IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
July 2020 Vol.:18, Issue:4

Extraction and Isolation of Diterpenoids Derivatives Andrographolide of *Andrographis paniculata* as Novel Anticancer Agents

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INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
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ISSN 2349-7203
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Submission: 23 June 2020
Accepted: 29 June 2020
Published: 30 July 2020

Keywords: Diterpenoids Derivatives, Andrographolide, *Andrographis paniculata*, Anticancer Agents

ABSTRACT

Cancer is dreadful disease and represents one of the biggest health care issues for the human race and demands a protective strategy for cure. Plants are reservoirs for novel chemical entities and provide a promising line for research on cancer. *Andrographis paniculata* Nees, *Acanthaceae* commonly known as Kalmegh, their extract and major diterpenoid lactone andrographolide has been proven to possess several important protective biological activities, including antioxidant, anti-inflammatory, immunomodulatory, antiseptic, antimicrobial, cytotoxic, hypolipidemic, cardioprotective, hepatoprotective, and neuroprotective effect. These pharmacological activities will be lost when lactone or its conjugated double bond is open. In addition proper modification on the functional groups at C-3, C-19 will significantly enhance the molecules pharmacological activity.

1. INTRODUCTION

Cancer is one of the severe metabolic syndrome and deadly diseases which are characterized by irregular cell proliferation. This uncontrolled proliferation of a normal cell which produces genetic instabilities and alterations accumulates within cells and tissues which transforms normal cell into a malignant cell. These genetic instabilities include mutations in DNA repair genes, tumor suppressor genes, oncogenes and genes involve in cell growth metabolism[1]. Both external factors (radiations, smoking, tobacco, pollutants in drinking water, food, air, chemicals, certain metals and infectious agents) and internal factors (genetic mutations, body immune system and hormonal disorders) can cause cancer. According to World Health Organization, more than 14 million people diagnosed with cancer and 8 million died in 2012. High mortality and incidence make it important public health and economic issue which requires effective prevention. Medicinal plants have various advantages over chemical products because plant derived compounds are more tolerant and non-toxic to the normal human cells[2]. Already available conventional therapies for the treatment of cancer are radiotherapy and chemotherapy which have various side effects like neurological, cardiac, renal and pulmonary toxicity, seriously affecting the health of the person. Therefore, an alternative method is required to develop that include less toxic and more potent anticancer drug as compare to the drugs available in the market[1]. Several studies have been made on naturally occurring compounds known to possess cytotoxicity effects, as they display potential to destroy cancer cells. Due to these advantages of medicinal plants they are in high demand and several species of medicinal plants have been investigated and selected for the preparation of cancer medicines. Recently, there has been an increased scientific interest in the study of materials from plant source as an anticancer compound. Several studies have found the role of medicinal plants in prevention and treatment of cancer. National Cancer Institute has approximately screened 35,000 plant species for their potential anticancer activities and they have found that among them about 3,000 plant species have shown reproducible anticancer activity. Emergence of important anti-cancer agents from natural source requires more research in order to develop more drugs to treat this disease. Medicinal plants contain wide ranges of secondary metabolites which include flavonoids, flavones, anthocyanins, lignans, coumarins, isocatechins and catechins. These bioactive compounds are mainly responsible for the antioxidant property of medicinal plants[3]. The increasing side effects and expensive medication has tilted the focus of researches on herbal medicines.

Therefore in this review, an effort has been made to provide information about the medicinal plants that possess anticancer activity.[4]

Andrographis paniculata, belonging to the family *Acanthaceae* extract and their major diterpenoid component significantly suppresses cell proliferation, induces cell cycle arrest, and induces cell apoptosis of various cancer cells.[4]

2. Experimental Work on *Andrographis paniculata*

The main objective of this study was to purify one of these major components (Andrographolide) from *Andrographis paniculata,* to analyze its quality and to calculate yield on weight to weight bases and exact percentage of Andrographolide for health care issues for the human race and demands a protective strategy for cure and treatment of cancer.[4]

2.1 Morphology of plant:

The traditional medicinal plants of *A. paniculata* and its derived drugs are the potential source of alternative medicine and are tremendously used to treat various health ailments related to GIT and digestion, vermicide, anticancer, analgesic, anti-inflammatory, antibacterial, antityphoid, antihyperglycemic and liver impairment. *A. paniculata* is an annual profusely branched, erect herb extremely bitter in taste[5]. It grows to a height of 30-110 cm in moist shady places with glabrous leaves and white flowers with rose purple spots on the petal. The stem dark green, 0.4-1.0 m in height, 2-6 mm in diameter, quadrangular with longitudinal furrows and wings on the angles of the younger parts, slightly enlarged at the nodes; leaves glabrous, up to 8.0 cm long and 2.6 cm broad, lanceolate, pinnate; flowers small and solitary, corolla whitish or light pink in color with hairs, in lax spreading axillary and terminal racemes or icles; capsules linear-oblong, acute at both ends, 1.9- 0.3 cm; seeds numerous, subquadrate, yellowish brown[5]. *A. paniculata* is an annual herb found in Sri Lanka, Pakistan, Java, Malaysia, Indonesia and throughout India, specifically in Maharashtra, Karnataka, Uttar Pradesh, Tamilnadu, Andhra Pradesh and Madhya Pradesh. It is cultivated to some extent in Assam and West Bangal.

Figure No. 1: Morphology of *Andrographis paniculata*

2.2 Chemical Properties:

Therapeutically active constituent of kalmegh found in aerial parts. The primary active constituent of *A. paniculata* is the Andrographolide. It is colorless bitter in taste and crystalline and known as diterpene lactone[4]. Investigation of *A. paniculata* showed that it is a rich source of 14-deoxy-11-oxoandrographolide ($C_{20}H_{28}O_5$), 14 deoxy-11, 12-didehydro andrographolide/andrographolide D ($C_{20}H_{30}O_4$), 14 deoxy-andrographolide($C_{20}H_{30}O_4$) and other non-bitter compounds neoandrographolide ($C_{26}H_{40}O_8$); homoandrographolide ($C_{22}H_{32}O_9$); andrographosterol ($C_{23}H_{38}O$); andrographone ($C_{32}H_{64}O$); andrographane ($C_{40}H_{82}O$); andrographosterin; andrograpanin; stigmasterol; α-sitosterol; andrographin ($C_{18}H_{16}O_6$); and dihydroxy-di-methoxy flavone. Andrographolide [$C_{20}H_{30}O_5$]; (3-[2-{decahydro-6-hydroxy-5-(hydroxymethyl)-5,8α-dimethyl-2-methylene-1 napthalenyl} ethylidene] dihydro-4-hydroxy 2(3H) furanone] is a colorless crystalline bicyclic diterpenoid lactone and present in all parts of the plant, Maximally in the leaves. On the orally consumption of andrographolide, appears to accumulate in organs throughout the viscera. Andrographolide are mainly identified as sulfonic acid adducts and sulfate compounds, as well as glucuronide conjugations. Some metabolites of andrographolide like sulfate ester compounds, sulfonates, and andrographolide analogoues were isolated from rat urine and feces. While those metabolites isolated from the human urine were like as sulfates cysteine S-conjugate, and glucuronide conjugates. One of the metabolites, 14- deoxy-12-sulfo-andrographolide was reported to be matching to the anti-inflammatory drug.[5]

2.3 MATERIALS AND METHOD

2.3.1 Plant material:

50 g of green plant extract is introduced in soxhlet extractor for extraction of main bioactive content that is andrographolide, concentrate the obtained extract and dissolve in ethanol pure crystals of andrographolide (2g) is obtained.[6]

2.3.2 Sample collection:

Andrographis paniculata plant resources were collected from the Jawaharlal Nehru Krishi Vishwa Vidyalaya Krishi Nagar, Jabalpur Madhya Pradesh (India) with voucher number 47 book no. 116. Plant materials were used for phytochemical and antimicrobial analysis. The collected plant leaves were air dried and powdered using mortar and pastel.[7]

2.3.3 Preparation of extract:

Preparation of Kalmegh (*A. paniculata*) leaves were dried and crushed to powder form. Dried (5 g) powder was soaked in double distilled water (500 mL) and refluxed for 5 h. The aqueous solution was filtered and concentrated to 100 mL. This extract was used to study the corrosion inhibition properties. Corrosion tests were performed on a mild steel of the following percentage composition: Fe 99.30%, C 0.076%, Si 0.026%, Mn 0.192%, P 0.012%, Cr 0.050%, Ni 0.050%, Al 0.023%, and Cu 0.135%, which were polished successively with fine grade emery papers from 600 to 1200 grade. The specimens were washed thoroughly with double distilled water and finally degreased with acetone and dried at room temperature. The aggressive solution 1 M HCl was prepared by dilution of analytical grade HCl (37%) with double distilled water and all experiments were carried out in unstirred solutions.[7]

2.3.4 Phytochemical Test

The obtained plant extract was analyzed for the presence of natural phytochemicals such as terpenoids, phlobatannins, flavonoids, reducing sugars, phenols and tannins etc.[8]

2.3.4.1 Terpenoids test

Standard plant extracts sample solution was prepared by 5 mg of plant extract and 5 ml of absolute methanol solution in a test tube[7]. Each extract solution was added 2 ml of chloroform solution followed by 1 ml of concentrated H_2SO_4. Formation of red color solution gives positive result and no color change gives negative result. A reddish-brown coloration of the interface indicates the presence of terpenoids.[9]

2.3.4.2 Phlobatannins test

Approximately 20 mg of plant extract sample was treated with 10 ml distilled water followed by 2ml of dilute HCL and then the sample was heated for 10 min at a temperature 80 °C. If the solution gives brown color precipitate then it is the sign of positive result and if no precipitation observed then it is showing negative result.[8,11]

2.3.4.3 Flavonoids test

Approximately 20 mg of plant extract sample was treated with 10 ml distilled water followed by 5 ml of Ammonia solution (35% v/v) and 1 ml of concentrated H_2SO_4. If the solution gives red color solution, then it is the sign of positive result and if no color changes observed then it is showing negative result.[7,11]

2.3.4.4 Phenol and tannins test

Plant extracts sample solution was prepared by taking 10 mg of plant extract with 10 ml of distilled water followed by 2 ml of 2% $FeCl_3$ solution. Formation of brown color precipitate gives positive result and no precipitate gives negative result.[10,11]

2.3.4.5 Iodine test

Plant extracts sample solution was prepared by taking 10 mg of plant extract with 10 ml of distilled water followed by 2 ml of iodine solution. If the solution gives blue color then it is the sign of positive result and no color changes observed and then it is the sign of negative result.[9,10,11]

2.3.4.6 Wagner's test

Plant extracts sample solution was prepared by taking 10 mg of plant extract with 10 ml of distilled water. Wagner's reagent was prepared by mixing 50 ml of Iodine solution (2 g in 100 ml distilled water) in to the 50 ml of KI solution (6 g in 100 ml distilled water). After preparation, Wagner's reagent was added in to the plant extract solution. Formation of red color precipitate gives positive result and no precipitate gives negative result.[11,12]

2.3.4.7 Mayer's test

Plant extracts sample solution was prepared by taking 10 mg of plant extract with 10 ml of distilled water. Mayer's reagent was prepared by mixing 50 ml of $HgCl_2$ solution (1.36 g in 100 ml distilled water) and 50 ml of KI solution (5 g in 100 ml distilled water). After that Mayer's reagent was added in to the plant extracts sample solution. If the solution forms in to red color precipitate, then it is the sign of positive result and if no precipitate observe then it is the sign of negative result.[11]

2.3.4.8 Keller kiliani test

Plant extracts sample solution was prepared by treating 2 mg of plant extract with 2 drops of 2% $FeCl_3$ solution followed by 2 ml of concentrated H_2SO_4. Formation of dark brown ring at the interface of the sample gives positive result and no ring formation gives negative result.[11]

2.3.5 Instrument and equipment

2.3.5.1 HPLC analysis:-

HPLC analysis and related chromatogram of pure andrographolide and the same compound isolated from the *Andrographis paniculata* plant is illustrated below to compare the purity of isolation which confirms the identity of Andrographolide. Their results are listed as below for their comparisons.[1,12]

Mode: LC

Detector: UV, 223nm

Column: 4.6mm x 25-cm ; 5µm packing L1

Flow rate : 1.5 Ml/min

Injection size : 20 µL

Sample: Standard solution A and Standard solution B.

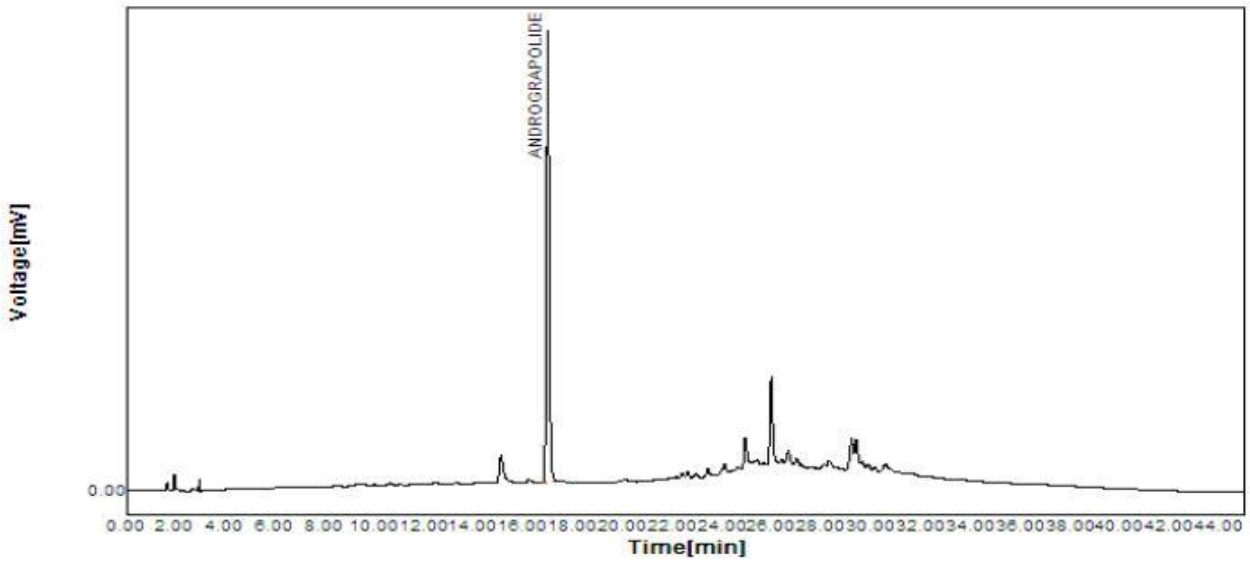


Figure No. 2: Chromatogram (Standard)

Table No. 1: HPLC Reading for (Stander)

Sr. No.	Name	RT [Min]	Area [Mv*s]	Height [Mv]	Amt
1.	Andrographolide	16.9500	1402.4926	177.2338	1.0000
Sum			1402.4926	177.2338	1.0000

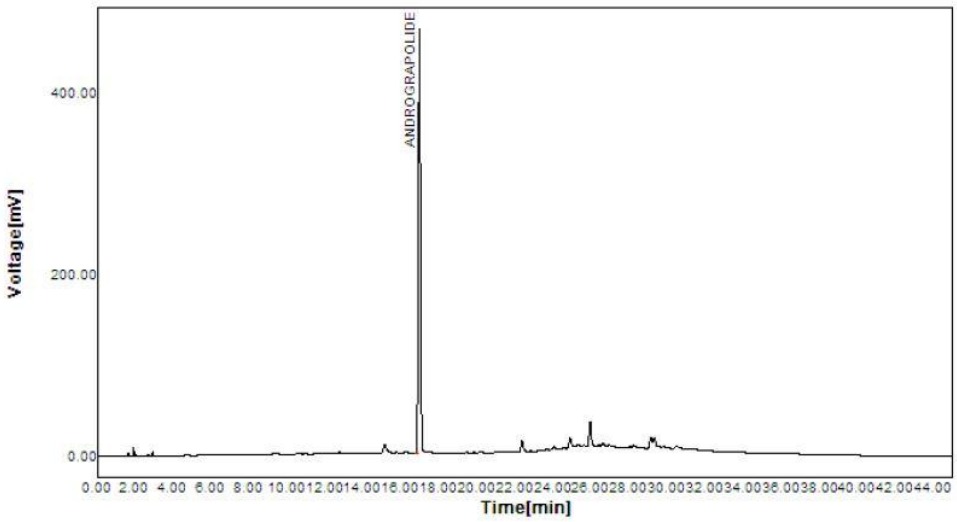


Figure No. 3: Chromatogram (Pure)

Table No. 2: HPLC Reading for (Pure)

Sr. No.	Name	RT [Min]	Area [Mv*s]	Height [Mv]	Amt
1.	Andrographolide	16.9433	3792.0465	456.4335	3.1123
Sum			3792.0465	456.4335	3.1123

2.3.5.2 FTIR analysis of Andrographolide:-

FTIR of isolated Andrographolide is measured by model Vertex 70 Bruker, at the range 4000-400cm-1 the various peaks in the spectra shows the presence of functional group in the isolated compound[1,12,13].

Table No. 3: Identification of functional group by FTIR

Wavenumber (cm-1)	Assignments
785.65	Alkynes
899.60	Aromatic
1.31.54	Alkenes
1221.45	Alcohols
1363.39	Alkanes
1731.23	Carbonyl group
3036.65	Alkanes

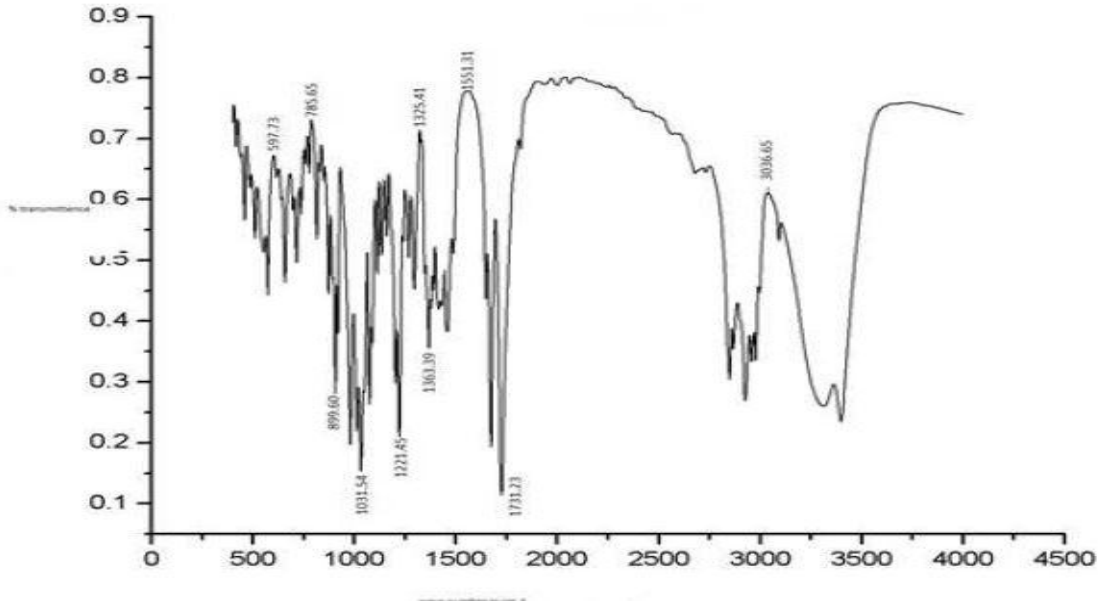


Figure No. 4: FTIR Spectra for isolated compound

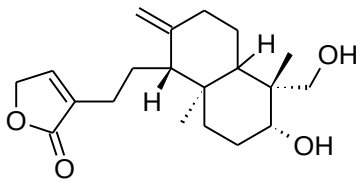
Table No. 4: Preliminary phytochemical analysis of screened medicinal plant Andrographis paniculata

Sr.No.	Test	Observation	Result
1.	Terpenoids test	Red color solution is observed	(+)ve
2.	Phlobatannins test	Brown color precipitate is not formed	(-)ve
3.	Flavonoids test	Red color solution is not observed	(-)ve
4.	Phenol and tannins test	Brown color precipitate is not formed	(-)ve
5.	Iodine test	Blue color is not appeared	(-)ve
6.	Wagner's test	Red color precipitate is not formed	(-)ve
7.	Mayer's test	Faint yellow color precipitate is not formed	(-)ve
8.	Killer Killani test	Dark brown color ring appeared	(+)ve

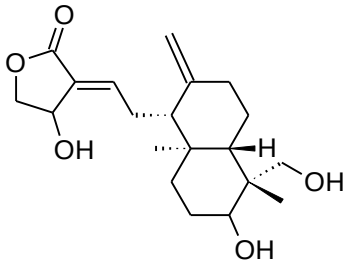
(+)ve = Indicate the presence of phytochemical

(-)ve = Indicate the absence of Phytochemical

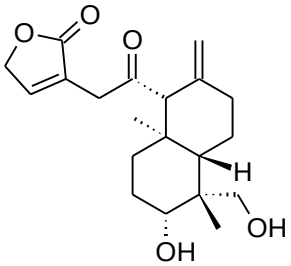
2.3 Chemical Structure



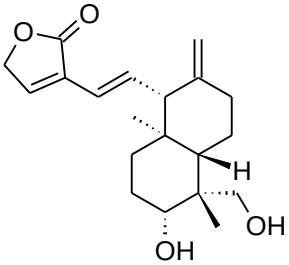
14-Deoxyandrographolide



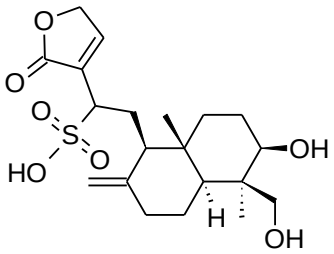
Andrographolide



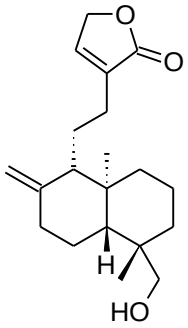
14-Deoxy-11-oxoandrographolide



14-Deoxy-11,12-Didehydroandrographolide

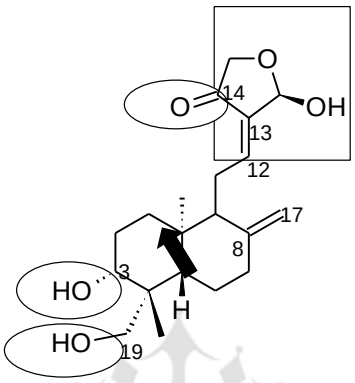


14-Deoxy-(12R)-Sulfo-Andrographolide



Andrograpanin

2.4 Structure activity relationship of Andrographolide



Several attempts have been made to enhance the bioavailability of AGP through structural modification. Additionally, AGP presents a good modifiable pharmacophore for numerous pharmacological activities. Some analogues have exhibited improved bioavailability and enhanced therapeutic efficacy[14]. Structure activity relationship (SAR) studies on AGP revealed that an intact g-butyrolactone ring, C12 = C13 and C8 = C17 double bonds, as well as the OH group at C-14 are crucial for sustenance of its cytotoxic activity. To improve the potency and selectivity of anticancer activity of AGP, the compound has been subjected to systematic structural modifications in its side chains such as acylation of hydroxyls and introduction of a benzylidene moiety at C-3 and C-19 Chemical structure of andrographolide (AGP). To increase bioavailability and functionality, chemical modifications are often performed on the a,b-unsaturated g-butyrolactone moiety (in square box), the two double bonds C-8/17 and C-12/13 (black arrows) and three hydroxyls (circles) at C-3, C-14 and C-19. Modifications at these positions have shown improvement of anticancer activity.[15,16]

2.5 Pharmacological activity of plant *A. paniculata*

Various anti-cancer agents inhibiting the proliferation of cancer cells by inducing apoptosis, necrosis, cell-cycle arrest or cell differentiation; others might involve immunomodulatory activity, by triggering body's own immune system against these cells. The compounds that inhibit multiple procancer events are of greater interest as they are more likely to inhibit a wider range of cancers under great variety of circumstances. In this review, andrographolide presents a strong candidature as a therapeutic anticancer Pharmacophore as it exhibits a dual property, acting both directly and indirectly on the cancer cells.[16,17]

Methanolic extract of *A. paniculata* had shown noteworthy toxicity against human epidermoid leukemia and lymphocytic leukemia cell lines. Potent cytotoxicity in a dose dependent manner towards various kinds of cancer cell lines including drug resistant cancer cells has also been reported in another excellent work. The cytotoxic property has been attributed to the ability of andrographolide to inhibit proliferation and induce apoptosis in cancer cells.[17]

2.5.1 Prostate cancer

AGP was found to attenuate tumour growth in prostate cancer (PCa) cells by modulating certain cell cycle regulators (e.g., cyclin-dependent kinases), proinflammatory cytokines [e.g., interleukin (IL)-6] and chemokines (e.g., CXCL11, CXCR3 and CXCR7) [18–20]. IL-6 has a dual role in prostate cancer cell growth and differentiation, by acting as a paracrine growth factor in androgen-dependent prostate cancer cells (such as LNCaP) and as an autocrine growth factor in androgen-independent PCa cells (such as PC-3 and DU145) [21]. Chun and colleagues [19] demonstrated selectivity of AGP towards inhibition of IL-6 in three PCa cell lines: DU145, PC-3 and LNCaP cell lines. DU145 and PC-3 express the constitutive IL-6 autocrine loop, whereas LNCaP cells lack IL-6 yet express its receptor. In the latter, AGP managed to suppress paracrine IL-6-stimulated signalling pathways including the Janus kinase/signal transducer and activators of transcription (JAK/ STAT) (Stat3 phosphorylation), the mitogen-activated protein kinase (MAPK)/Erk phosphorylation and the phosphoinositide 3-kinase (PI3K)/AKT (Akt phosphorylation) pathways. The sensitivity of these PCa cells (LNCaP, DU145 and PC-3) towards AGP might be attributed to their androgen dependency, prostate-specific antigen (PSA) expression and p53 status. Indeed, AGP displayed greater

cytotoxic selectivity towards PC-3 cells (GI50 = 1.5 mM), which is androgen-independent and lacking p53 yet expressing PSA.[17,18]

Administration of AGP to DU145-xenografted mice successfully delayed tumour growth with no obvious toxic effects. The compound (10 mM) significantly inhibited PCa cell growth by initiating apoptosis, through the intrinsic pathway, involving poly (ADP-ribose) polymerase (PARP), caspases and Bcl-2 family members. Almost 86% of PC-3 cells were found to be at the stages of early and late apoptosis when treated with 10 mM AGP. Similarly, AGP induced apoptotic cell death by activating the caspase cascade and increasing the expression of proapoptotic Bcl-2 family members, such as Bax and Bid. Furthermore, AGP arrested the cell cycle at G2/M phase by downregulating the expression of cyclin-dependent kinase (CDK) 1, without modulating CDK4 and cyclin D1 expression. Additionally, AGP also induced p53- and reactive oxygen species (ROS)- dependent TRAIL-mediated extrinsic apoptotic cell death by elevating the expression of DR4 and DR5 receptors and stimulating the caspase-8/caspase-3 cascade in PC-3 cells. A different study revealed the inhibition of cell growth and survival in AGP- treated PCa cell line C4-2b. AGP was shown to modulate and block cell cycle progression by differential alteration of cell cycle regulators, including cyclin B1, cyclin A2 and cyclin E2, as well as chemokine receptors (CXCR3 or CXCR7). In addition, the chemokine (C-X-C motif) ligand C-X-C chemokine receptor (CXCL11-CXCR3/7) axes in PCa cells was attenuated, thus diminished cell viability and hindered cell migration.[19,20]

To improve the antitumour activity of AGP, an analogue known as 3,19-(3-chloro-4-fluorobenzylidene) andrographolide was synthesised and proven to selectively inhibit the growth of PCa cells in the National Cancer Institute (NCI) *in vitro* screen. SRJ23 (GI50 = 0.4 mM) displayed a 50-fold improved inhibitory profile, compared with the parent compound AGP (GI50 = 19.95 mM), and induced G2/M-phase-specific cell cycle arrest with a concomitant decrease in CDK1 expression, as well as apoptotic cell death of PC-3 via activation of the mitochondrial caspase-cascade signalling system.

2.5.2 Lung cancer

There are two categories of lung cancer, namely small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC). SCLC is a highly malignant cancer which originated from fast-growing oat-like cells exhibiting neuroendocrine features and accounts for 15% of lung cancer cases, whereas NSCLCs make up the remaining 85% of lung cancers. NSCLC is further classified into three pathologic subtypes known as adenocarcinoma (the most prevalent type), squamous cell carcinoma and large cell carcinoma. NSCLC is usually associated with a relatively poor prognosis and high risk of tumour relapse. The relapse that occurs following first-line platinum-based chemotherapy, such as cisplatin treatment, is attributed to acquired resistance. The mechanism underlying cisplatin resistance is inconclusive, yet enhanced autophagy was reported to play a major part. It is factual that many clinically relevant anticancer therapeutic agents induce autophagy. In cancer cells, autophagy could promote cellular survival through inhibition of the negative autophagy regulator: the mammalian target of rapamycin complex 1 (mTORC1). Similarly, Mi et al. illustrated the inhibitory effect of AGP on pro-survival autophagic processes via activation of phosphatase and tensin homologue (PTEN)-dependent Akt/mTOR cascade in the cisplatin-resistant NSCLC cells (in vitro model: A549/DDP). Suppression of autophagy re-sensitised the NSCLC cells to cisplatin-mediated apoptosis. Meanwhile, the anticancer effect of the compound was further enhanced upon in vivo administration of AGP–cisplatin combination (5 mg/kg and 0.75 mg/kg, respectively). Synergistic antitumour activity was also observed in an in vivo study involving administration of the combination 100 mg/kg AGP plus 20 mg/kg paclitaxel to an A549 NSCLC xenograft. The underlying molecular mechanism of this anticancer synergy is not clearly understood but AGP was proposed to potentiate paclitaxel-mediated anti-proliferative and apoptotic cell death of NSCLC cells by increasing the intracellular ROS accumulation. AGP attenuation of pulmonary tumour formation in vascular endothelial growth factor (VEGF)-induced transgenic mice could occur via the suppression of angiogenic VEGF expression and blockage of the cell cycle at G2/M phase, with concomitant downregulation of cyclin A and cyclin B. The antiangiogenic potential of AGP is attributed to its ability to downregulate the expression of hypoxia-inducible factor-1 (HIF-1), which is a master regulator of cellular response to hypoxia that participates in tumour growth and angiogenesis, through sustained suppression of its upstream PI3K/Akt signalling pathway.[21,22] Apart from the suppression of tumour angiogenesis, AGP (<5 mM) was shown

to suppress migration and invasion of A549 lung cancer cells in wound-healing and in vitro trans-well assays.

Using the A549 NSCLC cell line, Lim et al. described a distinct inhibitory activity exhibited by an AGP analogue: 3,14,19- tripropionyl andrographolide (SRS06). It was found to promote apoptotic cell death by downregulating the levels of nuclear factor-kappa B (NF-kB) protein, a major regulator of DNA transcription, and inhibiting p65 DNA binding activity at a relatively low concentration (5 mM). Indeed, SRS06 showed higher potency relative to the parent compound. Another NSCLC cell line, H3255 harbouring an epidermal growth factor (EGFR) mutation, showed increased DNA fragmentation, decreased Na+-K+-ATPase and protein kinase C activity as well as decreased transforming growth factor (TGF)-b1 and VEGF levels upon treatment with AGP at concentrations below 5mM.[21,23]

2.5.3 Liver cancer

Hepatocellular carcinoma (HCC) is a chronic type of liver cancer. AGP has been reported in many studies associated with HCC and identified its effectiveness in impeding carcinogenesis through activation of several pathways including autophagic cell death, apoptosis and inhibition of tumour angiogenesis. AGP induced apoptosis by activating the antioxidant systems in vitro (in hepatoma Hep3B cells) and in vivo (liver tissues obtained from rats with diethylnitrosamine induced HCC) models. This included the upregulation of intracellular reduced glutathione (GSH), superoxide dismutase (SOD), glutathione- S-transferase (GST) levels and the downregulation of malondialdehyde (MDA) and nitric oxide (NO) levels. However, the augmentation of GSH level was reportedly temporary, owing to the potential of AGP to bind and deplete its cellular level, thereby causing an increased glutathione peroxidase activity, ROS production and eventual apoptotic cell death. Furthermore, AGP-induced cell death could be autophagy-mediated, via the activation of liver cancer cells (Huh-7, QGY-7703 and BEL-7402) cyclophilin-D-induced mitochondrial dysfunction and elevation of intracellular ROS levels, independently of caspase-associated apoptosis.[24]

The antiangiogenetic potential of AGP in hepatoma Hep3B cells and tumour-bearing Hep3B-xenografted nude mice occurred by blocking the expression of VEGFD and VEGFA, as well as phosphorylation of VEGF receptor 2 (VEGFR2) and its downstream targets such as the MAPK proteins crucial for cell growth, proliferation and survival. Yang and colleagues also revealed a more pronounced antiangiogenic activity of ADN-9, a 15-benzy-lidene-substituted

analogue of AGP (exact structure was not disclosed), on human umbilical vein endothelial cells (HUVECs). It produced a stronger inhibitory effect on the VEGF-induced capillary-like tube formation associated with attenuation of VEGF/ VEGFR2/AKT signalling and VEGF-induced nuclear translocation of NF-kB at non-toxic concentrations (1.25–5 mM). This analogue also exerted a higher antimetastatic effect against murine hepatoma H22 in orthotopic and subcutaneous xenograft models compared with AGP.[25]

Aberrant expression of microRNAs (miRNAs), which constitute a class of small noncoding RNAs participating in regulation of the expression of oncogenes or tumour suppressor genes, has been implicated in the initiation, progression and metastasis of HCC. Demonstrated the AGP-induced alteration of a miRNA expression profile in Hep3B and SMCC7721 liver cancer cells; whereas few crucial miRNAs were identified to possibly contribute to inhibition of hepatoma tumour growth. These include miR-222-3p, miR-23a-3p, miR-106b-5p and miR-30b-5p. The expression of these miRNAs was elevated in vivo and in vitro, with concomitant reduction in the expression of their downstream target genes involved in hepatoma tumour growth and development.[26]

2.5.4 Breast cancer

AGP has also shown activity towards breast cancer. This malignancy can be categorised into two groups: invasive (infiltrating), which accounts for ~80% of breast cancer patients, and noninvasive (in situ). AGP inhibited the survival of MDAMB- 231, a highly aggressive triple-negative breast cancer cell line. The compound had an IC50 of 30 mM and it induced this inhibition via the induction of ROS accumulation, caspase-dependent mitochondrial-mediated intrinsic apoptosis and cell cycle arrest at the G2/M phase. Interestingly, at a similar concentration, AGP did not induce cytotoxicity in normal human breast epithelial cells (MCF-10A). Zhai and colleagues revealed that AGP (10 mM) inhibition of MDA-MB-231 cellular proliferation occurred via suppression of inhibitor of kappa B-alpha (IkB-a) phosphorylation, prevention of NF-kB nuclear translocation and consequential abrogation of MMP-9 gene expression. The action of AGP in MDA-MB-231 cells was also reported to involve HIF-1 inactivation. Under hypoxic conditions (1% O2), AGP significantly suppressed tumour growth and angiogenesis by reducing HIF-1a activity and hypoxia-mediated VEGF expression, through inhibition of the upstream PI3k/Akt/mTOR signaling cascade at relatively low concentrations.[27]

In contrast to the finding, AGP reportedly induced non-phase-specific cell cycle arrest in MCF-7 cells, a breast cancer model expressing oestrogen receptors. AGP predomi-nantly arrested the cell cycle at G1- and G2/M phases at the early time point (24 h treatment period) but induced an S block at later time points (48 h and 96 h treatment periods). Interestingly, two AGP analogues, namely SRJ23and 3,19-(2-bromobenzy-lidene) andrographolide (SRJ09), were reported to induce G1 phase-specific cell cycle perturbation in MCF-7 cells, with a concomitant increase in p21 expression and decrease in CDK4 expression. This suggests that a substituent of benzylidene pharmacophore at 3-19-positions of the AGP structure improves the potency of the compound and cell cycle phase specificity. Further study on SRJ09 revealed that this analogue prompted MCF-7 breast cancer cell death through an extrinsic apoptotic pathway independent of Bcl-2 and p53.[28,29,30]

2.5.5 Oral cancer

Oral cancer is a type of head and neck cancer, wherein 95% of the cases are oral squamous cell carcinomas (OSCCs). OSCC is characterised by poor prognosis and low survival rate owing to its high invasive and metastatic properties. In addition, resistance to chemo- and radiotherapy complicates the effectiveness of treatments in advanced OSCC patients. Metastasis and resistance to chemo- and radiotherapy were believed to arise from the presence of cancer stem cells (CSCs). A previous study investigated the ability of AGP to reduce the cancer stemness and invasiveness in oral cancer stem cells (OCSCs). They discovered that the weakening effect was mediated through an over-expression of the tumour suppressor miRNA-218 – a miRNA that regulates self-renewal ability, epithelial–mesenchymal transition (EMT) and EMT-associated traits such as cell invasion, migration and chemoresistance. The downregulation of this miRNA has been implicated in different tumours including glioma, thyroid cancer and NSCLC. Furthermore, in this study, AGP was shown to exert a moderate effect on suppressing tumour-initiating activity, yet it played a sensitiser role in combination with radiation, evidenced by almost 80% synergistic reduction in invasion ability and clonogenicity which subsequently suppressed tumorigenesis in OCSCs. Another study reported the in vivo antioncogenic activity of AGP on OSCC by abolishing NF-kB activation and averting tumorigenesis. It is noteworthy that overexpression of miR-218 is associated with downregulation of its direct functional downstream target: EGFR-coamplified and overexpressed protein (ECOP), which governs the transcriptional activity of NF-kB and associated apoptotic response.[8,12,13]

2.5.6 Colorectal cancer

CRC is one of the most commonly diagnosed solid tumours and is usually associated with a high recurrence rate caused by development of acquired resistance to chemotherapies such as 5-fluorouracil (5-FU) and cisplatin. The anticancer potential of AGP has been widely evaluated in CRC cells and the results obtained were promising. In a study, AGP was shown to directly bind and stabilise Bax, without altering its mRNA level. Using an in-house developed 5-FU-resistant HCT116 cancer cell line (HCT116/5-FUR), treatment with AGP (10 mM) significantly increased Bax expression and synergistically enhanced 5-FU-induced apoptosis. The synergistic effect could also be mediated through other mechanisms, including an elevated ratio of proapoptotic Bax: antiapoptotic-Bcl-2 protein expression, activation of caspases and increased association of death ligand to receptors that augment release of cytochrome c. These proved that AGP could reverse chemotherapy resistance and act as a sensitiser in CRC cells towards chemotherapy-induced apoptosis, via either intrinsic (mitochondrial) or extrinsic (death receptor) pathways.[15]

Tumour invasion and metastasis are major concerns of CRC tumour relapse. MMP-2 is an example of proteins implicated in the control of tumour cell invasion and metastasis. At sub cytotoxic concentrations (<3 mM), AGP suppressed MMP-2 activity without affecting its expression. Inhibition of the MAPK signalling cascade, especially attenuation of Erk activation, could principally contribute to the anti-invasive activity of AGP in CRC [63]. Treatment of HCT-116 colon cancer cells with SRJ09 showed improved antiproliferative and apoptogenic effects. In contrast to the ability of AGP to arrest the cell cycle at G1/S and G2/M phases in colon cancer cells, SRJ09 significantly induced G1-phase-specific cell cycle arrest accompanied by an increase in p21 and decrease in CDK4 protein levels. Thenon-phase specificity of AGP in blocking cell cycle progression could be due to AGP-induced ROS generation and accumulated endoplasmic reticulum stress (ER stress), which entirely affect cell survival. Additionally, in vitro assessments have demonstrated the potential of SRJ09 to penetrate through the DLD-1 colon cancer multicell layer (MCL) by diffusion and induce greater cytotoxicity when compared with the parent compound (IC50 = 41 mM, which is fourfold lower than that of AGP). The anti-colorectal-cancer activities of two AGP analogues: 19-O-triphenylmethyl andrographolide (RS-PP-050) and 19-tert-butyldiphenylsilyl-8,17-epoxy andrographolide, were previously investigated. Both compounds were revealed to have acted via the Wnt/b-catenin signaling pathway in HT-29

colon cancer cells. Mechanistically, RS-PP-050 inhibits b-catenin phosphorylation in a GSK-3bindependent pathway, whereas 3A.1 functions through suppression of total b-catenin protein expression in a GSK- 3b-dependent pathway. The clinical relevance of AGP in combination with capecitabine is being studied; the trial started in 2014 and 52 colorectal cancer patients have joined the study.[30,31]

2.5.7 Gastric cancer

Gastric cancer (GC), commonly known as stomach cancer, is a leading cause of cancer-related deaths globally, with the highest incidence occurring in Asia, Latin America and the Caribbean. AGP is reportedly an effective cytotoxic agent for GC, owing to its potential to prevent GC cell proliferation. A study with human GC cell line SGC-7901 demonstrated a 36.6% decrease in G1 phase cells and significant increase in cells (>260%) at G2/M phase after 48 h of treatment with a high dose of 40 mg/ml AGP (equivalent to 114 mM). Similarly, the authors showed that AGP suppressed cell proliferation by modulating the levels of cell-cycle- and apoptosis-related proteins. Increasing concentrations of AGP (10, 20 and 40 mg/ml) led to upregulation of cell-cycle- inhibitory proteins (cyclin B1 and CDC2) and proapoptotic protein (Bax), as well as downregulation of antiapoptotic protein (Bcl-2). Similarly, AGP inhibited GC cell invasion by suppressing matrix metalloproteinase (MMP)-2 and MMP-9 activities through induction of tissue inhibitors of metalloproteinases (TIMP) expression (protein inhibitors of MMPs). Thus, inhibition of cancer cell proliferation by AGP could be achieved by blocking cell-cycle progression, promoting intrinsic apoptosis and/or repressing invasive activity.[32]

Conversely, AGP modulates the extrinsic apoptotic pathway by acting as a sensitiser for tumour necrosis factor (TNF) – related apoptosis-inducing ligand (TRAIL) expression. TRAIL, a member of the TNF family of ligands, is capable of selectively inducing apoptosis in cancer cells through interaction with the membrane death receptors: TRAIL-R1 (also known as DR4) and TRAIL-R2 (also known as DR5).[34,35] Lim and colleagues also reported TRAIL-induced apoptosis in human GC cell lines (either TRAIL-sensitive SNU601 and SNU638 cells or TRAIL-resistant AGS cells) treated with AGP. They observed an induced expression of DR5 (AGP concentration at 20 mM) and DR4 (AGP concentration at 30 mM), which subsequently activated the downstream caspase-8/caspase-3 pathway.[32,34] The cytotoxicity of an AGP analogue, 19-triisopropylsilyl-andrographolide, was determined using AGS and MKN- 45 GC cells. The analogue induced cytotoxic activity by inhibiting

topoisomerase IIa and increasing DNA damage marker g-H2A.X. This marker subjected the cancer cells to apoptotic cell death via activation of the caspase-3-dependent pathway. In addition, this AGP analogue has a logP value (octanol-water partition coefficient) that is 3.8-times higher than AGP, which confers better penetration into cells. However, the binding mechanism to topoisomerase IIa is not well understood.[33]

3. RESULT AND DISCUSSION

3.1 Result

The main constituent of *A. paniculata* leaves extract is andrographolide, their IUPAC name and structure are available in section named chemical structure and investigation, preliminary phytochemical testing has been done in the various extract of *A. paniculata* leaves. The extraction is done with ether as a solvent using soxhlet apparatus. It shows various type of phytochemicals in the extract which has been characterized by using FTIR analysis. Identity of andrographolide has been illustrated using HPLC and their value of RT is given in table 2 and table 3 which compare the purity of isolation which confirm the identity of andrographolide.

The ability of andrographolide to inhibit the cell growth of human tumor cells was assessed in a cell-based proliferation assay using protein staining as an estimate of relative cell member. This study need further new research and require to obtain better result.

3.2 Discussion

The medicinal value depends on the presence of chemical substance and their role in human body. Isolation, identification, characterization and quantification of phytochemicals and evaluation of their potential benefits to humans have become an important area of pharmaceutical research. Modification of the active pharmacophores from plants by analog synthesis to improve the therapeutic efficacy has resulted in the development of several therapeutically valuable drugs. Andrographis paniculata is a herb that is used in traditional Indian and Chinese medicine. The methanolic extract of Andrographis paniculata shows potent cell-differentiating activity in mouse myeloid leukemia (M1) cells (16) and reported to stimulate both the antigen-specific and non-specific immune responses in mice (8). Andrographis plant extract is known to contain diterpenoids, flavonoids, and steroids (6). The main components of Andrographis paniculata extract are the diterpene lactones of which

andrographolide is the major component. Andrographolide constitutes 70% of the plant extract fraction and was identified as the major chemical responsible for the cytotoxic activity of the extract (6). However, andrographolide's role as an anticancer and immunomodulatory agent in humans and its molecular mechanism of action has not been fully elucidated. The herb can be used to cure diseases and isolation and identification of active components which can lead to invention of new drug at less economic cost to patients.

4. CONCLUSION

The plant *A. paniculata* can be used as potent drug exploiting the anticancer in entire study. In view of the favourable *in vitro* and *in vivo* results discussed above, *A. Paniculata* analogues could be researched for the therapy of breast, lung, liver, colon and pancreatic cancers. More specifically, SRJ09 and SRJ23 with novel mechanisms of action targeting the oncogenic K-Ras are potential candidates that could have clinical benefits in the treatment of cancers that are addicted to Ras signalling, such as pancreatic, lung and colon cancers.

ACKNOWLEDGEMENT

The task of preparing the dissertation has been a fascinating experience and it is really a moment of great pleasure for me to express my hearty gratitude to those who have helped me in the successful completion of this dissertation.

I express my deep sense of gratitude to **Mr. Dilip kr. Patel**, Head, Chandra Shekhar Singh College of Pharmacy, Allahabad, for his guidance. It is with affection and reverence that I acknowledge my indebtedness to him and his astounding dedication, often for beyond the call of duty. It was my immense pleasure working under his guidance. His incessant encouragement and cogent comment always inspired me to be creative.

I am highly thankful to **Mrs. Roohi Kesharwani, Asst. Professor,** for his kind guidance and support throughout the course.

I am also thankful to **Ms. Jyoti Rawat, Mr. Vivek Dwivedi, Mr. Sudhakar Singh** for their moral support and endeavor, during my Research work.

I express my gratitude towards all my respected teachers, librarians and technical staff for their timely help during my course of study.

I would like to express my thanks and appreciation to **Mr. Shahnawaz shameem** (Research scholar, SHIATS, Naini, Allahabad), **Mr. Ritesh tiwari** for their generous help and precious suggestions.

I avail this rare opportunity to express my exult in extending genuine appreciation to my most cherished and treasured friends **Mr. Prince Srajal Kesharwani.**

Last but not least, I am immensely thankful to my respectable parents my father **Mr. Prakash Chandra Kesharwani** and my mother **Mrs. Urmila Kesharwani** who have taken a lot of pains during completion of my higher studies and thankful to my husband for their encouragement and support throughout the course of study.

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