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# ISOLATION AND CYTOTOXIC POTENTIAL OF SILVER NANOSUSPENSION OF THE ROOTS OF *APHANAMIXIS POLYSTACHYA*

R. Gude *, A. Joshi, A. Bhandarkar and D. Mayenkar

Goa College of Pharmacy, 18th June Road, Panaji - 403001, Goa, India.

**Keywords:**

*Aphanamixis Polystachya*, Isolation, Herbal mediated Silver Nanosuspension, SRB Assay, cytotoxic activity

**Correspondence to Author:**
**R. Gude**

Associate Professor,
Goa College of Pharmacy, 18th June Road, Panaji - 403001, Goa, India.

**E-mail:** rajigude@yahoo.com

**ABSTRACT: Objective:** The study was aimed to isolate the components from the methanolic extract of the root bark of *Aphanamixis polystachya* and to confirm their potential cytotoxicity of the methanolic extract, silver nanoparticles and silver nanosuspension of the root bark of *A. polystachya*. **Method:** Isolation from the methanolic extract of root barks of *A. polystachya* were performed using column chromatography technique. Cytotoxic activity on MCF-7 and MDA-MB cell lines by SRB Assay was conducted on silver nanosuspension of *A. polystachya*. **Results:** The components isolated from methanolic extract of root bark of *A. Polystachya* by column chromatography were confirmed as Rohituka 7, Rohituka 3, Amoorinin-3-o-α-l-Rhamnopyranosyl-(1→6)–β-D-Glucopyra-noside and 8-Methyl-7, 2′,4′-tri-O-Methylflavonone-5-O-α-L-Rhamnopyranosyl (1→4)-β-D- glucopyranosyl-(1→6)-β-D-glucopyranoside on the basis of IR, $^{1}$HNMR, $^{13}$C NMR and MS. The prepared silver nanosuspension, tested in concentrations ranging between 500-7.81 µM showed $IC_{50}$ value at 0.58 µM in MCF-7 cell line, and $IC_{50}$ in MDA-MB cell line being greater than 1000µM. **Conclusion:** Column chromatography of the methanolic extract of root barks of *A. polystachya* led to the isolation of Rohituka 7, Rohituka 3, Amoorinin-3-O-α-L-Rhamnopyranosyl-(1→6)–β-D Glucopyranoside and (methyl-7,2′,4′-tri-O-methyl flavonone-5-O-α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside. SRB Assay confirmed that silver nanosuspension of *A. polystachya* to be potentially cytotoxic. The isolated components have already proven to possess cytotoxic activity. Hence, the study suggests that silver nanosuspension exhibited better cytotoxic activity.

**INTRODUCTION:** Cancer is a major health problem globally, affecting 15% of the population. It is an uncontrolled growth and quick division of the abnormal cells in the body. It is projected that by 2020, the incidence of cancer levels will increase to 15 million cases, causing deaths [1].

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In the treatment of cancer, many synthetic and chemotherapeutic agents have been developed, having various side effects. Many of the plants traditionally have reported possessing antitumor activity [1].

The medicinal plant chosen for the work is *A. polystachya*, which is a highly valued species for mankind and has been thoroughly investigated for its high potential medicinal value [2]. The design and development of herbal nanoparticles have become frontier research in the field of nanotechnology. Development of this novel drug delivery system will help in overcoming various constraints like

bioavailability, solubility and stability of the herbal drug [3]. The present study is to isolate the components from the methanolic extract of the root bark of the plant *A. polystachya* having potential cytotoxic activity. The extract was formulated into silver nanoparticles and subsequently into silver nanosuspension and assessed for cytotoxic activity by SRB method.

## MATERIALS AND METHODS:

**Authentication of the Plant:** The root bark of *A. polystachya* were identified and obtained from Mangalore, Dakshina Karnataka, India. The plant part was identified and authenticated by Dr. Gopal Krishna Bhatt, Poorna Prajnya College Udupi-Karnataka, India, and Dr. Dinesh Nayak, Mangalore-Karnataka, India bearing number: GCP.Pharmacog.05/2013. The herbarium was deposited in the Department of Pharmacognosy, Goa College of Pharmacy.

**Description of the Plant:** *A. polystachya* is an evergreen medium-sized tree with a dense spreading crown and a straight cylindrical bole up to 15m in height and 1.5-1.8 m in width belonging to the family Meliaceae. In Sanskrit, it is known as Anavallabha, Ksharayogya, Lakshmi, Lakshmivana, Lohita [4].

| | | |
|---|---|---|
| Kingdom | : | Plantae |
| Phylum | : | Magnoliophyta |
| Class | : | Magnoliospida |
| Order | : | Sapindales |
| Family | : | Meliaceae |
| Genus | : | Aphanamixis |
| Species | : | *Aphanamixis polystachya* |
| Botanical Name | : | *Aphanamixis polystachya* (Wall) R.N. Parker |
| Synonyms | : | *Algaia polystachya* Wall & *Amoora rohituka* (Roxb.) Wt. & Arn. |

**Distribution:** *A. polystachya* is native to temperate Asia, tropical China, Indian subcontinent-Peninsula, Northeast India, Bhutan, Sri Lanka, Myanmar, Thailand, Malaysia, Indonesia, Papua, New Guinea, and the Philippines. In India, it is found distributed in the Sub-Himalayan tract from the Rapti river eastwards, Sikkim up to 6000 ft., Assam, Burma, Chota Nagpur, Konkan, W. Ghats, and adjoining hill ranges from the Poona district southwards to Tinnevelly up to 3500 ft [5].

**General Experimental Procedure:** Roots, along with the bark, were collected, washed, and dried in the shade. The dried material was then powdered (500 g) and exhaustively extracted by maceration with 3 liters of methanol for 3 days. After 3 days, the methanolic layer was decanted off. The process was repeated thrice. The solvents from the total extract were distilled off using a rotary vacuum evaporator (Roteva) and concentrated to a syrupy consistency and then evaporated to dryness (150g).

**Preliminary Phytochemical Screening (Qualitative Analysis):** The preliminary phytochemical studies were performed to confirm the availability of different phytoconstituents present in the methanolic extract [2, 6].

**Synthesis of Herbal mediated Silver Nanoparticles of *A. polystachya*:** The $10^{-3}$ mM Silver nitrate solution was prepared. 10 ml of herbal extract was taken in 250 ml conical flask/ beakers separately, and to this 90 ml of $AgNO_3$ solution was added. The conical flasks were incubated at room temperature. A color change of the leaf extracts from pale yellow to dark brown was checked periodically. The brown color formation indicates that the silver nanoparticles were synthesized from the herbal extract, and they were centrifuged at 5000 rpm for 15 min in order to obtain the pellet that is used for further study. The supernatant was used for characterization [6].

**Extraction and Isolation of Components from the Methanolic extract of the Roots of *A. polystachya*:** The methanol soluble fraction (150g) was suspended in 1.5 L of water and extracted with petroleum ether (60:80) to remove the fatty components completely. The defatted crude extract was further partitioned with chloroform (3 × 1L) to give a chloroform soluble fraction (90g). 90g of chloroform fraction was mixed with silica gel (30g, #60-120). The sample was loaded on a column packed with 500g of silica gel (Molychem, #60 × 120) prepared in petroleum ether (60-80). The column was subjected to elution with different solvent system starting first with 100% petroleum ether followed by petroleum ether: ethyl acetate graded mixtures (95:5, 90:10, 80:20, 70:30, 50:50), 100% ethyl acetate and finally with graded mixtures of ethyl acetate: methanol (99:1, 98:2, 97:3, 96:4, 95:5).

The elutions were monitored by TLC (silica gel G, visualization by UV at 254nm, 366nm and vanillin sulphuric acid reagent heated at 110 °C). Each time 10ml of elutes were collected and identical elutes were combined (TLC monitored) and concentrated to 5ml and kept aside. The elutions carried out with petroleum ether: ethyl acetate (90:10 to 50:50) resulted in 4 fractions containing mixture of compounds and having identical $R_f$ values (7g).

7g was mixed with flash grade silica (3g, #200-400) using mortar and pestle. This mixture was subjected for rechromatography with column (1ft in length and 2cm i.d.). The sample was loaded onto a column packed with 150g of flash grade silica (Molychem, #60 × 120). The mixture was loaded onto this column and the elutions were carried out with 100% petroleum ether, petroleum ether: ethyl acetate graded mixture (90:10, 85:50, 80:20, 75:25, 70:30, and 65:35). The elutions were monitored by TLC (silica gel G, visualization by UV at 254nm, 366nm and vanillin sulphuric acid reagent heated at 110 °C). Each time 10ml of the elutes were collected and identical elutes were combined (TLC monitored) and concentrated to 5ml and kept aside. The elutions carried out with petroleum ether: ethyl acetate (80:20) resulted in a single spot on TLC prepared in petroleum ether (80:20).

After removing the solvent, a light yellow solid was obtained which was designated as RG-APE2 (78mg). The elutions carried out with petroleum ether: ethyl acetate (65:35) resulted in a single spot on TLC prepared in petroleum ether: ethyl acetate (65:35). After removing the solvent, a light-yellow solid was obtained which was designated as RG-APE1 (74 mg). Elutions carried out with 100% ethyl acetate, ethyl acetate: methanol (99:1 and 98:2) resulted in a mixture of compounds having identical $R_f$ values [TLC monitored, ethyl acetate: methanol (98:2)]. After removing the solvent, a light brown amorphous powder resulted (185 mg).

**Purification of Compounds using Flash Chromatography (Combi Flash Chromatograph):** 185 mg of the powder was taken and mixed by triturating with flash grade silica (600 mg, #200-400) using mortar and pestle. Pre-packed silica column (Redisep RF, 1 gm) was used. All the parameters were set and monitored using peak track software.

The compounds which are UV absorbing *i.e.* at 254 nm and 366 nm, are only detected by flash chromatography. The elutions were carried out with ethyl acetate: methanol (98:2) and collected in a test tube. Each time, 8ml of elutes were collected and identical elutes were combined (TLC monitored, ethyl acetate: methanol 98:2), concentrated and kept aside. The test tubes from 3-18 resulted in a single spot on TLC (ethyl acetate: methanol, 98:2).

After removing the solvent, light brown flakes resulted and the compound was designated as RG-APE3 (68 mg). The test tubes from 22-44 resulted a single spot on TLC (ethyl acetate: methanol, 98:2). After removing the solvent, it resulted in a light yellow powder and the compound was designated as RG-APE4 (77 mg). The elutions carried out with other solvent systems resulted a mixture of compounds or resinous mass, which were not processed further.

**Preparation of Silver Nanosuspension of *A. polystachya*:** Silver Nanosuspension of *A. polystachya* (AgNS) was prepared by nano-precipitation method. The prepared *A. polystachya* silver nanoparticles (10 mg) were added to sodium lauryl sulphate (0.125%) in 10 ml of deionised water with continuous stirring at 500 rpm for 1 h. The solvent was allowed to evaporate to a dry nanosuspension [7].

**Cytotoxic Assay of Nanosuspension of *A. polystachya*:** The $IC_{50}$ of AgNS was determined by performing the Sulforhodamine B (SRB) Assay for cytotoxicity. The SRB assay was performed according to the described method [7, 8, 10] with slight modifications using concentrations of compounds ranging from 500-7.81 µM.

Absorbance was read at 540 nm on a scanning multi-well plate reader (EL × 800, BioTek Instruments Inc., Winooski, VT, USA), the percentage cell viability was calculated using excel sheet and $IC_{50}$ values were determined using graph pad prism. All the experiments were conducted in triplicates. The percentage cell death was calculated using the formula:

% Cell death = [(OD of control − OD of test) / (OD of control)] × 100

**Characterization of Isolated Compounds:**
**Spectral Data:**

**RG-APE 1:** Light yellow solid; $R_f$ = 0.060 (solvent system $CHCl_3$: EtOAc 80:20); m.p.: 123 °C;

**IR (KBr):** 3334.92 $cm^{-1}$, 2924.25 $cm^{-1}$, 1724.36 $cm^{-1}$, 1442.75 $cm^{-1}$, 1377.17 $cm^{-1}$, 1132.21 $cm^{-1}$.

**$^1$HNMR ($CDCl_3$):** δ 7.5509 (d, 1H, H-1), δ 6.4724 (s, 1H, H-2), δ 2.1816 (d, 1H, H-5), δ 2.5108 (s,1H, H-6),δ 3.1835 (s, 1H, H-9), δ 5.1943 (s,1H, H-11),δ 5.8707 (t,1H, H-12), δ 5.5848 (d,1H, H-13), δ 2.0304 (t,1H, H-16), δ 3.7364 (t,1H, H-17), δ 0.9843 (s, 3H, H-18), δ 1.0638 (s,3H, H-19), δ 7.3975 (d,1H, H-21), δ 6.8561 (s, 1H, H-22),δ 7.3869 (s, 1H, H-23), δ 1.6665 (s,3H, H-28),δ 4.8607 (d, 1H, -H29), δ 5.2234 (s, 1H, H-30), δ 3.4367 (s, 1H, H-2'), δ 1.4091 (s, 1H, H-3'), δ 1.2339 (d, 1H, H-4'), δ 0.7841 (t, 1H, H-5'), δ 0.8359 (d, 3H, H-3'-Me),δ 8.1664 (s, 1H, COOH), δ 2.0470 (s, 3H, $CH_3COO$).

**$^{13}$CNMR ($CDCl_3$):** δ 153.08 (C-1), δ 122.42 (C-2), δ 169.0 (C-3), δ 81.93 (C-4), δ 74.06 (C-5), δ 31.25 (C-6), δ 174.83 (C-7), δ 138.50 (C-8), δ 50.70 (C-9), δ 39.55 (C-10), δ 74.06 (C-11), δ 80.37 (C-12), δ 61.20 (C-13), δ 81.90 (C-14),δ 72.30 (C-15), δ 39.34 (C-16), δ 39.14 (C-17), δ 11.53 (C-18), δ 28.67 (C-19), δ 123.05 (C-20) δ 110 (C-22), δ 143.0 (C-23), δ 28.90 (C-28), δ 74.06 (C-29), δ 119.30 (C-30), δ 174.83 (C-1'), δ 74.06 (C-2′), δ 39.01.25 (C-3′), δ 22.06 (C-4′), δ 11.39 (C-5′), δ 15.30 (C-3′-Me), δ 150.08 (C-HCOO), δ 159.08 (C-$CH_3COO$), δ 20.7 (C-$CH_3COO$).

**Mass Spectra: (LC-MS):** $C_{35}H_{44}O_{13,}$ mol. wt: 672; LC-MS (m/z): 672.16 $(M+)^+$. The other peaks appeared at 672.27, 643.28, 511.20, 451.18, 433.17, 339.12, 269.12, 165.07, 152.08. From the m.p, IR, $^1$HNMR, $^{13}$CNMR, and MS, compound RG-APE1 was designated as Rohituka 7.

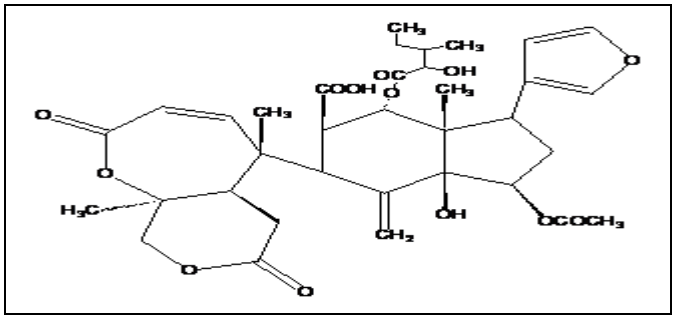

**FIG. 1: CHEMICAL STRUCTURE OF RG-APE1 (ROHITUKA 7)**

**RG-APE2:** Light yellow solid; $R_f$ value: 0.065 (solvent system $CHCl_3$: EtOAc 80:20); m.p.: 122.4 °C; positive response for Liebermann- Burchard test for triterpenoids.

**IR (KBr):** 3389.10 $cm^{-1}$, 2931.25 $cm^{-1}$, 1741.57 $cm^{-1}$, 1448.90 $cm^{-1}$, 1379.09 $cm^{-1}$, 1031.08 $cm^{-1}$.

**$^1$HNMR ($CDCl_3$):** δ 3.7364 (t, 1H, H-1), δ 2.9528 (d, 1H, H-2), δ 2.3029 (d 1H, H-5), δ 2.8218 (m, 1H, H-6), δ 3.1835 (s, 1H, H-9), δ 4.8607 (s, 1H, H-11), δ 5.8708(t,1H, H-12), δ 2.841, 2.3360 (t,1H, H-16), δ 3.7277 (t,1H, H-17), δ 0.8596 (s,1H, H-18), δ 1.1986 (s, 3H, H-19), δ 7.3975 (d,1H, H-21), δ 6.4724 (s,1H, H-22), δ 7.5509 (s, 1H, H-23),δ 1.6665 (s, 1H, H-28), δ 4.9521, 3.7227 (t,1H, H-29),δ 6.8561(s, 1H, H-30), δ 3.4367 (s, 1H, H-2'), δ 1.7908 (d, 1H, H-3'), δ 1.1606, 0.9428 (t, 1H, H-4'), δ 0.6985 (s, 1H, H-5'), δ 0.8934 (t, 3H, H-3'-Me).

**$^{13}$CNMR ($CDCl_3$):** δ 78.26 (C-1), δ 28.98 (C-2), δ 167.06 (C-3), δ 80.37 (C-4), δ 39.34 (C-5), δ 31.25 (C-6), δ 169.86 (C-7), δ 123.05 (C-8), δ 54.70 (C-9), δ 50.70 (C-10), δ 74.06 (C-11),δ 78.26 (C-12), δ 39.55 (C-13), δ 81.93 (C-14),δ 204.08 (C-15), δ 39.14 (C-16), δ 28.67 (C-17), δ 15.30 (C-19), δ 119.32 (C-20), δ 139.55 (C-21) δ 111.22 (C-22), δ 143.20 (C-23), δ 22.06 (C-28), δ 74.06 (C-29), δ 122.42 (C-30), δ 174.83 (C-1'), δ 74.06 (C-2′), δ 31.25 (C-3′), δ 20.70 (C-4′), δ 11.39 (C-5′), δ 11.53 (C-3′-Me).

**Mass Spectra (LC-MS):** $C_{32}H_{40}O_{11}$, Mol. wt: 600, $(M+)^+$. The other peaks appeared at 599.36, 557.35, 497.33, 479.32, 407.26, 189.07, 152.06. From the m.p, IR, $^1$HNMR, $^{13}$CNMR and Mass spectra, compound RG-APE2 was designated as Rohituka 3.

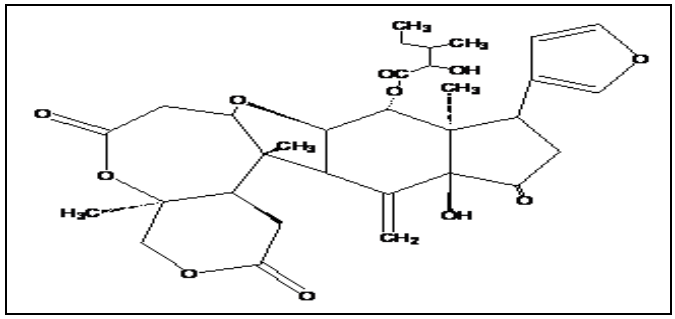

**FIG. 2: CHEMICAL STRUCTURE OF RG-APE2 (ROHITUKA 3)**

**RG-APE3:** Light brown flakes; $R_f$ value: 0.371 (solvent system 100% EtOAc); m.p.: 189 °C;

positive response for Molisch's test for carbohydrates and belongs to the limonoid class.

**IR (KBr):** 3456.11 $cm^{-1}$, 1745.0 $cm^{-1}$, 1641.22 $cm^{-1}$, 820.39 $cm^{-1}$, 860.37 $cm^{-1}$, 1244.4 $cm^{-1}$, 893.29 $cm^{-1}$, 820.39 $cm^{-1}$

**$^{1}$HNMR (DMSO):** δ 0.96 - δ 1.05 (s, 12H, H-18, H-19,H-24, H-25), δ 1.06, 1.33, 1.40,1.17 (s,4H, H-11,12), δ 2.26(d,1H, H-5), δ 2.27 (s,2H, H-6),δ 2.28 (s,1H, H-9), δ 3.39 (s,1H, H-3),δ 3.78 (s,3H, H-27), δ 4.88 (s,1H, H-15),δ 4.91, δ 5.09 (each s,2H H-26), δ 5.63 (s,1H, H-17), δ 5.68 (s,1H, H-1),δ 5.86 (s,1H, H-2,), δ 6.43-δ 7.59 (m,3H, H-21,22,23 of furan), δ 1.27 (s, 3H, H-6″), δ 3.80- δ 4.49(m, 4H, H-2′,3′,4′5′), δ 4.52 (s,1H, H-1″), δ 4.54- δ 4.68(m,4H, H-2″,3″,4″,5″),δ 4.70-4.87 (m,6H, H-2′,3′,4′,2″,3″,4″-OH), δ 4.93 (s,2H, H-6′), δ 5.13 (s,1H, H-1′).

**$^{13}$CNMR (DMSO):** δ 136.14 (C-1), δ 124.59 (C-2), δ 105.44 (C-3), δ 37.51 (C-4), δ 39.93 (C-5), δ 39.51 (C-6), δ 172.93 (C-7), δ 149.50 (C-8), δ 40.14 (C-9), δ 28.96 (C-10),δ 27.21 (C-11), δ 28.96 (C-12), δ 39.72 (C-13),δ 78.50 (C-14),δ 59.64 (C-15), δ 170.14 (C-16), δ 86.50 (C-17), δ 11.41 (C-18), δ 14.00 (C-19), δ 120.14 (C-20) δ 101.59 (C-21), δ 140.16 (C-22) δ 142.93 (C-23), δ 20.66 (C-24), δ 20.41 (C-25), δ 114.14 (C-26) δ 49.85 (C-27), δ 110.16 (C-1′), δ 90.50 (C-2″), δ 70.14 (C-3′), δ 79.03 (C-4′), δ 79.16 (C-5′) δ 39.30 (C-6′), δ 112.12 (C-1″) ,δ78.83 (C-2′) δ 87.59 (C-3″), δ 88.03 (C-4″) δ 68.16 (C-5″), δ 15.44 (C-6″).

**Mass Spectra (ESI-MS):** $C_{39}H_{54}O_{16}$, mol.wt.: 778.84, 778.1 $(M+)^{+}$. The other peaks appeared at 712.4, 677, 623.3, 560.4, 482, 390.7, 278.8, 144.9.

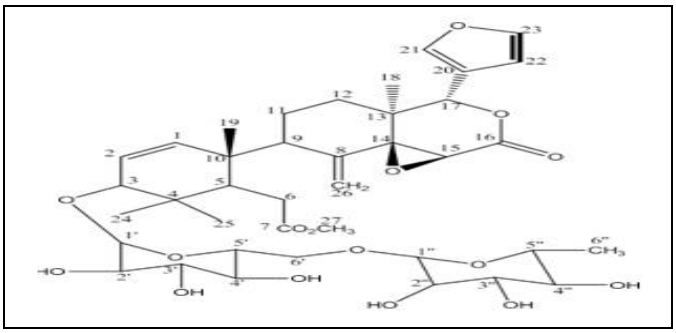

**FIG. 3: CHEMICAL STRUCTURE OF COMPOUND RG-APE3(Amoorinin-3-O-α-L-Rhamnopyranosyl-(1,6) –β-D- Glucopyranoside)**

From the m.p., IR, $^{1}$HNMR, $^{13}$CNMR, and MS, compound RG-APE3 was designated as Amoorinin-3-O-α-L-Rhamnopyranosyl–(1→6) –β-D-Glucopyranoside.

**RG-APE4:** Yellow Solid; $R_f$ value: 0.06 (solvent system $CHCl_3$: EtOAc 60:40); m.p.: 212 °C; positive test for flavanone glycoside.

**IR (KBr):** 3447.18 $cm^{-1}$, 2963.12 $cm^{-1}$, 2877.24 $cm^{-1}$, 1644.29 $cm^{-1}$, 1461.18 $cm^{-1}$, 1377.10 $cm^{-1}$, 1234.4 $cm^{-1}$, 1147.6 $cm^{-1}$, 800.4 $cm^{-1}$, 828.41 $cm^{-1}$.

**$^{1}$HNMR (DMSO):** δ1.24 (s, 3H, H-6‴- Me), δ 2.13 (s, 3H, H-8 Me), δ 2.53 (s,1H, H-5″), δ 3.39 (s,1H, H-3″), δ 3.58 (s,2H, H-6″), δ 3.69 (s,2H, H-6‴), δ 3.72 (s,1H, H-2⁗), δ 3.77 (s,2H, H-3), δ 3.82-3.87 (m,9H, H-2″,3″,4″,2‴,3‴,6‴,2⁗,3⁗4⁗-OH), δ 3.80, 3.93, 4.00 (s, 9H, H-2′, 4′, 7-3xOMe) δ 6.04 (d, 1H, H-1″), δ 6.05 (d, 1H, H-1‴), δ 5.07 (d,1H, H-1⁗), δ 5.38 (m,1H, H-2), δ 6.63 (s,1H, H-6), δ 6.95 (s,1H, H-3′), δ 7.30 (d,1H, H-5′), δ 7.32 (d,1H, H-6′), δ 4.02 (s,1H, H-2″), δ 4.10 (s,1H, H-4″), δ 4.20 (s,1H, H-2‴), δ 4.54 (s,1H, H-3‴), δ 4.55 (s,1H, H-3⁗), δ4.56 (s,1H, H-4‴), δ 4.58 (d, 1H, H-4⁗), δ 5.04 (d, 1H, H-5‴), δ5.07 (m, 1H, H-5⁗).

**$^{13}$CNMR (DMSO):** δ 83.03 (C-2), δ 40.15 (C-3), δ 195.15 (C-4), δ 102.89 (C-4a), δ 164.86 (C-5) δ 130.13 (C-6), δ 168.37 (C-7), δ 115.37 (C-8) δ 165.67 (C-8a), δ 11.68 (C-8-Me) δ 129.13 (C-1′),δ 155.67 (C-2′), δ 114.86(C-3′),δ 159.03 (C-4′) δ 116.52 (C-5′), δ119.03 (C-6′), δ 55.67, 56.62, 50.15 (C-2′,4′,7-$OCH_3$ x3), δ 103.03 (C-1″), δ 78.37 (C-2″), δ 39.52 (C-3″), δ 31.94 (C-4″), δ 81.30 (C-5″), δ 38.89 (C-6″), δ 94.71 (C-1‴), δ 39.94 (C-2‴), δ 79.03 (C-3‴), δ 31.30 (C-4‴), δ 28.71 (C-5‴), δ 25.02 (C-6″), δ 99.94 (C-1⁗), δ 39.73 (C-2⁗), δ 39.31 (C-3⁗), δ 29.03 (C-4⁗), δ 28.62 (C-5⁗), δ 18.79 (C-6⁗).

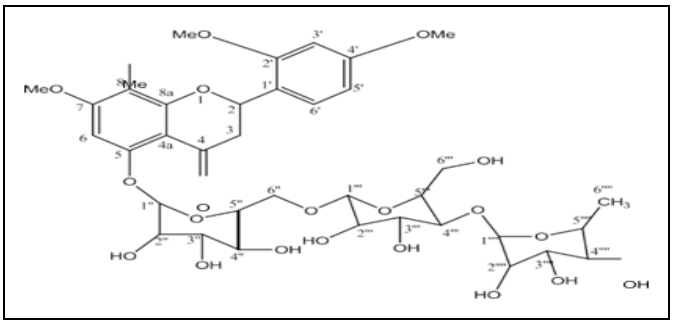

**FIG. 4: CHEMICAL STRUCTURE OF COMPOUND RG-APE4 – (methyl-7,2′,4′-tri-O-methyl flavonone-5-O-α-L-rhamnopyranosyl (1→4)-β-D-glucopyranosyl-(1→ 6)-β-D-glucopyranoside)**

M**ass Spectra (ESI-MS):** $C_{37}H_{50}O_{20}$, mol. wt.: 814.78, 816.1 (M+, 2H) $^{+}$. The other peaks are observed at 751.1, 572.4, 332.7, 159.8, 145.9, 128, 113.9. From the m.p., IR, $^{1}$HNMR, $^{13}$CNMR and MS, compound RG-APE4 was designated as 8-methyl- 7, 2′, 4′- tri- O- methylflavonone-5-O-α-L-rhamno-pyranosyl (1 4)-β-D-glucopyranosyl-(1 6)-β-D-glucopyranoside.

**RESULTS:** Extensive column chromatography isolated four compounds, Rohitika 7 **Fig. 1**, Rohituka 3 **Fig. 2**, Amoorinin-3-O-α-L-Rhamno-pyranosyl-(1→6) –β-D- Glucopyranoside **Fig. 3** and Methyl-7,2′,4′-tri-O-methyl flavonone-5-O-α-L-rhamnopyranosyl (1→4)- β- D-glucopyranosyl-(1→6)-β-D-glucopyranoside **Fig. 4**. The structures of the isolated compounds were established by interpretation of their spectroscopic data, by IR, $^{1}$HNMR, $^{13}$CNMR and MS with those published in the reference papers.

The formulated *A. polystachya* nanosuspension was tested in concentration ranges between 500-7.81 µM on MCF-7 and MDA-MB cell lines. It was found that the $IC_{50}$ value obtained was 0.58 µM in MCF-7 cell line, and the $IC_{50}$ in MDA-MB is greater than 1000.

**DISCUSSION:** In this study, an attempt was made to isolate the components from the methanolic extract of the root bark extract of *A. polystachya.*

The compound RG-APE1 showed an m.p. at 123.0 °C and positive response for Liebermann- Burchard test for triterpenoids. The IR spectrum showed a strong absorption at 3334.92 $cm^{-1,}$ indicating the presence of the hydroxyl group. The band at 2924.25 $cm^{-1}$ indicated C-H stretching in $CH_3$. The prominent peak at 1724.36 $cm^{-1}$ shows carbonyl stretching of ester. The $^{1}$HNMR displayed exhibited a singlet at δ 5.2234, indicating methyl protons at C-30. The doublet at δ 7.3975 (C-21) and two singlets at δ 6.8561 (C-22) and δ 7.3869 (C-23) indicated the presence of protons in the furan moiety. The peak at δ 8.1664 indicated the presence of the carboxylic group. A singlet at δ 2.0470 indicated three methyl protons of $CH_3COO$. A singlet at δ 3.4367 indicated the presence of proton at C-2'. The value at δ 1.4091 appeared as a singlet and δ 1.2339 a doublet indicating two protons at c-3,' and C-4'.δ0.8359 appeared as a doublet indicating three protons at C-3'-Me. The $^{13}$CNMR spectrum exhibited values at δ 174.83 (C-7), δ 169.0 (C-3), indicating the presence of two ester groups. δ 150.08 indicated the carbon for COOH. The value at δ 159.08 indicated the presence of carbon for $CH_3COO$.

The LC-MS spectrum displayed the molecular ion peak at m/z 672 correspondings to the molecular formula $C_{35}H_{44}O_{13}$. From the above evidence, the compound RG-APE1was designated as Rohituka 7.

The compound **RG-APE2** showed a m. p. at 122.4 °C. It showed a positive response for Liebermann-Burchard test for triterpenoids. The IR spectrum showed a strong absorption at 3389.10 $cm^{-1,}$ indicating the presence of the hydroxyl group. The band at 2931.25 $cm^{-1}$ indicated C-H stretching in $CH_3$. The prominent peak at 1741.57 $cm^{-1}$ shows carbonyl stretching of ester. The $^{1}$HNMR of this compound exhibited a singlet at δ 6.8561indicating methyl protons at C-30. The value at doublet δ 7.3975, singlets δ6.4724 and δ 7.5509 indicated the presence of furan moiety. Here it is clearly noted that the value of COOH and $CH_3COO$ are absent when compared to Rohituka 7. A singlet at δ 3.4367 and δ 1.7908 indicated the presence of protons at C-2' and C-3' and d 1.1606 at C-4'. The proton at C-5 appeared at 0.6985 as a single. A triplet appeared at δ 0.8934, indicating 3 methyl groups at 3'- Me. The $^{13}$CNMR spectrum exhibited a peak at δ 167.06 (C-3), δ 169.0 (C-7), indicating the presence of two ester groups. The LC-MS spectrum displayed the molecular ion peak at m/z 600 correspondings to the molecular formula $C_{32}H_{40}O_{11}$. From the above evidence, the compound RG-APE2was designated as Rohituka 3.

The compound RG-APE3 showed a m. p. at 189 °C and a positive response to Molisch's test for carbohydrates and belong to the limonoid class. The IR spectra showed a peak at 3456.11 $cm^{-1,}$ indicating the presence of the hydroxyl group. The peak at 1745.0 $cm^{-1}$ corresponds to the carbonyl group of the ester. The peaks at 1641.22 $cm^{-1}$ and 820.39 $cm^{-1}$ indicated a trisubstituted double bond. The peak at 860.37 $cm^{-1}$ is due to the presence of furan ring. The peak at 1244.4 $cm^{-1}$ corresponds to the epoxide moiety. The peak at 820.29 $cm^{-1}$ is due to the glycoside moiety.

In the proton NMR spectra, the singlets at δ 0.96, δ 0.99,δ 1.09,δ 1.05 corresponds to 12 hydrogens of 4 methyl groups at C-18, 19, 24, 25. The singlet at δ 1.27 corresponds to the methyl protons of the rhamnose at C-6″. The value at δ 5.13 corresponds to 1 hydrogen of C-1′ of a glucose molecule. The multiplets at δ 6.43- δ 7.59 is due to three hydrogens of the furan moiety. The singlet at δ 4.91, δ 5.09, corresponds to methylene protons at C-26.

The $^{13}$CNMR spectra showed signals at δ 172.93 (C-7), δ 170.14 (C-16) suggesting the presence of two ester groups. The signals at δ 142.93 (C-23), δ 140.16 (C-22), δ 101.59 (C-21), δ 120.14 (C-20) are characteristic of furan ring. The LC-MS spectrum indicated the molecular ion peak at 778.1 [M+] corresponding to the molecular formula $C_{39}H_{54}O_{16}$.

From the above evidences the compound RG-APE3 was designated as Amoorinin-3-O-α-L-rhamno-pyranosyl – (1→6) –β-D-glucopyranoside.

The compound RG-APE4 showed m.p. at 212 °C and a positive test for flavanone glycoside. The IR spectra showed peaks at 3447.18 $cm^{-1,}$ indicating the presence of hydroxyl group. The peak at 2963.12 $cm^{-1}$ corresponds to C-H stretching of $CH_3$. The peak at 2877.24 $cm^{-1}$ corresponds to the C-H stretching of in $CH_3$ of methoxy group. The peaks at 1644.29 $cm^{-1}$, 1461.18 $cm^{-1}$, 1377.10 $cm^{-1}$, 1234.4 $cm^{-1}$, 1147.6 $cm^{-1}$, 800.4 $cm^{-1}$ is due to C-H stretching in flavonone nucleus. The peak at 828.40 $cm^{-1}$ is due to a glycoside. In the proton NMR spectra, the singlet at δ 1.24 is due to methyl protons of the third glucose moiety at C-6′′′′. The doublet at δ 5.07 corresponds to the protons of C-1′′′′of glucose, the singlet at each δ 3.80, 3.93, and 4.00 is due to the protons of the 3 methoxy group in the structure. The multiplets at δ 3.82-3.87 account for hydroxyl protons. The singlet at δ 2.13 accounts for the methyl protons at C-8.

The $^{13}$CNMR shows signals at δ 55.67, 56.62, and 50.15 representing the 3 methoxy groups. The value at δ 195.15 indicates one carbonyl group. The value at δ 11.68 was indicative of one methyl group at C-8. The LC-MS spectrum indicated the molecular ion peak at 816.11 [M+] corresponding to the molecular formula $C_{37}H_{50}O_{20}$. From the above evidences the compound RG-APE4was designated as 8- methyl-7, 2′, 4′-tri-O-methyl-flavonone-5-O-α-L- rhamnopyranosyl (1→4)-β-D-glucopyranosyl- (1→6) -β- D-glucopyranoside.

The isolated compounds from the methanolic extract of root bark of *A. polystachya* have been reported to have potential anti-cancer activity on MCF-7 and MDA-MB cell lines [8]. The cytotoxic studies were performed on Silver Nanosuspension of *A. polystachya*. It was seen that the formulation showed potent cytotoxic activity. This activity can be attributed to the isolated components which have been already reported to possess potential cytotoxic effects against human cell lines [9]. It can thus be concluded that the silver nanosuspension of *A. polystachya* can be suggested for breast cancer activity, which is eco-friendly, cost-effective, and stable.

**CONCLUSION:** The investigation justifies that the isolated components from the methanolic extract of the root bark of *A. polystachya*, RG-APE1, RG-APE2, RG-APE3, and RG-APE4 have been proven to have cytotoxic activity [8]. Based on these preliminary investigations, we formulated the root bark extract of *A. polystachya* into silver nano-particles and subsequently into silver nanosuspension which exhibited better cytotoxic activity than the root bark extract of the plant. The use of AgNP has emerged as a novel approach in cancer therapy. These studies suggest that formulating into AgNS can be further used in drug targeting the anti-cancer cells and create an impact on the human health

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## REFERENCES:


1. http://www.who.int/mediacentre/news/releases/2003/pr27/en/.
2. Shweta SS, Rani WC, Tapadiya GG and Khadabadi SS: *Aphanamixis polystachya* (wall.) Parker - an important ethnomedicinal plant. Int J Pharm Sci Rev Res 2014; 24(1): 25-28.
3. Thirumurugan G: Nanotechnology: an effective tool for enhancing bioavailability and bioactivity of phytomedicine. Asian Pac J Trop Biomed 2014; 4(1): S1-S7.
4. http://biofuelpark.com/aphanamixis-polystachya-wall-r-parker/.
5. Kirtikar KR, Basu BD, Indian Medicinal Plants. Vol I. 2nd ed. Dehradun: International Book Distributor 2005: 551-53.
6. Gude R, Joshi AB, Bhandarkar A, Shirodker A and Bhangle S: Synthesis and characterisation of silver nanoparticles from the roots of *Aphanamixis polystachya*. World Jour of Pharm Research 2016; 2(5): 1399-408.
7. Jahan N, Aslam S, Rahman KU, Fazal T, Anwar F and Saher R: Formulation and characterisation of nanosuspension of herbal extracts for enhanced antiradical potential. Journal of Experimental Nanoscience 2016; 11(1): 72-80.
8. Houghton P, Fang R, Techatanawat I, Steventon G, Hylands PJ and Lee CC: The sulphorhodamine (SRB) assay and other approaches to testing plant extracts and derived compounds for activities related to reputed anticancer activity. Methods 2007; 42(4): 377-87.
9. Zhang Y, Wang JS, Wang XB, Gu YC and Kong LY: Polystanins A–D, four new protolimonoids from the fruits of *Aphanamixis polystachya*. Chem Pharm Bull 2013; 61(1): 75-81.
10. Vichai V and Kirtikara K: Sulforhodamine B colorimetric assay for cytotoxicity screening. Nature Protocols 2006; 1(3): 1112-16.
11. Shanker K, Mohan GK, Hussain MA, Jayarambabu N and Pravallika PL: Green biosynthesis, characterization, in vitro antidiabetic activity, and investigational acute toxicity studies of some herbal-mediated silver nanoparticles on animal models. Pharmacogn Mag 2017; 13(49): 188-92.
12. Mohanta YK, Panda SK, Jayabalan R, Sharma N, Bastia AK and Mohanta TK: Antimicrobial, antioxidant and cytotoxic activity of silver nanoparticles synthesized by leaf extract of *Erythrina suberosa* (Roxb.). Frontiers in Molecular Biosciences 2017; 4(14): 1-9.
13. Sultana N, Rahman MO, Tahia F, Hassan MA and Rashid MA: Antioxidant, cytotoxicity and antimicrobial activities of *Aphanamixis polystachya* (wall.) R. N. Parker. Bangladesh J Bot 2017; 46(4): 1381-87.
14. Wang GW, Jin HZ and Zhang WD: Constituents from Aphanamixis species and their biological activities. Phytochemistry Reviews 2013; 12(4): 915-42.
15. Zhang HP, Wu SH, Luo XD, Ma YB and Wu DG: A new limonoid from the seed of *Aphanamixis polystachya*. Chinese Chemical Letters 2002; 13(4): 341-42.
16. Brown DA and Taylor DA: Limonoid extractives from *Aphanamixis polystachya*. Phytochemistry 1978; 17(11): 1995-9.
17. Agarwal SK, Verma S, Singh SS and Kumar S: A new limonoid from *Aphanamixis polystachya*. Indian J Chem 2001; 40B: 536-38.
18. Srivastava SK, Srivastava SD and Srivastava S: New biologically active limonoids and flavonoids from *Aphanamixis polystachya*. Indian J Chem 2003; 42B; 3155-58.
19. Agnihotri VK, Srivastava SD and Srivastava SK: A new limonoid, amoorinin, from the stem bark of Amoorarohituka. Plantamedica 1987; 53(03): 298-9.
20. Samir KS, Panadda P, Choudhuri SC, Takashi OM and Ishibashi M: A new lignan from *Aphanamixis polystachya*. J Nat Med 2006; 60: 258-60.
21. Ragasa CY, Aguilar ML, Ng VA, Bugayong ML, Jacinto SD, Li WT and Shen CC: Terpenoids and sterol from *Aphanamixis polystachya*. J Chem Pharm Res 2014; 6(6): 65-8.
22. Lin CJ, Lo IW, Lin YC, Chen SY, Chien CT, Kuo YH, Hwang TL, Liou SS and Shen YC: Tetranortriterpenes and Limonoids from the roots of *Aphanamixis polystachya*. Molecules 2016; 21(9): 1167.
23. Wang GW, Jin HZ and Zhang WD: Constituents from Aphanamixis species and their biological activities. Phytochemistry reviews 2013; 12(4): 915-42.
24. Zhang H, Chen F, Wang X, Wu D and Chen Q: Complete assignments of 1H and 13C NMR data for rings A, B-seco limonoids from the seed of *Aphanamixis polystachya*. Magn Reson Chem 2007; 45(2): 189-92.
25. Zhang Y, Wang JS, Wang XB, Gu YC, Wei DD, Guo C, Yang MH and Kong LY: Limonoids from the fruits of *Aphanamixis polystachya* (Meliaceae) and their biological activities. J Agric Food Chem 2013; 61(9): 2171-82.
26. Lakshmi V, Pandey K, Kapil A, Singh N, Samant M, Dube A: *In-vitro and in-vivo* leishmanicidal activity of *Dysoxylum binectariferum* and its fractions against Leishmania donovani. Phytomedicine2007; 14(1):36-42.
27. Valan MF: Isolation and characterisation of the active principle from the bark of *Aphanamixis polystachya*. Asian Journal of Pharmaceutical Research 2014; 4(1): 19-23.
28. Mishra AP, Saklani S, Chandra S, Mathur A, Milella L and Tiwari P: *Aphanamixis polystachya* (wall.) Parker, Phytochemistry, Pharmacological properties and medicinal uses: An overview. World Journal of Pharmacy and Pharmaceutical Sciences 2014; 3(6): 2242-52.
29. Sarker AA, Chowdhury FA and Farjana Khatun: Phytochemical screening and *in-vitro* evaluation of pharmacological activities of *Aphanamixis polystachya* (Wall) Parker fruit extracts. Tropical Journal of Pharmaceutical Research 2013; 12(1): 111-16.
30. Rani WC, Shweta S, Tapadiya GG and Khadabadi SS: Evaluation of Phytochemical and Anticancer Potential of *Aphanamixis polystachya* (Wall.) Parker Stem Bark using *in-vitro* models. International Journal of Pharmaceutical Sciences Review & Research 2014; 28(1): 138-42.
31. Hegde K and Moitra G: Evaluation of anticancer activity of extract of *Aphanamixis polystachya* (Wall.) Parker leaves. International Journal for Pharmaceutical Research Scholars 2014; 3(2): 876-81.
32. Khan TR, Karmarkar P, Das A, Banik R and Sattar MM: Evaluation of cytotoxic and anthelmintic activities of bark extract of *Aphanimixis poystachya* (Wall). International Research Journal of Pharmacy 2013; 4(4): 126-29.
33. Md AAS, Md RK, Md AK, Sudhir K, and Mohammad AR: *In-vitro* membrane stabilizing activity, total phenolic content, free radical scavenging and cytotoxic properties of *Aphanamixis polystachya* (Wall.). Bangladesh Pharmaceutical Journal 2010; 13(2): 55-9.
34. Sarker AA and Pathan AH: Phytochemical Analysis and Bioactivities of *Aphanamixis polystachya* (Wall) R. Parker Leaves from Bangladesh. Journal of Biological Sciences 2013; 13(5): 393-99.

35. Enamala N, Hemashenpagam N, Vimal SS and Vasantha RS: Characterization of *Coriander sativum* mediated silver nanoparticles and evaluation of its antimicrobial and wound healing activity. Indo American Journal of Pharmaceutical Research 2013 3(7): 5404-09.
36. Konwar R and Baquee AA: Nanoparticle: An overview of preparation, characterisation and application. International Research Journal of Pharmacy 2013; 4(4): 47-57.
37. Saklani S and Mishra AP: Pharmacognostic, phytochemical and antimicrobial screening of *A. polystachya*, an endangered medicinal tree. International J of Pharmacy and Pharmaceutical Sci 2012; 4(3): 235-40.
38. Gude R, Joshi A and Bhandarkar A: Anti-oxidant, cytotoxic and apoptotic studies of root bark assisted silver nanoparticles from *Aphanamixis polystachya*. Int J Res Pharm Sci 2019; 10(2): 943-50.

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