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Research article

Isolation of a new iso-quinoline alkaloid and cytotoxicity studies of pure compounds and crude extracts of *Ravenia spectabilis* engl

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ABSTRACT

A new 2-quinolone alkaloid, iso-oligophyline (**1**), and two very unusual C_{34} terpenoids, proposed names ravespanol (**2**) and ravespanone (**3**), along with two known compounds, β-sitosterol (**4**), and methyl linoleate (**5**), were isolated from the leaf extract of *Ravenia spectabilis engl.* Methyl linoleate constitutes the first report of isolation from this species. We have already reported the isolation of atanine (**6**), oligophyline (**7**), ravenoline (8), and arborinine (**9**) from the plant. Based on nuclear magnetic resonance (NMR) spectroscopy and mass spectrometric analysis, the structure of the isolated chemicals was determined. The crude fractions and four compounds (**6**,**7**,**8** and **9**) were evaluated for a cytotoxicity study on a panel of six human stomach cancer cell lines (SCL, SCL-6, SCL-37′6, SCL-9, K-3, N21) by MTT assay. Among the plant extracts and isolated compounds, petroleum ether fraction and compound **7** exhibited the highest cytotoxic activity against SCL and SCL-6 cells, where the IC_{50} values were 17.9 and 16.56 μM, respectively.

1. Introduction

The emergence of new threats or the development of drug resistance requires the development of new medications. For the discovery of potential bioactive chemicals or lead structures for novel drugs, medicinal plants should be emphasized [1,2]. According to an assessment by the World Health Organization (WHO), around 80 % of the world's population primarily relies on traditional medicines for their healthcare needs [3]. The valuable properties of plant-based products have led to a greater focus on biological activity screening as well as the isolation and identification of plant-based bioactive compounds. This has been driven by the rising demand for novel compounds to afford healthcare support for various human ailments, including inflammation, diabetes, cancer, and neurological disorders [4]. Recent advancements in isolation, identification, and testing technologies have significantly contributed to medicinal plant research. These have led to the isolation of antimalarial medications such as artemisinin (Artemisia annua) and

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quinine (Cinchona spp.), anti-AIDS glycyrrhizin (from Glycyrrhiza species), vinblastine (from Catharanthus roseus), hypercin (from Hypericum species), and taxol (from Taxus bravifolia). All of these compounds were discovered in natural products [5]. Therefore, the study of plant chemical components and their pharmacological screening could serve as a foundation for the developing of lead compounds in the drug discovery procedure. However, little research has been done on the possible use of higher plants as a source of new drugs. Among the predictable four lakh plant species, only 6 % have been assessed for their activity, and very few, not more than 20 %, have been studied phytochemically [6]. Thus, there is a requirement to investigate the various promising bioactive fractions of medicinal plants, perform phytochemical analysis, and perform phytopharmacological evaluations for drug discovery.

There are many pharmacologically active chemicals found in rutaceous plants, including those with anti-inflammatory, anti-implantation, anti-neoplastic [7], and anti-mutagenic properties [8]. The Rutaceous family is widely known for producing a variety of secondary metabolites, complex furo- and pyranocoumarins, phenanthridine, acridone, and furo- and pyranoquinoline alkaloids [9]. It consists of over 2070 species and 160 genera, including a variety of woody shrubs, trees, and perennial herbaceous plants. The family

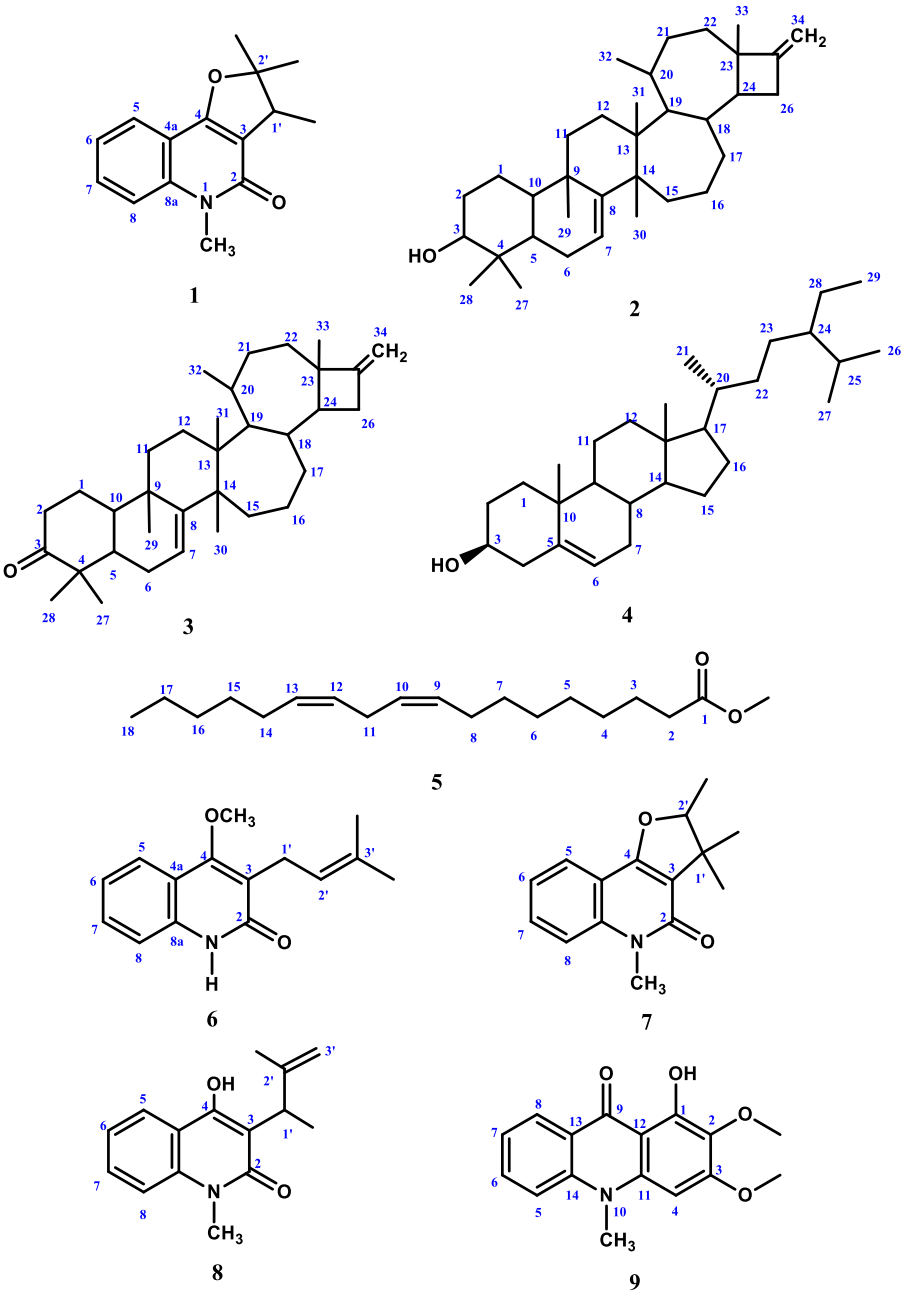

Fig. 1. Structures of compounds (**1–9**) isolated from *R. spectabilis* engl.

has members all throughout the world, but it is most prevalent in tropical and temperate areas. *Ravenia spectabilis* engl. belonging to the rue family is a medium sized shrub is found throughout the South America and some Asian nations [10]. According to a literature review, *R. spectabilis* has cytotoxic and antibacterial properties [11]. Numerous compounds, including paraensine, ravesilone, spectabiline, ravenine, ravenoline, atanine, g-fagarine, arborinine, stigmasta-4,22-dien-3-one, and stigmasterol, have been identified in this plant through previous phytochemical studies. G-fagarine, arborinine, and atanine were found to have some biological activity. Two previously unidentified indole alkaloids were recently recovered, namely, 3-prenyl-5(3-keto-but-1-enyl) indole and 3-prenyl-indole-5-carbaldehyde, which have the similar structure as 3,5-diprenyl indole [12].

Among various life-threatening diseases, cancer is regarded as a major public health burden all around the globe [13]. It is considered as one of the major causes of death [14,15]. Despite significant progress in its diagnosis and treatment options [16,17], this disease is spreading rapidly and is projected to affect 21 million people by the year 2030 [18]. Because of the high mortality accompanying with cancer, billions of dollars have been spent to find effective cancer therapies [19]. One of the major challenges encountered during the development of anticancer medications is the high occurrence of cancer being detected in advanced stages [20]. Traditional medicine, which includes the use of medicinal plants, nutraceuticals, and functional foods, has long been employed as a fundamental healthcare strategy in addressing tissue impairments and bodily disorders at the molecular level, such as cellular signaling. For instance, the isolation of vinblastine and vincristine from the Madagascar periwinkle, *Catharanthus roseus* G. Don. (Apo-Cynaceae), first initiated the concept of using medicinal plant extracts as anticancer agents [21]. Thus, almost two-thirds of existing anticancer drugs have been obtained from naturally occurring secondary metabolites, as well as some derivatives that are used in traditional medicinal practices [22]. As a part of our continuous investigation into novel bioactive compounds derived from plants, *Ravenia spectabilis* engl., we report the isolation of a new secondary metabolite called iso-oligophyline (**1**), two other new terpenoids with proposed structures (**2**) and (**3**), along with two known compounds. We also analyse the cytotoxic potential of leaf extracts and previously reported compounds on several stomach cancer cell lines.

2. Results

The methanolic extract of the leaves of *R. spectabilis* yielded five compounds: iso-oligophyline (**1**), ravespanol (**2**), ravespanone (**3**), β-sitosterol (**4**), and methyl linoleate (**5**). Iso-oligophyline (**1**), is a new 2-quinolone alkaloid (**1**) has been reported first time. Compound **2,** ravespanol, and compound **3,** ravespanone, are two new unusual C_{34} terpenoids (proposed structures), and methyl linoleate constitutes the first report of isolation from this species. The cytotoxic properties of the remaining four compounds i.e. atanine (**6**), oligophyline (**7**), ravenoline (**8**), and arborinine (**9**) has been also stated (Fig. 1).

Compound **1** was obtained as a yellowish mass, and gave an orange-red colour with Dragendorff's reagent. The ^{1}H NMR (Fig. S1, Table 1) spectrum showed signals at δ_H 7.72 d (J = 7.2 Hz), 7.17 dd (J = 8.0,7.8 Hz), 7.52 dd (J = 8.5,8.0), and 7.32 d (J = 8.5 Hz), assignable to H-5, H-6, H-7, and H-8, respectively. The presence of four aromatic proton multiplets suggests the presence of an ortho disubstituted aromatic ring of the 2-quinolones. This information indicates that the compound likely contains a 2-quinolone core with substitution at the ortho position of the aromatic ring, specifically at positions 5, 6, 7, and 8. The coupling constants (J values) provide additional information about the coupling between neighboring protons, which aids in assigning the positions of the substituents on the aromatic ring.

A singlet representing three protons resonating at δ_H 3.67 could be assigned to the *N*-methyl group. The spectrum displayed two methyl singlets at δ_H 1.40 and 1.44, a methyl doublet at δ_H 1.33 (J = 6.2 Hz), and a methine multiplet at δ_H 3.25. All ^{1}H NMR signals of iso-oligophyline resemble those observed in oligophyline [23]. The significant difference lies in the position of the methine signal. In oligophyline, it appears at a low field (δ_H 4.60) due to the presence of oxygen in the same carbon (likely indicating an alcohol or ether functionality). However, in iso-oligophyline, the absence of this oxygen results in the methine signal appearing at a higher field (δ_H 3.25), placing its position at C-1′. From this analysis, it can be inferred that iso-oligophyline is structurally similar to oligophyline, with

Table 1
NMR spectroscopic data for compound **1** in $CDCl_3$ (δ in ppm, J in Hz).

Position	δ_C	δ_H	HMBC
2	c	–	–
3	114.5	—	—
4	160.7	–	–
4a	113.2	–	–
5	123.2	7.72 d (J = 7.2 Hz)	140.5 (C-8a), 130.8 (C-7), 160.7 (C-4)
6	121.5	7.17 dd (J = 8.0, 7.2 Hz)	113.2 (C-4a)
7	130.8	7.52 dd (J = 8.5, 8.0 Hz)	140.5 (C-8a), 123.2 (C-5)
8	114.5	7.32 d (J = 8.5 Hz)	121.5(C-6), 113.2 (C-4a)
8a	140.5	–	–
1′	44.7	3.25 1H m	–
Me-1′	14.2	1.33 3H d (J = 6.2 Hz)	92.7 (C-2′), 44.7 (C-1′)
2′	92.7	–	–
Me-2′	22.5	1.44 3H s	92.7 (C-2′), 44.7 (C-1′), 28.9 (Me-2′)
Me-2′	28.9	1.40 3H s	92.7 (C-2′), 44.7 (C-1′), 22.5 (Me-2′)
N–Me	29.0	3.67 3H s	–

c = not observed.

the main difference being the absence of an oxygen-containing functional group, resulting in a different chemical environment for the methine carbon and thus a shift in its NMR signal to a higher field. In oligophyline, the methine appeared at a low field at δ_H 4.60 due to the presence of oxygen in the same carbon.

The ^{13}C NMR (Fig. S2) spectrum exhibited an *N*-methyl carbon at δ_C 29.0 and three methyl carbons at δ_C 22.5, 28.9, and 14.2, a methyline carbon at δ_C 44.7. The HSQC (Fig. S3) and HMBC (Fig. 2 and S4) experiments showed all expected ^1J, ^2J, and ^3J couplings among the carbons and protons. All expected ^1J, ^2J, and ^3J couplings were observed among the carbons and protons. The information from the ^{13}C NMR spectrum confirms the presence of different types of carbons in the molecule, including methyl, methylene, and *N*-methyl carbons. The results of the HSQC and HMBC experiments provide crucial coupling information, which aids in confirming the connectivity between different carbon and proton atoms in the molecule. Combining the data from the ^{1}H and ^{13}C NMR spectra and the results of the HSQC and HMBC experiments allows for a comprehensive structural elucidation of the compound. The COSY (Fig. 2 and S5) spectrum revealed the coupling between H-1′ protons to Me-1′ protons and also between the protons as expected for the disubstituted benzene ring. Thus, compound **1** was identified as a new 2-quinolone alkaloid and was given the trivial name iso-oligophyline. The ^{1}H and ^{13}C NMR spectra are provided in Table 1, which presumably contain the detailed chemical shifts and coupling constants for further analysis of the compound's structure. HR-ESIMS [M+H] Showed + *m/z* 244.1326, which matches well with the calculated value for $C_{15}H_{17}NO_2$ (244.1332) (Fig. S6). The provided information confirms the structural elucidation of iso-oligophyline as a new 2-quinolone alkaloid. The consistency between the experimental data (NMR spectra and mass spectrometry) and the calculated values supports the proposed molecular formula.

When examined under UV light on a TLC plate, compound **2** separated as tiny needle-shaped crystals that were invisible. When sprayed with vanillin in sulfuric acid reagent and heated for 5 min, the compound developed a brown hue. The only difference between compound **2** and compound **3**, which were found to be highly uncommon and closely related to C-34, was in position 3. The 1D and 2D spectroscopic data show that compound **2** has a hydroxyl group and compound **3** has a keto group at C-3.

For the determination of structure of compound **2** the ^{1}H, ^{13}C NMR, ^{1}H–^{1}H COSY, HSQC, and HMBC spectra were available in both $CDCl_3$ and C_5D_5N. The ^{1}H NMR spectrum (Table 2, Figs. S7, S8, S9) displayed an olefinic proton, two exomethylene protons, an oxymethine proton, six methyl singlets, and a methyl doublet at δ_H 5.33 d (J = 5.1 Hz), 4.98 and 4.94 (br s, each), 3.70, 0.90, 0.95, 1.11, 1.12, 1.21, 1.25 and 0.96 d (J = 6.6 Hz)., 34 carbons were showed by ^{13}C NMR spectrum (Fig. S10), where two quaternary carbons (unsaturated) at δ_C 156.8 and 158.0, a methine carbon (unsaturated) at δ_C 118.8, a methylene carbon (unsaturated) at δ_C 107.4, indicating the presence of a tri-substituted olefinic group and an exomethylene group, a carbinol carbon at δ_C 75.8 and seven methyl carbons at δ_C 13.4–26.5. This spectral data provides valuable structural information about compound **2** that the presence of an olefinic proton, exomethylene protons, and a tri-substituted olefinic group in the ^{1}H NMR spectrum suggests the presence of a complex unsaturated system. The ^{13}C NMR spectrum confirms the presence of unsaturation, with quaternary, methine, and methylene carbons indicative of an olefinic group. The presence of a carbinol carbon suggests the presence of a hydroxyl group. Further analysis of the ^{1}H–^{1}H COSY, HSQC, and HMBC spectra in both $CDCl_3$ and C_5D_5N, along with consideration of the molecular formula and connectivity information, would aid in determining the complete structure of compound **2**.

Twelve methylene protons had ^1J connectivity to the methylene carbons at δ_C20.9, 30.0, 25.1, 32.8, 34.4, 34.3, 30.3, 28.8, 31.4, 38.4, 24.0, and 107.4 in the HSQC spectrum (Fig. S11). Additionally, the spectrum demonstrated eight methine protons' ^1J connection to methine carbons at δ_C 75.8, 35.8, 118.8, 47.8, 28.7, 54.6, 37.7, and 28.0. Additionally, the methyl carbons were assigned using the HSQC.

The methyls at δ 1.25 and 0.90 in the HMBC spectrum (Fig. S12) showed frequent associations with a methine carbon at δ_C 35.8 (C-5), a quaternary carbon at δ_C 39.6 (C-4), and a carbinol carbon at δ_C 75.8 (C-3). These two methyls at positions 27 and 28, respectively,

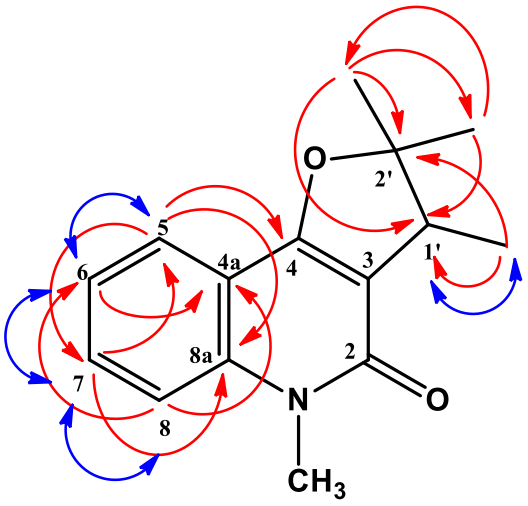

Fig. 2. HMBC (red) and COSY (blue) correlations for compound **1**.

Table 2
NMR spectroscopic data for compound **2** and compound **3.**

	Compound 2 (C_5D_5N)			Compound 2 ($CDCl_3$)	Compound 3 ($CDCl_3$)		
Posit[n]	δ_C	δ_H	HMBC	δ_C	δ_C	δ_H	HMBC
1	20.9	1.89, 1.54	–	19.9	25.7	1.85 m, 1.93 m	–
2	30.0	1.90, 1.95	–	28.6	37.1	2.57 m, 2.32 m	25.7 (C-1), 216.5 (C-3)
3	75.8	3.70 br s	20.9 (C-1)	76.5	216.5	–	–
4	39.6	–	–	38.7	48.9	–	–
5	35.8	2.48	20.4 (C-28), 39.6 (C-4), 47.8 (C-10),	35.3	43.6	1.85 m	–
6	25.1	2.31, 2.03	39.6 (C-4), 156.8 (C-8),	24.2	24.2	2.23 m, 1.96 m	43.6 (C-5), 117.8 (C-7), 156.3 (C-8)
7	118.8	5.33 d (J = 5.1 Hz)	25.1 (C-6), 35.8 (C-5), 37.0 (C-9), 53.4 (C-14)	117.8	117.8	5.28 dd (J = 6.6, 1.8 Hz)	24.2 (C-6), 36.5 (C-9), 43.6 (C-5), 52.7 (C-14)
8	156.8	–	–	156.2	156.3	–	–
9	37.0	–	–	36.3	36.5	–	–
10	47.8	1.28	–	46.8	46.5	1.50 m	–
11	32.8	1.76 m, 1.49 m	22.0 (C-29), 43.4 (C-13), 53.4 (C-14), 47.8 (C-10) 34.4 (C-12), 156.8 (C-8)	32.2	32.4	–	–
12	34.4	1.92 m, 1.75 m	24.3 (C-31), 32.8 (C-11), 37.0 (C-9), 37.0 (C-9), 47.8 (C-10)	33.8	33.7	1.76 m	20.6 (C-29), 32.4 (C-11), 52.7 (C-14)
13	43.4	–	–	42.8	42.8	–	–
14	53.4	–	–	52.7	52.7	–	–
15	34.3	1.52 m, 1.49 m	53.4 (C-14), 28.7 (C-18)	33.6	33.6	–	–
16	30.3	1.32 m, 1.32 m	–	29.7	29.7	–	–
17	28.8	1.97, 1.28	28.0 (C-24)	28.1	28.1	–	–
18	28.7	1.14 m	24.0 (C-25),	28.2	28.2	–	–
19	54.6	1.54 m	–	53.9	53.9	1.52 m	–
20	37.7	1.36 m	–	37.0	37.0	–	–
21	31.4	0.95 m, 1.36 m	–	30.7	30.6	–	–
22	38.4	1.27 m, 1.53 m	28.0 (C-24), 158.0 (C-26)	37.8	37.8	–	–
23	39.7	–	–	39.2	39.2	–	–
24	28.0	1.10 m	24.0 (C-25),	27.8	27.8	1.08	23.4 (C-25)
25	24.0	2.06, 2H m	13.4 (C-33), 107.4 (C-34), 158.0 (C-26)	23.4	23.4	2.01, 2H m	13.1 (C-33), 106.4 (C-34), 157.8 (C-26)
26	158.0	–	–	157.8	157.8	–	–
27	26.5	1.25 3H s	20.4 (C-28), 35.8 (C-5), 39.6 (C-4), 75.8 (C-3)	25.3	22.0	1.05 3H s	19.2 (C-28),43.6 (C-5), 48.9 (C-4), 216.5 (C-3)
28	20.4	0.90 3H s	26.5 (C-27), 35.8 (C-5), 39.6 (C-4), 75.8 (C-3)	19.7	19.2	1.01 3H s	22.0 (C-27), 43.6 (C-5), 48.9 (C-4), 216.5 (C-3)
29	22.0	1.21 3H s	32.8 (C-11), 37.0 (C-9), 47.8 (C-10), 156.8 (C-8)	21.2	21.2	1.06 3H s	32.4 (C-11), 36.5 (C-9), 46.5 (C-10), 156.3 (C-8)
30	28.4	1.12 3H s	34.3 (C-15), 43.4 (C-13), 53.4 (C-14), 156.8 (C-8)	27.8	27.5	1.103H s	33.6 (C-15), 42.8 (C-13), 52.7 (C-14), 156.3 (C-8)
31	24.3	0.95 3H s	34.4 (C-12), 43.4 (C-13), 53.4 (C-14)	23.7	23.7	0.89 3H s	33.7 (C-12), 42.8 (C-13), 52.7 (C-14)
32	19.4	0.96 3H d (J = 6.6 Hz)	31.4 (C-21), 37.7 (C-20)	18.8	18.8	0.90 3H d (J = 6.9 Hz)	30.6 (C-21), 37.0 (C-20)
33	13.4	1.11 3H s	28.0 (C-24), 38.4 (C-22), 39.7 (C-23), 158.0 (C-26)	13.1	13.1	1.04 3H s	27.8 (C-24), 37.8 (C-22), 39.2 (C-23), 157.8 (C-26)
34	107.4	4.98, 4.94 br s, each	24.0 (C-25), 39.7 (C-23)	106.4	106.4	4.82, 4.80 br s, each	23.4 (C-25), 39.2 (C-23),

Spectra were measured on a 400 MHz instrument. **Posit [n] = Position.**

also showed a correlation with one another, suggesting that they are germinal methyls. The olefinic proton at δ_H 5.33 may be ascribed to H-7 since it showed ^3J correlations to δ 35.8 (C-5), 37.0 (C-9), and 53.4 (C-14).

The methyl at δ 1.21, which was potentially related to H-29, showed $^{2/3}J$ correlations to δ_C 32.8 (C-11) and ^3J correlations to C-8, C-15, and C-12, ultimately validating their placements at 30 and 31. There were ^2J correlations between the methyl doublet at δ_H 0.96 and δ_C 37.0 (C-9), 47.8 (C-10), and 156.8 (C-8). There was shared connectivity between two methyl groups that were resonant at δ_H 1.12 and 0.95 and C-13 and C-14.

The former additionally displayed a connection of δ_C 37.7 and ^3J to δ_C 31.4, to which a shielded proton resonating at δ 0.95 is linked. The latter may be ascribed to H-21, and the methyl doublet to H-32, since it showed a ^3J correlation to δ_C 54.6 (H-19). The H-21 proton in the COSY spectrum (Fig. S13) correlated with protons at δ_H 1.52 and 1.27 (putting them at 22), indicating direct linkage to the carbon at δ 38.4. A ^3J association was seen between the residual methyl at δ_H 1.11 and the unsaturated carbon at δ_C 158.0 (C-26), quaternary carbon at δ_C39.7 (C-23), methine carbon at δ_C 28.0 (C-24), and methylene carbon at δ_C 38.4 (C-22). A ^3J connection

between the exomethylene protons and C-23 and C-26 was found. The H-15 proton demonstrated linkage to H-16 and the latter to H-17 protons in the COSY spectra (Fig. S13). The H-17 and H-18 protons in the HMBC spectrum exhibited a ^{3}J association with C-24 and C-26, respectively. As a result, compound **2**'s structure was estimated and given the name ravespanol (Fig. 3).

Compound **3** was acquired as a sticky mass. When sprayed with vanillin in sulfuric acid reagent and heated for 5 min, it formed a brown hue and displayed a purple chromatographic spot and no luminescence under a UV lamp.

In terms of ^{1}H NMR (Fig. S14), ^{13}C NMR (Fig. S15), ^{1}H–^{1}H COSY, HSQC, and HMBC (Fig. S16) spectral data, compound **3** is similar to compound **2** with the exception that the carbinol carbon vanished in the ^{13}C NMR spectrum and the oxymethine proton vanished in the ^{1}H NMR spectrum, indicating the absence of the hydroxyl group at position 3. The carbinol carbon at δ_C 75.8 was replaced by a carbonyl carbon resonating at δ_C 216.5, suggesting that a keto group had taken the place of the hydroxyl group. The ^{1}H NMR spectrum of compound **3** revealed the proton signals of six methyl singlets, a methyl doublet, two exomethylene protons, and an olefinic proton (Table 2, Fig. S14). These protons were resonating at δ_H 4.82, 4.80 (br s, each), 0.89, 1.01, 1.04, 1.05, 1.06, 1.10, and at 0.90 d ($J = 6.9$ Hz), respectively. All 34 carbons, including seven methyls, twelve methylenes, seven methines, and eight quaternary carbons, were identified by the ^{13}C NMR spectra. The HSQC and HMBC (Fig. S16) spectra showed all of the predicted correlations. Thus, Fig. 1 depicts the approximate structure of ravespanone. HMBC and HSQC Spectra revealed all the expected correlations between protons and carbons. Based on this data, the tentative structure of ravespanone is proposed, likely involving an olefinic group, exomethylene groups, and methyl substituents. The structural elucidation is supported by the correlation data obtained from the HMBC and HSQC spectra. Further analysis, including consideration of the molecular formula and connectivity information, would refine the proposed structure of compound **3**.

β-sitosterol (**4**): White crystal; ^{1}H NMR (400 MHz, $CDCl_3$): H-3, 3.53 1H m, H-6, 5.35 1H d ($J = 5.0$ Hz), H-18, 5.35 1H d ($J = 5.0$ Hz), H-19, 1.00 3H s, H-21, 0.92 d ($J = 6.5$ Hz), H-26, 0.82 d ($J = 7.2$ Hz), H-27, 0.84 d ($J = 7.2$ Hz), H-29, 0.85 t ($J = 7.2$ Hz) [24].

Methyl linoleate (**5**): Yellowish mass; ^{1}H NMR (400 MHz, $CDCl_3$) (Fig. S22):5.36 (4H, m, H-9, H-10, H-12, H-13),3.65 (3H, s, $COOCH_3$), 2.79 (2H, m, H-11), 2.37 (2H, t, $J = 7.2$, H-2), 2.07 (4H, m, H-8, 14), 1.63 (2H, m, H-3), 1.26–1.32 (14H, m, H-4 to H-7, H-15 to H-17), 0.89 (3H, t, $J = 6.8$, H-18) [25].

2.1. Cytotoxicity study

Among the different fractions of the extracts, the petroleum ether fraction exhibited good cytotoxic activity (IC_{50} 17.9 μM) against SCL and moderate activity (IC_{50} 34.26 μM) against SCL-9. Out of the four compounds tested, two were cytotoxic in multiple cancer cell lines. However, compared to vincristine sulfate, compounds **7** and **8** possessed moderate cytotoxicity with compound **7** showing the highest cytotoxicity (IC_{50} 16.56 μM). Compounds **7** and **8** were found cytotoxic against three and five of the cancer cell lines tested, respectively (Table 3). Compound **7** showed reasonable cytotoxicity (IC_{50} from 16.56 to 30.8 μM) except against the N-21 line, while compound **8** also possessed moderate cytotoxicity (IC_{50} from 26.9 to 41.86 μM) against SCL-6, SCL-37′6, and SCL-9. On the other hand, no cytotoxic activity was observed for compounds **6** and **9** (Fig. S23).

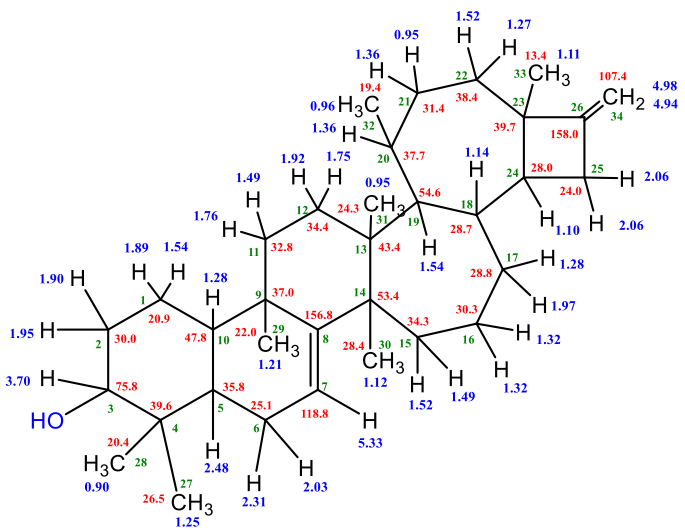

Fig. 3. Ravespanol (^{1}H and ^{13}C are from C_5D_5N Spectra).

Table 3
Cytotoxicity assay results for leaf extracts, compounds **6**–**9**, and Vincristine sulfate against various cell lines (IC_{50} in μM).

Compounds/Extracts	SCL	SCL-6	SCL-37′6	SCL-9	K-3	N21
Methanol leaf extract	276.46 ± 7.4	275.26 ± 6.2	269.86 ± 12.4	274.3 ± 9.6	270.23 ± 7.5	292.13 ± 8.2
Ethyl acetate leaf extract	281.3 ± 8.3	288.56 ± 4.5	279.53 ± 7.1	275.56 ± 7.3	266.35 ± 5.2	270.5 ± 6.3
Dichloromethane leaf extract	294.56 ± 3.3	376.93 ± 7.4	288.5 ± 6.1	272.7 ± 5.3	265.23 ± 6.2	316.53 ± 8.4
Petroleum ether leaf extract	17.9 ± 3.13	Nil	466.96 ± 10.6	34.26 ± 6.2	266.43 ± 6.5	274.2 ± 5.4
6	Nil	Nil	Nil	Nil	Nil	Nil
7	30.8 ± 2.3	16.56 ± 3.6	36.43 ± 6.4	22.06 ± 3.3	20.36 ± 1.8	307.86 ± 9.7
8	422.16 ± 9.7	34.26 ± 4.2	41.86 ± 1.9	26.9 ± 2.8	320.13 ± 6.2	289.83 ± 3.2
9	Nil	Nil	Nil	Nil	Nil	415.8 ± 11.6
Vincristine sulfate	5.9 ± 0.6	6.1 ± 0.8	5.3 ± 0.6	5.3 ± 0.6	6.1 ± 0.7	5.3 ± 0.5

3. Materials and methods

3.1. General experimental procedures

Preparative TLC was performed on glass plates coated with silica gel 60 PF254 (0.5 mm thickness, Merck, Rahway, NJ, USA), and the separated compounds were identified using a vanillin H_2SO_4 spray reagent. Gel permeation chromatography was carried out using Sephadex LH-20. Using a 400 MHz Ultrashield NMR Spectrophotometer (Ultra Shield Race Products, Flint, TX, USA) and a Bruker Avance100 (Bruker, Billerica, MA, USA), ^{1}H NMR spectra were acquired in $CDCl_3$. (δ values were re-ported in reference to $CHCl_3$ at 7.25 ppm).

3.2. Plant material

The fresh plant materials (leaves) of *R. spectabilis* were obtained from the premises of Dhaka University, and a voucher specimen (Accession no. 34694) of the plant has been deposited at the Bangladesh National Herbarium (BNH), Dhaka. The leaves were shade-dried for around 15 days, and after that, the dried plants materials were further subjected to drying in a hot air oven at 40°–45° for 4–5 h. Using a grinding machine, the leaves were milled into course powder.

3.3. Extraction and isolation

About 1.2 kg of the powdered leaf plant material was immersed in 3.5 L of methanol for 20 days at room temperature. The methanol extract was then concentrated (30.7 g) and fractionated by VLC over Silica gel 60H using a sequence with petroleum ether, dichloromethane, ethyl acetate, and methanol mixtures of increasing polarity. A total of 42 fractions (100 mL each) were collected.

Sephadex LH-20 column chromatography was employed for the fractionation of VLC fraction 10 (70 % dichloromethane in petroleum ether), and 16 fractions were obtained, whereas 20 % petroleum ether in chloroform was used as the eluting solvent. From Sephadex fractions 4 and 5, compound **3** (3.3 mg, R_f 0.56, n-Hexane/toluene, 5:95) was obtained as a gummy mass, which was not visible when observed under UV light on a TLC plate and showed a brown spot with vanillin/H_2SO_4. Again, the same process was employed for VLC fraction 12 (using 100 % dichloromethane) and obtained 22 fractions. Compound **2** (6.2 mg, R_f 0.51, n-Hexane/toluene, 5:95) was isolated as fine needle-shaped crystals from Sephadex fractions 9 and 10 of this VLC fraction. The crystals were invisible under UV light and produced a brown color when sprayed with vanillin/H_2SO_4. By employing the same eluting solvent, VLC fraction 14 (5 % dichloromethane in ethyl acetate) was further fractionated by Sephadex LH20 column chromatography to give 31 fractions. Sephadex fractions 10–12 displayed a bright pinkish-purple spot with vanillin/H_2SO_4; these fractions were combined and then subjected to preparative TLC (silica gel, EtOAc/toluene, 15:85, multiple developments) to obtain compound **1** (3.5 mg, R_f 0.56, EtOAC/toluene, 1:99). Sephadex fractions 20–25 of the same VLC fraction showed a light brown colour on UV visualization, and the fractions were mixed, and the mixture was then employed for preparative TLC (silica gel, EtOAc/toluene, 3:97), and thus compound **5** (4.5 mg, R_f 0.50, EtOAC/toluene, 1:99) was obtained. Fraction 15 (10 % dichloromethane in ethyl acetate) was fractionated by Sephadex LH20 column chromatography to give 35 fractions, using the same eluting solvent used for the previous VLC fraction. Compound **4** (4.2 mg, R_f 0.55, EtOAC/toluene, 15:85) was obtained as colourless crystals from Sephadex fractions 27–31, which were mixed and were subjected to purification by n-Hexane with a few drops of ethyl acetate.

3.4. Preparation of sample for bioassay

The crude methanolic leaf extract of *R. spectabilis* was then partitioned by following the protocol designed by Kupchan [26] and modified by Van Wagenen et al. [27]. To make the mother solution, 5 g of crude leaf extract was dissolved in 10 % aqueous methanol, which was successively partitioned in sequence with petroleum ether, carbon tetrachloride, chloroform, and water in order of increasing polarity using a separating funnel.

3.5. Cytotoxicity assay

The cytotoxicity of the different fractions of the leaf extracts and pure compounds was evaluated using a panel of six human stomach cancer cell lines: SCL, SCL-6, SCL-37′6, SCL-9, Kato-3, and N21. The MTT test developed by Mosmann [28] was used to calculate cell mortality. Cell lines were studied using a series of serial dilutions (250, 125, 62.5, 31.25, and 15.63 μg/mL) of the plant extracts, pure compounds, and the control. For each concentration, three replicate analyses were carried out. For each concentration, the percentage of cell mortality was assessed. The IC_{50} values in μM units were then determined. In this study, vincristine sulfate was employed as a positive control. RPMIC (RPMI-1640 complete medium, used to culture the cancer cells for their confluent growth) and RPMIC-DMSO (RPMIC containing 0.25 % DMSO, used to prepare the test materials and to culture the cells in the presence of the test materials) were selected as negative controls. Cells grown in RPMIC and RPMIC-DMSO were found to be the same and were considered to have 100 % cell survival (i.e., cell mortality was nil) for the evaluation of cell mortality and to determine the IC_{50} for the extracts and compounds tested.

4. Conclusions

Among the five compounds elucidated from *Ravenia spectabilis* engl., one of the compounds, a 2-quinoline alkaloid named iso-oligophyline, is new. Additionally, two very unusual C_{34} terpenoids, compounds **2** and **3**, are also proposed to be new. The petroleum ether fraction, along with compounds **7** and **8,** demonstrated strong anticancer activity against some of the stomach cancer cell lines tested. Among the extracts and isolated pure compounds, compound **7,** oligophyline exhibited the most potent cytotoxic activity.

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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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Appendix A. Supplementary data

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