RESULTS

7. RESULTS

7.1 COLLECTION AND AUTHENTICATION OF PLANT
The plant material was collected from Tehri (Garwal), Utrtrakhand, India and authenticated by Dr. Sunita Garg from NISCAIR, New Delhi (Voucher specimen no-NISCAIR/RHMD/Consult/2013/2233/14).

7.2 PHYSICOCHEMICAL PARAMETERS
The results of ash values & extractive values of plant material are given in Table 5 and Table 6.

Table 5: The percentage ash values of Zanthoxylum alatum Roxb. stem bark

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Type of ash</th>
<th>Ash values (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Total ash</td>
<td>8.50 ± 0.53</td>
</tr>
<tr>
<td>2.</td>
<td>Acid insoluble ash</td>
<td>2.00 ± 0.67</td>
</tr>
<tr>
<td>3.</td>
<td>Water soluble ash</td>
<td>3.50 ± 1.01</td>
</tr>
</tbody>
</table>

Table 6: The percentage extractive values of Zanthoxylum alatum Roxb. stem bark

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Type of Extractive value</th>
<th>Hot (% w/w)</th>
<th>Cold (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ethanol soluble</td>
<td>19.20 ± 1.04</td>
<td>17.40 ± 2.76</td>
</tr>
<tr>
<td>2.</td>
<td>Water soluble</td>
<td>14.10 ± 1.39</td>
<td>12.40 ± 2.51</td>
</tr>
</tbody>
</table>

7.3 PREPARATION OF EXTRACTS
The yield, colour and consistency of the extracts are illustrated in Table 7.

Isolation and Characterization of Anticancer Principles from Zanthoxylum alatum Roxb.
RESULTS

Table 7: The colour, consistency and percentage yield of the extracts of *Zanthoxylum alatum* Roxb. stem-bark

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Extract</th>
<th>Colour</th>
<th>Consistency</th>
<th>Yield (%w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Petroleum ether</td>
<td>Dark green</td>
<td>Semi solid</td>
<td>5.20</td>
</tr>
<tr>
<td>2</td>
<td>Chloroform</td>
<td>Green</td>
<td>Solid</td>
<td>1.80</td>
</tr>
<tr>
<td>3</td>
<td>Ethyl acetate</td>
<td>Brown</td>
<td>Semi solid</td>
<td>6.50</td>
</tr>
<tr>
<td>4</td>
<td>Methanol</td>
<td>Reddish brown</td>
<td>Solid</td>
<td>2.80</td>
</tr>
</tbody>
</table>

7.4 QUALITATIVE PHYTOCHEMICAL SCREENING

The saponins and protein were found to be absent in all the extracts. The phenolic compounds were present in all the extracts. The carbohydrates was found only in methanolic extract whereas glycosides and flavonoids were present in ethyl acetate and methanolic extracts. However, steroids were present in both petroleum ether and ethyl acetate extracts. Results are shown in Table 8.
RESULTS

Table 8: Results of phytochemical screening of various extracts of *Zanthoxylum alatum* Roxb. stem bark

<table>
<thead>
<tr>
<th>Category</th>
<th>Petroleum ether</th>
<th>Chloroform</th>
<th>Ethyl acetate</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Glycoside</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Steroids and triterpenoids</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

+ indicates presence, - indicates absence

7.5 CYTOTOXIC ACTIVITY

The petroleum ether and ethyl acetate extracts has shown more cytotoxic potential as compared to other extracts (Figure 12, 13, 14, 15). They caused significant inhibition of viability of cancer cell lines. Results were expressed in IC\textsubscript{50} values. The IC\textsubscript{50} values of all
RESULTS

extracts (petroleum ether, ethyl acetate, methanolic and chloroform) in MIA-PaCa, A-549, MCF-7 and CaCo-2 cancer cell lines are shown in Table 9. As petroleum ether and ethyl acetate extracts showed most promising results in MIA-PaCa and A-549 cancer cell lines so, both these extracts were subjected to silica gel column for isolation of bioactive compounds. Compounds isolated from extracts were also subjected to MIA-PaCa and A-549 cancer cell lines for checking their cytotoxic potential.
RESULTS

Figure 12: Effect of Petroleum ether, ethyl acetate, methanol, chloroform extract of *Zanthoxylum alatum* stem bark and standard drug docetaxal on MIA-PaCa human Pancreatic cancer cell lines. Values were represented as mean ± SD, (n=3), *p<0.05 compared to control.
RESULTS

Figure 13: Effects of Petroleum ether, ethyl acetate, methanol, chloroform extract of *Zanthoxylum alatum* stem bark and standard drug docetaxal on A-549 human Lung cancer cell lines. Values were represented as mean ± SD, (n=3), *p*<0.05 compared to control.
Figure 14: Effects of Petroleum ether, ethyl acetate, methanol, chloroform extract of *Zanthoxylum alatum* stem bark and standard drug docetaxal on MCF-7 human Breast cancer cell lines. Values were represented as mean ± SD, (n=3), *p<0.05 compared to control.
RESULTS

Figure 15: Effects of Petroleum ether, ethyl acetate, methanol, chloroform extract of *Zanthoxylum alatum* stem bark and standard drug docetaxal on CaCo-2 human Colon cancer cell lines. Values were represented as mean ± SD, (n=3), *p<0.05 compared to control.
RESULTS

Table 9: IC\textsubscript{50} values of various extracts on A-549, MIA-PaCa, MCF-7, CaCo-2 cancer cell lines

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Extract</th>
<th>MIA-PaCa cell line</th>
<th>A-549 cell line</th>
<th>MCF-7 cell line</th>
<th>CaCo-2 cell line</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Petroleum ether</td>
<td>63.13 ± 1.15</td>
<td>68.13 ± 1.20</td>
<td>96.66 ± 1.53</td>
<td>133.33 ± 3.21</td>
</tr>
<tr>
<td>2.</td>
<td>Ethyl acetate</td>
<td>78.00 ± 1.63</td>
<td>85.33 ± 1.52</td>
<td>113.66 ± 2.08</td>
<td>138.00 ± 2.64</td>
</tr>
<tr>
<td>3.</td>
<td>Methanol</td>
<td>92.43 ± 1.52</td>
<td>118.04 ± 1.73</td>
<td>142.34 ± 3.21</td>
<td>172.34 ± 2.30</td>
</tr>
<tr>
<td>4.</td>
<td>Chloroform</td>
<td>136.58 ± 2.88</td>
<td>141.34 ± 2.05</td>
<td>153.77 ± 2.51</td>
<td>186.94 ± 1.53</td>
</tr>
<tr>
<td>5.</td>
<td>Docetaxel</td>
<td>11.12 ± 2.20</td>
<td>12.01 ± 2.10</td>
<td>19.67 ± 1.15</td>
<td>8.89 ± 1.52</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SD, (n=3).

7.6 ANTIOXIDANT ACTIVITY

The extracts having maximum cytotoxic activity has also shown significant antioxidant potential. The antioxidant activity of extracts were increased with increase in concentration. The % age inhibition of ascorbic acid, petroleum ether and ethyl acetate extracts in DPPH and NO assays are shown in Figure 16 a, b. Both extracts has shown significant activity in different models. IC\textsubscript{50} values of standard and extracts in DPPH and NO assays are given in Table 10. Petroleum ether and ethyl acetate extracts has shown reducing power as shown in Table 11.
RESULTS

Figure 16: a) DPPH radical scavenging effect and; b) NO scavenging activity of standard and extracts of Zanthoxylum alatum stem bark.

Table 10: IC\textsubscript{50} values of standard and extracts in DPPH and NO assay

<table>
<thead>
<tr>
<th>Extract</th>
<th>DPPH</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>57.30 ± 1.90</td>
<td>42.80 ± 2.61</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>85.16 ± 1.05</td>
<td>72.39 ± 1.53</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>99.25 ± 2.53</td>
<td>94.81 ± 2.56</td>
</tr>
</tbody>
</table>

Values were represented as mean ± SD, (n=3).
RESULTS

Table 11: Reductive ability of standard and extracts of Zanthoxylum alatum stem bark

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>Absorbance</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ascorbic acid</td>
<td>Petroleum ether</td>
<td>Ethyl acetate</td>
</tr>
<tr>
<td>25</td>
<td>0.127 ± 0.005</td>
<td>0.092 ± 0.006</td>
<td>0.062 ± 0.007</td>
</tr>
<tr>
<td>50</td>
<td>0.212 ± 0.008</td>
<td>0.196 ± 0.002</td>
<td>0.128 ± 0.008</td>
</tr>
<tr>
<td>100</td>
<td>0.467 ± 0.006</td>
<td>0.367 ± 0.006</td>
<td>0.317 ± 0.008</td>
</tr>
<tr>
<td>200</td>
<td>0.897 ± 0.005</td>
<td>0.669 ± 0.009</td>
<td>0.569 ± 0.009</td>
</tr>
<tr>
<td>400</td>
<td>2.121 ± 0.007</td>
<td>1.537 ± 0.004</td>
<td>1.287 ± 0.009</td>
</tr>
</tbody>
</table>

7.7 TOTAL PHENOLIC CONTENT

The total phenolic content found in the petroleum ether extract was 5.12 mg/g GAE and ethyl acetate extract was 4.36 mg/g GAE which was determined on the basis of its gallic acid equivalent (GAE) from calibration curve of gallic acid (Figure 17).
RESULTS

Figure 17: The calibration curve of standard gallic acid.

7.8 CHARACTERIZATION OF ISOLATED COMPOUNDS

The petroleum ether and ethyl acetate extracts of dried stem bark of ZA were chromatographed on silica gel column and thus isolated and purified three lignans; sesamin, kobusin and 4’O demethyl magnolin and one steroid β-sitosterol from petroleum ether and two flavonoids; apigenin and kaempferol-7-O-glucoside from ethylacetate extract.

7.8.1 Compound A

Compound A was isolated in the form of white powder, 40 mg (0.5% w/w yield); Rf 0.55 (Hexane:acetone, 80:20); m.p. 136-138°C. Presence of β-sitosterol was detected on TLC plates as the pink spot on staining with 50% H₂SO₄. It showed no absorption at UV in the range between 200-400 nm. IR (KBr, cm⁻¹) spectrum showed a broad peak at 3450.94
RESULTS

cm⁻¹ for –OH group, 2931.20 is due to aliphatic C-H streching, 1670.82 for C=C (trisubstituted), 1124.87, 1055.14 for C-O- (Figure 18).

¹H NMR (CDCl₃, 400 MHz, δ with TMS = 0) showed signals at 5.35 (t, 1H, H-5, J = 5.2), 3.51 (m, 1H, H-3), 0.998 (s, 3H, CH₃-19), 0.91 (d, 3H, CH₃-21, J = 6.7), 0.86 (t, 3H, CH₃-29, J = 5.2), 0.84 (d, 3H, CH₃-26, J = 6.8), 0.82 (d, 3H, CH₃-27, J = 6.8), 0.68 (s, 3H, CH₃-18) (Figure 19).

¹³C NMR (CDCl₃, 100 MHz, δ with TMS = 0) shows signals at C-1 (δ 37.06), C-2 (δ 31.27), C-3 (δ 72.09), C-4 (δ 44.8), C-5 (δ 135.5), C-6 (δ 116.4), C-7 (δ 31.2), C-9 (δ 50.8), C-18 (δ 14.1), C-19 (δ 19.06), C-20 (δ 34.5), C-21 (δ 26.5), C-26 (δ 20.8), C-27 (δ 19.06), C-28 (δ 26.4), C-29 (δ 15.84) (Figure 20).

MS ES+: m/z 437 [M+ Na]⁺ and 415 [M+1]. So the mass of the compound was 414. (Figure 21). Thus, on the basis of above physical, chemical and spectral data compound A was characterized as β-sitosterol (Figure 22) having molecular formula C₂₉H₅₀O.
RESULTS

Figure 18: IR spectra of β-sitosterol.

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Figure 19: $^1\text{H}$-NMR spectra of β-sitosterol.
RESULTS

Figure 20: $^{13}$C-NMR spectra of β-sitosterol.

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Figure 21: Mass spectra of β-sitosterol.
RESULTS

Figure 22: Structure of β-sitosterol isolated from petroleum ether extract of Zanthoxylum alatum stem bark.

7.8.2 Compound B

Compound B was isolated as colorless crystals, 40 mg (0.5% w/w yield) Rf 0.74 (ethylacetate:toluene 30:70); m.p. 120-122°C. Compound gave positive Dragendorff’s reagent test; as some lignans give false positive Dragendorff’s test. UV \( \lambda_{\text{max}} \) (chloroform): 278 nm (Figure 23); IR (KBr) spectrum showed str. for C-O at 1239.30 cm\(^{-1}\) for ethers. The stretching for C-H at 2922.5 and C=C at 1609, 1502.16 and 1488.29 cm\(^{-1}\) (Figure 24).

Compound B showed classical \(^1\)H NMR signals indicating tetrahyrofuran type of lignan. The chemical shift signals for only nine hydrogens was observed instead of eighteen hydrogens in \(^1\)HNMR as the compound appears to be symmetrical. \(^1\)HNMR (CDCl\(_3\), 400 MHz, δ with TMS = 0). δ 6.84 (dd, 2H, H-6’ and H-6’’, J = 1.24, 7.85), 6.79 (d, 4H H-2’, H-2’’, H-5 and H-5’’, J = 7.85), 5.95 (s, 4H, H-3’b and H-3’’b), 4.7 (d, 2H, H-2 and H-6, J = 4.36), 4.23 (m, 2H, H-4 and H-8), 3.86 (dd, 2H, H-4 and H-8, J = 3.64, 9.24), 3.04 (m, 2H, H-1 and H-5) (Figure 25).
RESULTS

The structure was further confirmed by the study of $^{13}$C NMR (CDCl$_3$, 100 MHz, δ with TMS = 0) showed signals at C-4''',4'′(δ 147.98), C-3''',3′′(δ 147.12), C-1''',1′′(δ 135.04), C-6''',6′(δ 119.39), C-5''',5′(δ 108.21), C-2''',2′(δ 106.51), C-3′′′b,3′′b (δ 101.10), C-2,6 (δ 85.80), C-4,8 (δ 71.72), C-1,5 (δ 54.33) (Figure 26). The DEPT was performed to find out methine, methylene and methyl carbons. MS ES+: $m/z = 378 \ [M^++1+Na]^+$ (Figure 27).

Thus on the basis of above physical, chemical and spectral data compound B was characterized as tetrahydrofuran type of lignan named as sesamin (Figure 38a), having molecular formula C$_{20}$H$_{18}$O$_6$. 

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Figure 23: UV Spectra of Sesamin.
RESULTS

Figure 24: IR spectra of sesamin.
RESULTS

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Figure 25: $^1$H-NMR spectra of sesamin.
RESULTS

Figure 26: $^{13}$C-NMR spectra of sesamin.

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7.8.3 Compound C

Compound C was isolated as colorless crystals; 50 mg (0.6% w/w yield); \( R_f 0.54 \) (ethylacetate:toluene, 30:70); m.p. 116-118°C. Compound gave positive FeCl\(_3\) test and false positive test with Dragendorff’s reagent; UV \( \lambda_{\text{max}} \) (methanol): 281 nm (Figure 28); IR (KBr) showed str. for C–H at 2854.88 cm\(^{-1}\) for methoxy, C–O stretching at 1137.06 cm\(^{-1}\), 1238.93 cm\(^{-1}\) for ethers, C=C stretching at 1608 cm\(^{-1}\), 1504.15 cm\(^{-1}\), 1443.17 cm\(^{-1}\) and C-H stretching at 2925.38 cm\(^{-1}\) for aromatic ring (Figure 29).

Compound C showed \(^1\text{H NMR} \) (CDCl\(_3\), 400 MHz, \( \delta \) with TMS = 0) signals at 6.93 (s, 2H, H-2’ and H-2’’), 6.85 (s, 2H, H-5’’ and H-6’’), 6.81 (d, 1H, H-6’, \( J = 1.52 \)), 6.77 (d,
RESULTS

1H, H-5, $J = 7.92$), 5.95 (s, 2H, H-3’’b), 4.86 (d, 2H, H-6 and H-2, $J = 5.48$), 4.42 (d, 2H, H-4 and H-8 axial protons, $J = 9.6$), 4.1 (d, 2H, H-4 and H-8 equitorial protons), 3.91 (s, 3H, H-4’a), 3.88 (s, 3H, H-3’a), 2.87 (m, 2H, H-1 and H-5) (Figure 30).

$^{13}$C NMR (CDCl$_3$, 100 MHz, δ with TMS = 0) displayed signals at C-3’ (δ 148.85), C-4’’ (δ 148.02), C-4’ (δ 147.96), C-3’’ (δ 147.21), C-1’’ (δ 135.17), C-6’’ (δ 119.57), C-6’ (δ 117.71), C-5’ (δ 108.96), C-2’ (δ 108.16), C-3’’b (δ 101.05), C-6 (δ 87.68), C-8 (δ 71.01), C-4 (δ 69.76), C-4’a (δ 55.94), C-3’a (δ 55.91), C-1,5 (δ 50.16) (Figure 31). MS ES+: m/z 393 [M+Na]$^+$. So the mass of the compound was 370 (Figure 32).

Thus, on the basis of above physical, chemical and spectral data compound C was characterized as tetrahydrofuran type of lignan named as Kobusin (Figure 38b) having molecular formula C$_{21}$H$_{22}$O$_6$. 

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Figure 28: UV spectra of kobusin.

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Figure 29: IR spectra of kobusin.

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Figure 30: $^1$H-NMR spectra of kobusin.
RESULTS

Figure 31: $^{13}$C-NMR spectra of kobusin.

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Figure 32: Mass spectra of kobusin.

7.8.4 Compound D

Compound D was isolated as resinous yellow colored mass; 45 mg (0.56% w/w yield); R$_f$
0.41 (ethylacetate:toluene, 30:70); Compound gave FeCl$_3$ test and false positive test with
Dragendorff’s reagent. Various efforts were made to recrystallize but it fails and
compound was found to be hygroscopic as well as phenolic; UV \( \lambda_{\text{max}} \) (methanol): 279.8
nm, 241.6 nm (Figure 33); IR (KBr) showed O–H stretching at 3424.03 cm$^{-1}$ for
hydroxyl group, C–H stretching at 2847.67 cm$^{-1}$ for methoxy, C–O stretching at 1234.75
cm$^{-1}$, 1127.85 cm$^{-1}$ for ethers, C=C stretching at 1591.10 cm$^{-1}$ and C-H streching at
2924.88 cm$^{-1}$ for aromatic ring (Figure 34).

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$^1$H NMR (CDCl$_3$, 400 MHz, δ with TMS = 0) showed signals at 6.7-6.9 (m, 3H, H-2’’, H-5’’ and H-6’’), 6.5 (s, 2H, H-2’and H-6’), 4.7 (dd, 2H, H-6’ and H-2’, J = 6.96), 4.2 (m, 2H, H-4 and H-8 axial protons), 3.9 (s, 2H, H-4, H-8 equitorial protons of tetrahydrofuran rings), 3.85 (s, 6H, H-3’a and H-5’a), 3.84 (s, 3H, H-3’’), 3.90 (s, 3H, H-3’a), 3.11 (m, 3H, H-1, H-5) (Figure 35).

$^{13}$C NMR (CDCl$_3$, 100 MHz, δ with TMS = 0) shows signals at C-3’,5’ (δ 153.43), C-3’’ (δ 149.17), C-4’’ (δ 148.62), C-4’ (δ 137.39), C-1’ (δ 136.82), C-5’ (δ 133.47), C-1’’ (δ 133.38), C-6’’ (δ 118.51), C-5’’ (δ 110.96), C-2’’ (δ 109.14), C-6’ (δ 102.72), C-2,6 (δ 85.80), C-4,8 (δ 71.76), C-3’a (δ 55.95), C-5’a,3’’a (δ 55.92), C-4’’a (δ 54.09) (Figure 36).

Since $^1$H NMR shows signs for 4 protons indicating a symmetrical nature of the molecule with two phenyl ring in equatorial as also confirmed by their appropriate chemical shifts. The DEPT was performed to find out methine, methylene and methyl carbons. MS ES+: M$^+$ at 402.12 (Figure 37).

Thus, on the basis of above physical, chemical and spectral data compound D was characterized as 4’O demethyl magnolin (Figure 38c) having molecular formula C$_{21}$H$_{22}$O$_7$. This appears to be first report from a natural source.
RESULTS

Figure 33: UV spectra of 4’O demethyl magnolin.
RESULTS

Figure 34: IR spectra of 4’O demethyl magnolin.
RESULTS

Figure 35: $^1$H-NMR spectra of 4’O demethyl magnolin.

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Figure 36: $^{13}$C-NMR of 4’O demethyl magnolin.

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Figure 37: Mass spectra of 4’O demethyl magnolin.

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Figure 38: Structures of lignans isolated from petroleum ether extract of *Zanthoxylum alatum* stem bark.

### 7.8.5 Compound E

Compound E was isolated as yellow colored crystals, 45 mg (0.45% w/w yield); R<sub>t</sub> 0.46 (Chloroform: methanol, 90:10); m.p. 342-348°C. Compound gave intense yellow colour with dil. KOH. UV λ<sub>max</sub> (methanol): 268 and 332 nm (**Figure 39**). After addition of AlCl<sub>3</sub> λ<sub>max</sub> (methanol) shifts to 275 and 381 nm. The shift is stable after the addition of HCl; indicating the presence of OH at C-5 position. On addition of sodium acetate it showed the usual shift of benzoylate band indicating presence of OH’s at C-7 and C-4’ positions. IR (KBr, cm<sup>-1</sup>) spectrum showed stretching for –OH at 3284, for C=O at 1653.74, 1607.56 for aromatic, C-O stretching at 1354, C=C stretching at 1557.56 (**Figure 40**).
RESULTS

$^1$H NMR (DMSO, 400 MHz, δ with TMS=0) showed signals at 7.925 (d, 2H, H-2', H-6', $J = 8.8$), 6.8 (s, 1H, H-3), 6.927 (d, H-3',5', $J = 8.2$), 6.489 (d, 1H, H-8, $J = 2.1$), 6.199 (d, 1H, H-6, $J = 2.1$) (Figure 41).

$^{13}$C NMR (DMSO, 100 MHz, δ with TMS=0) displayed signals at C-4, >C=O (δ 181.72), C-8 (δ 164.09), C-2 (δ 163.71), C-5 (δ 161.41), C-7 (δ 161.13), C-6 (δ 157.27), C-2',6'(δ 128.45), C-1' (δ 121.13), C-3', 5' (δ 115.92) (Figure 42). MS ES-: $m/z$ 270 and $M^+$-2 at 268 (Figure 43).

Thus, on the basis of above physical, chemical and spectral data compound E was characterized as apigenin (Figure 47a) having molecular formula C$_{15}$H$_{10}$O$_{5}$.

Figure 39: UV spectra of apigenin.
RESULTS

Figure 40: IR spectra of Apigenin.
Figure 41: $^1$H-NMR spectra of apigenin.
Figure 42: $^{13}$C-NMR of apigenin.
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Figure 43: Mass spectra of apigenin.

7.8.6 Compound F

Compound F was isolated as pale yellow colored crystals, 35 mg (0.35% w/w yield); Rf 0.34 (Chloroform: methanol, 90:10); m.p. 268-271°C; UV $\lambda_{\text{max}}$ (methanol): 266 and 351 nm (Figure 44). After addition of AlCl$_3$ $\lambda_{\text{max}}$ (methanol) shifts to 270 and 390 nm. The shift is stable after the addition of HCl; indicating the presence of OH at C-5 position.

$^1\text{H NMR}$ (DMSO, 400 MHz, $\delta$ with TMS=0) showed signals at 8.0 (d, 2H, H2’, H6’, $J$ = 8.68 Hz), 7.3 (d, 2H, H5’, H3’, $J$ = 8.68 Hz), 6.1 (s, 1H, H6), 6.3 (s, 1H, H8), 5.3 (s, 1H, H1”; glucose anomeric proton), 4.47-5.22 (m, remaining sugar proton) (Figure 45). The sugar was identified after hydrolysis by boiling with 2% HCl followed by basification. Then it was concentrated and run on paper chromatography ($n$-butanol:acetic acid:water, 4:1:5) and compared with standard glucose. Thus identified as
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glucose. Mass spectral analysis of Compound F showed intense peak at 286 of aglycone along with major fragments \(m/z\) at 152 \([M^+ - C_7H_4O_4]\), 134 \([M^+ - C_8H_6O_2]\) and one sugar attached making it monosaccharide i.e. \([M^+ + 1]\) at \(m/z\) 385. Since flavonoid glycoside is fragile molecule, we could not get a molecular ion peak at \(m/z\) 448. However, it appears that fragmentation pattern shows the loss of two molecules of water (2x18) and CO (28) as indicated by fragment at \(m/z\) 385 \([M^+ + 1]\) (Figure 46).

Thus, on the basis of above physical, chemical and 7l9spectral data compound B was characterized as \textbf{Kaempferol-7-O-glucoside} (Figure 47b) having molecular formula \(C_{21}H_{20}O_{11}\).

![Figure 44: UV spectra of Kaempferol-7-O-glucoside.](image-url)
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Figure 45: $^1$H-NMR spectra of Kaempferol-7-O-glucoside.
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Figure 46: Mass spectra of Kaempferol-7-O-glucoside.

Figure 47: Structures of flavonoids isolated from ethyl acetate extract of Zanthoxylum alatum stem bark.
RESULTS

Table 12: Characteristic features of isolated phytoconstituents from the petroleum ether and ethyl acetate extract of *Zanthoxylum alatum* stem bark

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Compound</th>
<th>Molecular formula</th>
<th>Melting point</th>
<th>Rf value</th>
<th>Chemical test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>β-sitosterol</td>
<td>C&lt;sub&gt;29&lt;/sub&gt;H&lt;sub&gt;50&lt;/sub&gt;O</td>
<td>136-138°C</td>
<td>0.55</td>
<td>50% H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt; test</td>
</tr>
<tr>
<td>2.</td>
<td>Sesamin</td>
<td>C&lt;sub&gt;20&lt;/sub&gt;H&lt;sub&gt;18&lt;/sub&gt;O&lt;sub&gt;6&lt;/sub&gt;</td>
<td>120-122°C</td>
<td>0.74</td>
<td>Dragendorff’s and FeCl&lt;sub&gt;3&lt;/sub&gt; test</td>
</tr>
<tr>
<td>3.</td>
<td>Kobusin</td>
<td>C&lt;sub&gt;21&lt;/sub&gt;H&lt;sub&gt;22&lt;/sub&gt;O&lt;sub&gt;6&lt;/sub&gt;</td>
<td>116-118°C</td>
<td>0.54</td>
<td>Dragendorff’s and FeCl&lt;sub&gt;3&lt;/sub&gt; test</td>
</tr>
<tr>
<td>4.</td>
<td>4’O demethyl magnolin</td>
<td>C&lt;sub&gt;22&lt;/sub&gt;H&lt;sub&gt;26&lt;/sub&gt;O&lt;sub&gt;7&lt;/sub&gt;</td>
<td>-</td>
<td>0.41</td>
<td>Dragendorff’s and FeCl&lt;sub&gt;3&lt;/sub&gt; test</td>
</tr>
<tr>
<td>5.</td>
<td>Apigenin</td>
<td>C&lt;sub&gt;15&lt;/sub&gt;H&lt;sub&gt;10&lt;/sub&gt;O&lt;sub&gt;5&lt;/sub&gt;</td>
<td>342-348°C</td>
<td>0.46</td>
<td>Dil. KOH test</td>
</tr>
<tr>
<td>6.</td>
<td>Kaempferol-7-O-glucoside</td>
<td>C&lt;sub&gt;21&lt;/sub&gt;H&lt;sub&gt;20&lt;/sub&gt;O&lt;sub&gt;11&lt;/sub&gt;</td>
<td>268-271°C</td>
<td>0.34</td>
<td>Shinoda test</td>
</tr>
</tbody>
</table>

7.9 CYTOTOXIC POTENTIAL OF ISOLATED COMPOUNDS

Compounds isolated from petroleum ether and ethyl acetate extracts also subjected to MIA-PaCa and A-549 cancer cell lines for checking their cytotoxic potential. Isolated compounds has also shown cytotoxic potential in different ranges but the most potent inhibition of cell proliferation was observed with lignan 4’O demethyl magnolin in both cell lines. So, only 4’O demethyl magnolin was subjected to apoptosis study. The isolated...
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compounds showed more cytotoxicity than extracts (Figures 48, 49). IC₅₀ values of isolated compounds are shown in Table 13.

Figure 48: Effects of β-sitosterol, sesamin, kobusin, 4’O demethyl magnolin, apigenin and kaempferol 7-O-glucoside from Zanthoxylum alatum stem bark on MIA-PaCa human Pancreatic cancer cell line. Values were expressed as mean ± SD.

*Isolation and Characterization of Anticancer Principles from Zanthoxylum alatum Roxb.*
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Figure 49: Effects of β-sitosterol, sesamin, kobusin, 4’O demethyl magnolin, apigenin and kaempferol 7-O-glucoside from Zanthoxylum alatum stem bark on A-549 human Lung cancer cell lines. Values were expressed as mean ± SD.
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Table 13: IC\textsubscript{50} values of isolated compounds on A-549 and MIA-PaCa cancer cell lines

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of sample</th>
<th>IC\textsubscript{50} value ((\mu)g/ml)</th>
<th>A-549 cell line</th>
<th>MIA-PaCa cell line</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>(\beta)-sitosterol</td>
<td>112.33 ± 3.78</td>
<td>138.00 ± 2.64</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Sesamin</td>
<td>37.46 ± 1.09</td>
<td>34.04 ± 1.76</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Kobusin</td>
<td>34.71 ± 2.33</td>
<td>32.86 ± 2.02</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>4'O demethyl magnolin</td>
<td>26.47 ± 1.87</td>
<td>21.72 ± 1.50</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Apigenin</td>
<td>53.66 ± 4.72</td>
<td>44.33 ± 1.52</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Kaempferol-7-O-glucoside</td>
<td>35.34 ± 3.51</td>
<td>48.43 ± 2.08</td>
<td></td>
</tr>
</tbody>
</table>

7.10 APOPTOSIS STUDY

To find out whether the inhibition of cell proliferation by the most potent compound 4’O demethyl magnolin (compound D) was because of the induction of apoptosis, it was subjected to acridine orange/ethidium bromide method. MIA-PaCa cells were exposed to 4’O demethyl magnolin and after one day of exposure the cells were treated with acridine orange/ethidium bromide to determine the apoptosis. Fluorescence microscopy images showed the number of alteration in the morphology of cell such as reduction in its size and volume, contraction of cell, blebbing of cell membrane, chromatin condensation, fragmentation of nucleus and development of apoptotic bodies of the cell which were treated (Figure 50). Results of AO/EB clearly indicates that 4’O demethyl magnolin...
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enhance the apoptosis at IC$_{50}$ dose (21.72 µg/ml), however showing necrotic cell death at higher dose after 24 h.

Figure 50: Micrographs (Magnificationx400) of acridine orange/ethidium bromide stained MIA-PaCa (human pancreatic cancer cells); A: cells without drug (control) have normal nucleus of green colour representing live cells B: cells exposed to IC$_{50}$ drug (21.72 µg/ml) for 24 hrs. shows bright green nucleus; condensed or fragmented chromatin signifying apoptosis, C: cells exposed to highest dose (150 µg/ml) for 24 hours have a structurally normal red coloured nucleus indicating cell necrosis.