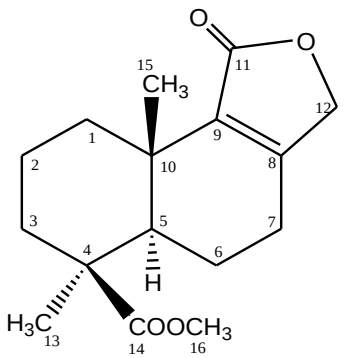

Figure 5. Polygonumate (2).

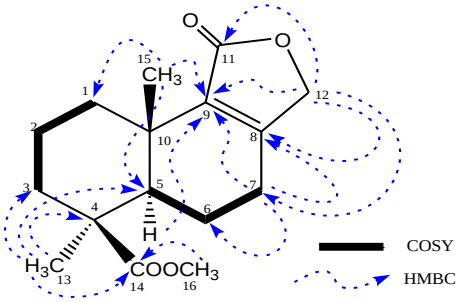

Figure 6. Key COSY and HMBC correlation of 2.

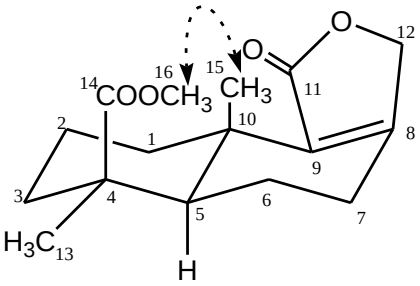

Figure 7. Key NOESY correlation of 2.

The structure of the compound 2 was characterized as Polygonumate and it was found as a new compound. IUPAC name of this compound as {(5aR,6S,9aS)-6, 9a-dimethyl-1-oxo-1,3,4,5,5a,6,7,8,9, 9a-decahydronaphtho [2,1-c]furan-6-carboxylic acid}, and it was also observed that one of the tertiary methyl (CH_3-14) in compound 2 get oxidized to carboxylic acid which then converted to methyl ester [δ_C 177.2 (CO), δ_H/δ_C 3.64/51.4 (-OCH_3)] seems to be an artifact of its de-esterified analogue Polygonumic acid (Figure 8).

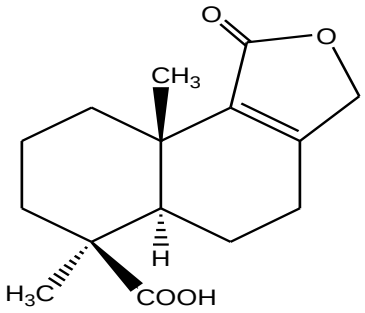

Figure 8. Polygonumic acid.

Table 2. ^{1}H and ^{13}C-NMR chemical shift and multiplicity of compound 2.

C No	δ_C (‡)	Mult.	δ_H (m, *J* Hz) (†)	HMBC (‡)
1	34.7	CH_2	2.56 (d, 13.2 Hz), 1.12 (d, 4.2 Hz)	C- 2,10,9,5
2	18.8	CH_2	1.55, 1.49 (overlap)	C- 1,3,4,10
3	37.9	CH_2	2.24 (overlap) 1.08 (d, 4.2 Hz)	C- 4,13,14,5,2
4	43.5	C	-	-
5	53.3	CH	1.38 (dd, 18.6, 1.8 Hz)	C- 9,7,6,4,14
6	20.03	CH_2	2.18(d, 6 Hz), 1.80 (d, 1.8 Hz)	C- 5,4,7,8
7	25.7	CH_2	2.35 (d, 5.4 Hz), 2.25 (brs)	C- 6,8,9,5
8	159.5	C	-	-
9	134.5	C	-	-
10	35.2	C	-	-
11	172.4	C	-	-
12	70.6	CH_2	4.53 (d,16.8 Hz), 4.61 (d,16.8 Hz)	C- 8,7,9,11
13	28.6	CH_3	1.22 (s)	C- 4,3,5,14
14	177.2	C	-	-
15	17.4	CH_3	0.95 (s)	C- 1,9,5
-OCH_3	51.4	CH_3	3.64 (s)	C- 14

(‡) 150 MHz and (†) 600 MHz, solvent: $CDCl_3$, some multiplicities are not clear.

3.1.3. Hydropiperoic acid (3)

Compound 3 was isolated as a colorless crystal. The structure of this compound was unambiguously determined by single crystal X-ray diffraction study. A colorless crystal of 0.48 x 0.21 x 0.18 mm was mounted on *Bruker SMART Apex II* diffractometer at 298 K. Cu Kα radiations of 0.7 Å (λ=0.71073Å) with a graphite monochromator were used to collect the diffraction pattern. Compound **3** was crystallized in monoclinic system with P21/c space group, having one molecule in asymmetric unit. The cell dimensions were a=7.6291(13) Å,

b=17.983(3) Å, c=9.1880(15) Å, α=90°, β=107.385(4)°, γ=90° and the volume was found to be 1203.0(4) $Å^3$. The final cell parameters were determined by full-matrix least square on F^2 refinement method of 2233 reflections out of 6947, with *R* = 0.0720 (for all data *R* = 0.0850). The data reduction was done by SAINT program. SHELXTL (Sheldrick, 1997) program was used to refine the data, whereas SHELXTL software was used to prepare the graphical work, finally SHELXTL and PLATON program were used to process the publication material. H atoms were positioned at their places geometrically and constrained to ride on their parent atoms with U~iso~ related to the atoms ridden on. Bond distances and angles (Table 3) were found to be in the normal range. Finally the structure (Figures 9 & 10) of compound 3 was identified as Hydropiperoic acid (3).

Table 3. Crystal data and structure refinement for Hydropiproic acid (3).

Compound	3
Empirical formula	$C_{14}H_{16}O_2$
Formula weight	216.27
Temperature	298(2) K
Wavelength	0.71073 Å
Crystal system, space group	Monoclinic, P21/C
Unit cell dimensions	a=7.6291(13) Å, b=17.983(3) Å, c=9.1880(15) Å
	α = 90°, β = 107.385(4)°, γ = 90°
Volume	1203.0(4) $Å^3$
Z, Calculated density	4, 1.194 mg/m^3
Absorption coefficient	0.078 mm^{-1}
F(000)	464
Crystal size	0.48 x 0.21 x 0.18 mm
Theta range for data collection	2.27 to 25.50°.
Limiting indices	-9<=h<=9, -18<=k<=21, -11<=l<=11
Reflections collected / unique	6947 / 2233 [R(int) = 0.0248]
Completeness to theta = 25.50	99.9 %
Max. and min. transmission	0.9860 and 0.9633
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2233 / 0 / 149
Goodness-of-fit on F^2	1.034
Final R indices [I>2sigma(I)]	R1 = 0.0720, wR2 = 0.1978
R indices (all data)	R1 = 0.0850, wR2 = 0.2105
Largest diff. peak and hole	0.378 and -0.227 $e.A^{-3}$

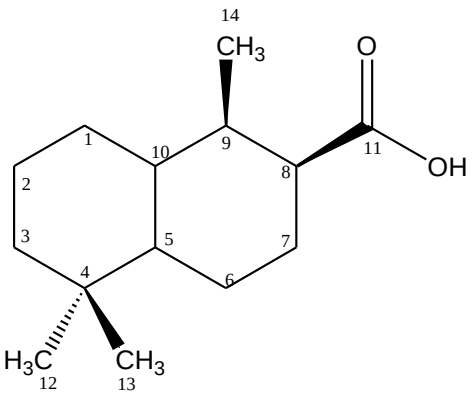

Figure 9. Hydropiproic acid (3).

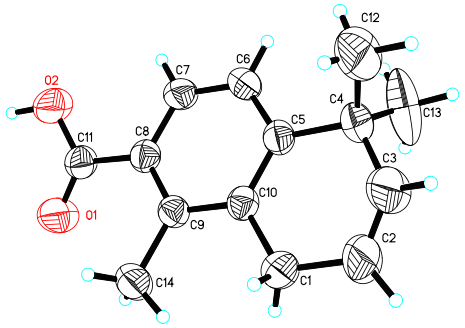

Figure 10. Single crystal X-ray structure of compound 3.

3.1. Biological activity

The development of antimicrobial resistance in many pathogenic microbes possesses one of the most serious problems in the control of infectious diseases. All the test organisms are phytopathogenic, for that all steps of the work were done with high precaution and aseptic condition. And the percentage inhibition of mycelia growth of the test fungus/bacteria was calculated by using following equation:

Percentage of inhibition = (CT/C) × 100

Here, C= Diameter of the fungal/ bacterial colony in the control
T = Diameter of the fungal/ bacterial colony in the treated

3.1.1. Antibacterial activities

To measure antibacterial activity *Bacillus subtilus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella Flexneri* and *Staphylococcus aureus* are selected as test Organisms. Antibacterial activities of Polygonolic acid, Polygonumate and Hydropiperoic acid are summarized in Table 4.

Table 4. Antibacterial activities of Polygonumate, Polygonolic acid and Hydropiperoic acid.

Compounds	Diameter of zone of inhibition in mm after 24 hours					
	Bacillus subttlis	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella typhi*	*Shigella Flexneri*	*Staphylococcus aureus*
Polygonolic acid	9	8	12	0	12	13
Polygonumate	4	0	0	-	0	3
Hydropiperoic acid	0	13	11	0	9	0

- means not done

The results showed that though Polygonolic acid, Hydropiperoic acid show different antibacterial activities to a measurable extent but the Polygonumate did not show any such activity.

3.1.2. Anti-fungal activities

Anti-fungal activities of Polygonumate, Polygonolic acid and Hydropiperoic acid are summarized in Table 4. Screenings were conducted against selective phytopathogenic fungi, *Aspergillus flavus, Candida albicans, Candida glabarata, Fusarium solani, Microsporum canis,*and *Trichophyton longifusis*. These fungi are phytopathogens of important crop plants such as jute, chilli, brinjal etc. Control of such pathogens by non-hazardous fungicides has been a major concern, especially as fungi gradually develop resistance to known fungicides. It is evident from the results presented in Table 5 that these compounds showed some anti-fungal activity.

Table 5. Anti-fungal activities of Polygonolic acid, Polygonumate and Hydropiperoic acid.

Compounds	% inhibition of mycelial growth					
	Aspergillus flavus	*Candida albicans*	*Candida glabarata*	*Fusarium solani*	*Microsporum cani*	*Trichophyton longifusis*
Polygonolic acid	30	26	40	36	-	51
Polygonumate	28	10	-	12	13	17
Hydropiperoic acid	-	47	35	38	47	-

- means not done

It was observed that different compound had different effects on these organisms. These observations suggested that these compounds played a significant role in the inhibition of micelial growth.

4. Conclusions

Polygonolic acid, Polygonumate and Hydropiperoic acid were identified first time in this study from *Polygonum hydropiper*. Inhibition power of the newly isolated compounds on a particular bacterial growth was measured in these researches. It is found that this plant is very important considering its medicinal and insecticidal activities. Some of the compounds are found to exhibit higher antibacterial activities than their analogous compounds. However for a clear understanding of the functions responsible for antibacterial activities of these compounds, more studies are needed to be performed with a series of analogous compounds against a series of bacteria.

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Conflict of interest

None to declare.

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