Self assembled arjunolic acid acts as a smart weapon against cancer through TNF-α mediated ROS generation

Subhankar Mannaa, Aditi Deya, Rakhi Majumdarb, Braja Gopal Bagb,**, Chandradipa Ghoshc, Somenath Roya,*

a Immunology and Microbiology Laboratory, Department of Human Physiology with Community Health, Vidyasagar University, Midnapore 721 102, West Bengal, India
b Department of Chemistry and Chemical Technology, Vidyasagar University, Midnapore, 721102, West Bengal, India
c Microbiology Laboratory, Department of Human Physiology with Community Health, Vidyasagar University, Midnapore 721 102, West Bengal, India

ARTICLE INFO

Keywords:
Biochemistry
Cell biology
Immunology
Arjunolic acid
Self assemble
Cancer cell
Apoptosis
Cytokines

ABSTRACT

Arjunolic acid (AA) a plant derived pentacyclic triterpenoid which showed effective anticancer activity against MCF-7 and HeLa cells as well as no significant toxic effect was observed against normal lymphocytes. In the current study the self assemble property of arjunolic acid gives an extra emphasis on anticancer activity which was proved by several fluorescence studies like ROS generation, EtBr/AO and DAPI staining. At a selected dose of 50μg/ml AA disrupt the redox balance inside the cancer cells by producing reactive oxygen species. The apoptotic event was mediated by two key regulator proteins TNF-α and NF-κβ which was proved here. The increment of the pro-inflammatory cytokines indicates the ROS mediated pathway of cancer cell apoptosis.

1. Introduction

In recent era the plant metabolites are used for their medicinal properties in various forms. Triterpenoids are the key plant derived secondary metabolites with its unique C30 subset along with chiral structure, arjunolic acid a 6,6,6,6,6-pentacyclic triterpenoid, containing a rigid pentacyclic backbone with functional groups like hydroxyl and carboxyl groups at opposite trimmings of the pentacyclic framework particularly two hydroxyl and one hydroxymethyl group attached to the 1st ring. In Pentacyclic backbone the 1-2, 2-3 & 3-4 rings are trans fused while the 4-5 rings are cis fused [1, 2, 3]. Arjunolic acid (AA) mainly derived from the Terminaliaarjuna (arjuna), a plant known for its beneficial effect as ayurvedic plant from ancient times to this modern times for its diverse biological aspects. AA acts as an anti-oxidant, free-radical-scavenger, anti-inflammatory, anti-platelet aggregation, hypolipidemic, anti-necrotic, anti-apoptotic, wound healing, antimicrobial, cardio protection, anti tumour etc. [4, 5].

Beside these above mentioned properties the AA was found from other sources likes Syzygiumguineense [6], Campsisgrandiflora [7], Combretumnelsonii [8], Cochlospermumtinctctorium [9], Leadchlaetodon [10], Cornuscapitata [11], Combretumleprosum [2, 12]. etc.as a secondary metabolites.

Cancer is a worldwide budding disease and the one of leading case of human mortality. It showed uncontrolled cell growth associated with loss of normal cellular function and deregulation of apoptosis. In 2018 there are 9.6 million people were deadly the cancer and was a major cause of 1 of 6 death, and new cancer cases took place on that year was 18.1 million due to our lifestyle and environmental changes [13]. In woman population throughout the world breast and cervical cancer are very common and foremost problem. There are the many chemotherapeutic agents in market to treat the cancer but the toxicity of these drugs towards normal cell is very high. So introduce of a novel drug which is derived from plant may be a potant candidate to treat the cancer [13].

The titerpenoids of T. arjuna. showed anticancer activity againstand human oral (KB), ovarian (PA1) and liver (HepG-2 and WRL-68) cancer cell line in vitro model and inhibiting different type of solid tumors (Leukemia P 388, Brain SF-295, Renal A-549, Gastric HGC-27,ovary SK-OV-3, SKMEL-2, HCT 15, XF-498) [14]. AA the active ingredients of T. arjuna. showed anticancer role against Ehrlich ascites carcinoma (EAC) and Dalton’s lymphoma (DL) in mice model [15].

* Corresponding author.
** Corresponding author.
E-mail addresses: braja@mail.vidyasagar.ac.in (B.G. Bag), sroy.vu@hotmail.com (S. Roy).

https://doi.org/10.1016/j.heliyon.2020.e03456
Received 18 September 2019; Received in revised form 31 October 2019; Accepted 18 February 2020
2405-8440/© 2020 Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
2. Methods & materials

2.1. Culture media and chemicals

Arjunolic acid (AA), Ethidium Bromide (EtBr), Acryldinoragace (AO) 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT reagent), fetal bovine serum (FBS), Histopaque 1077, were procured from Sigma (St. Louis, MO, USA). Minimum Essential Medium (MEM), RPMI 1640, sodium chloride (NaCl), sodium carbonate (Na2CO3), penicillin, streptomycin, sucrose, Hanks balanced salt solution, and ethylene diamine tetra acetate (EDTA) were purchased from Himedia, India. Commercially available dimethyl sulfoxide (DMSO) was procured from Himedia, India and all other chemicals were purchased from Merck Ltd., SRL Pvt. Ltd., and Mumbai, India. Commercially available dimethyl sulfoxide (DMSO) was procured from Himedia, India and all other chemicals were from Merck Ltd., SRL Pvt. Ltd. of its highest grade (purity) available.

2.2. Preparation of renewable AA nanoparticles & Physical measurements of drug

Preparation of renewable AA nanoparticles & Physical measurements of drug

AA was obtained in pure form following a procedure developed in our laboratory [1]. In brief, arjuna-bromolactone was dissolved in glacial acetic acid (105 ml) and the solution was treated with zinc dust (8.34 g, 127.6 mM) and stirred at room temperature. The development of the reaction was done by HPLC. After stirring for 30 min, the reaction mixture was filtered and washed with glacial acetic acid (15 ml). Cold water was added to the filtrate resulting precipitate further filtered and washed with water then allowed to dry which comes to white solid arjunolic acid (3.0 g, 97%). The self-assembled arjunolic acid was characterized by Transmission electron microscopy (TEM), scanning electron microscopy (SEM), Optical microscopy. The hollow structure of the vesicular self-assemblies of renewable nano sized arjunolic acid was proved by optical microscopy [16, 17].

2.3. Collection of peripheral blood lymphocytes (PBL)

Peripheral blood Lymphocytes (PBL) were collected from six human volunteers with physically healthy, disease free and not going through any medication. As per Helsinki the study was done [18].

As per Hudson and Hey description, 5 ml of blood collected from the volunteers by vein puncture process and took on a EDTA containing 1 ml centrifuge tube, then Histopaque 1077 (Himedia) was added carefully at a ratio of 1:3 and allowed to centrifuge with 1450 rpm for 40 min at 37 °C. After centrifugation the buffy coat are separated by using pipette and washing with PBS three times and re suspend on RPMI culture media and kept in CO2 incubator to achieve its exponential population by which the further experimental process was done [19].

3. Cell lines culture and maintenance

Two types of cancer cell lines, Breast cancer (MCF-7) & cervical cancer (HeLa) cell lines were obtained from Jadavpur University (India) and cultured in vitro in DMEM media supplemented with heat inactivated PBS(10%), antibiotic solution and kept in a CO2 incubator containing a mixture of 5% CO2 with 95% humidified air to achieve its exponential population by which the further experimental process was done [20].

3.1. Selection of dose & duration by In vitro cell viability assay

The Cell viability experiment was done by MTT assay with the cell population of \(2 \times 10^6\) by using tetrazolium salt which is known as MTT (3-[4,5-dimethylthiazol-2-yl] -2,5-diphenil-tetrazolium bromide), it is known for its staining activity of live mitochondria. After 24 h cell culture was performed with AA at different doses (1, 5, 10, 25, 50, 100 μg/ml). The cultivated cells were allowed to expose MTT solution for 2h 30 min, after that all cells were lysed using 15 min treatment of DMSO. Then the O.D. was measured by using ELISA reader at 570 nm [21].

The death percentage calculations were done by using the following formula:- % Cell death = [(OD control – OD sample)/OD control] X 100.

All the experiments were done in triplicate.

3.2. Drug uptake assay

Drug uptake assay was done by tagging the fluorescence dye Rhodamine B with the 1 ml AA of 50 μg/ml in DMSO-H2O mixture was mixed with 25μl (1 mg/ml) of Rh-B was allowed to stirrer in magnetic stirrer for 24hrs then the conjugated AA was collected by centrifugation at 4 °C and washed with de-ionized water to remove the un-conjugated Rh-B. Finally the Rh-B labelled AA was allowed to incubate with the cells. After the incubation period the cells were washed with PBS and observed under the fluorescence microscope (Nikon) [22].

3.3. Determination of GSH

To estimate the reduce GSH the treated cells were lysed with triton mixed with trichloroacetic acid (25%) and after the mixing allowed to centrifuge 15 min at 2000 rpm to precipitate the protein and the supernatant was collected to diluted with PBS (sodium phosphate buffer, 0.2 M, pH 8.0). Make the volume 1ml, 2ml (0.6mM) was added and kept for 10 min at room temp. At that time O.D. was measured at 405nm [23].

3.4. Determination of oxidized glutathione (GSSG) level

To estimate the oxidized glutathione or GSSG the treated cells were lysed and added 2μl of vinylpyidine, allowed to incubate for 1 h at 37 °C then 4% sulfosalicylic acid was added with the mixture to de-proteinize the cells. Then centrifugation at 2000rpm for 10 min performed to settle down the protein. The supernatant mixed with DTNB(0.6mM) and allowed to take O.D. immediately at 405nm [23].

3.5. ROS measurements

For determination of intercellular reactive oxygen species generation we incubated DCFH2DA 1 μg/ml stain with the treated cells for 30 min. After treatment the cells were washed 3 times with PBS and the DCF fluorescence intensity was measure under the fluorescence microscope [24].

3.6. Pre-treatment with N-Acetyl- L-Cysteine and pentoxifylline

To check out the involvement of ROS for the cell death, we treated the cells with N-acetyl- L-cysteine (NAC) in 5 mM for 5hr independently in another group pre treatment with 1 mMpentoxifylline (POF- a potent TNF-α inhibitor) for 24hr before drug treatment was conducted [25]. After pre treatment the cells were underwent through 24 h treatment schedule with AA (50 μg/ml) then the cell viability assay was done as previously said.

3.7. Morphological analysis by double staining with acridine orange (AO)–Ethidium bromide (ETBR)

To find out the possible mechanisms of cell death after the completing the treatment the cells were at first washed with the PBS and stained with double stain by adding 10 μl each of acridine orange (AO-50 μg/ml) and...
ethidium bromide (EtBr-50 μg/ml). After staining the cells were washed with PBS and observed under fluorescence microscope [26].

3.8. Nuclear morphology assessment using DAPI stain

The nuclear morphology changes were detected by using DAPI stain, in brief treated cells were incubated for 5 min with 1 μg/ml DAPI at 37 ºC. Then cells were washed with PBS and examined under the fluorescence microscope [27].

3.9. Measurement of pro-inflammatory and anti-inflammatory cytokine level by ELISA

According to the manufacturer’s procedure (Human ELISA Ready-SET-Go, E-bioscience, India) the different cytokines level (TNF-α, IL-12, IL-10 and TGF-β) were measured using ELISA kit after drug treatment in serum/FBS free 1ml culture media for 24hr. After treatment the supernatant 100μl from culture plate was taken for cytokine level estimation by ELISA method. The results (O.D. of different groups) were taken by using Bio-Rad ELISA micro plate reader [28].

3.10. Protein estimation

Proteins were estimated as per Lowey method and BSA taken as standard [29].

3.11. Statistical analysis

The all data were presented as mean value ± SEM, n = 6, Comparison between two groups were analysed by using Origin 6.1, p < 0.05 as a limit of significance.

4. Results

4.1. Cell viability assay

In this experiment in vitro cell viability study by MTT assay showed that the self assembled arjunolic acid (SA-AA) exhibited as a potent anticancer drug by decreasing of the intensity of stained live mitochondria is directly indicate the increase amount of cell death. The SA-AA killed breast cancer (MCF-7) (Figure 1a) and cervical cancer (HeLa) (Figure 1b) in a dose respective manner. SA-AA treated with 25 μg/ml, 50 μg/ml and 100 μg/ml respectively killed MCF-7 cells by 57.77%, 66.98%, 87.79% and HeLa cells by 66.04%, 79.34%, and 88.72%. Due to impressive anticancer activity simultaneously the normal cell toxicity was assessed because there are many anticancer drugs but their main drawback is cellular toxicity or side effects to the other organ or other non cancerous cell. We observed that nano sized SA-AA is good bio compatible drug (Figure 1c) and at 25 μg/ml, 50 μg/ml and 100 μg/ml dose the viable lymphocytes were 92.27%, 90.45%, 88.76% respectively. So this drug showed low toxicity towards

Figure 1. a) Does dependent anticancer efficacy of Arjunolic acid against MCF-7. b) Does dependent anticancer efficacy of Arjunolic acid against HeLa. c) Normal cell toxicity of Arjunolic acid in adoes dependent manner. Values has been expressed as a Mean ± SEM where P value < 0.05. All the experiments have done in triplicate.
lymphocytes. From this study we assumed that the 50 μg/ml concentration of SA-AA can be used for anticancer activity.

4.2. Drug uptake

In this study we observed that the Rhodamin B (Rh-B) conjugated self assembled nano size Arjunolic acid (SA-AA) successfully entered into both cancer cells MCF-7 & HeLa cell. The images showed that radish vesicle structure in the cells observed under fluorescence microscope which was Rh-B conjugated SA-AA (Figure 2).

4.3. Examination of cellular redox status (GSH and GSSG level)

SA-AA treated MCF-7 and HeLa cells showed the imbalance of cellular redox balance, decreased of GSH and increased of GSSG level compared with the control group significantly where P < 0.05.

From result it was found that in MCF-7, SA-AA (50 μg/ml) treatment significantly (p < 0.05) decreased GSH level (Figure 3a) by 8 folds and significantly (p < 0.05) elevated GSSG level (Figure 4a) by 5 folds as compared to control. Treatment with SA-AA (50 μg/ml) in HeLa showed decreased GSH level (Figure 3b) by 7.9 folds and increased GSSG level (Figure 4b) by 4.28 folds as compared with control and the in normal cells there is no significant change on GSH and GSSG (Figures 3a and 4a).

4.4. Estimation of cellular ROS level

Intracellular reactive oxygen species level was shown in Figure 5. It was found that the treatment with SA-AA (50 μg/ml) elevated cellular ROS level than the control group in MCF-7 (Figure 5b) & HeLa (Figure 5d) cells respectively. It means SA-AA treatment elevated the ROS generation in cellular level.

4.5. Et Br-AO double staining for cell morphology study

The double staining by EtBr/AO showed that the Control group of both cancer cells MCF-7 and HeLa cells remained in greenish fluorescence which indicates the live cell population where as when the cancer cell treated with the SA-AA in 50 μg/ml does for the cell population emitted orange redish-orange colour which exhibited the most of cancer cells are underwent to apoptosis due to exposure of SA-AA.

Morphological study seemed that the AA treated group on MCF-7 (Figure 6b) & HeLa (Figure 6d) cancer cells showed orange coloured cells and significantly presence of apoptotic bodies and membrane blabbing that of the reddish colour and disappearing of greenish cell population which primarily indicated the cell killing through the apoptosis not necrosis.

4.6. Nuclear morphological changes using DAPI staining

The nuclear specific fluorescence stain DAPI, binds strongly to A-T rich regions of DNA and emitted blue fluorescence light under fluorescence microscope when placed under uv light, which directly shows the morphological condition of the nucleus. This staining represented the, changes associated with nuclear damage and apoptosis occurred in MCF-7 & HeLa cells treated with the SA-AA. In fluorescence study it was evidently proved that the treatment with SA-AA caused morphological changes like nuclear fragmentation and chromatin condensation upon MCF-7 (Figure 7b) & HeLa cells (Figure 7d).

4.7. Changes in Cytokine level

After treatment with the SA-AA it was noted that the SA-AA reduced the anti apoptotic cytokines IL-10 and TGF-β in case of two types of cancer cell lines. IL-10 reduced by 2.8 folds in MCF-7 and 3.1 folds in

Figure 2. Rhodaminconjugated Drug uptake of MCF-7 and HeLa cells (respectively upper and Lower groups) where A,C were control group and B,D were treated group.
HeLa cells compare with the untreated group, TGF-β decreased 2.6 folds in MCF-7 and 2.9 folds in HeLa cells compared with the untreated group. Whereas SA-AA elevated pro-apoptotic cytokines levels. IL-12 increased 1.8 folds in MCF-7 and 2 folds in HeLa cells compared with the untreated group, TNF-α increased 2.2 folds in MCF-7 and 2.1 folds in HeLa cells compared with the untreated group (Figure 8).

4.8. Pathway detection through NAC & POF treatment

In both two type of cancerous cell the study showed that the pre treatment with NAC protects the cell to avoid cellular death. After exposure of SA-AA (50 μg/ml) in both group Pre treatment with NAC which is a ROS quencher, reduced the cancer cell death to 47.45% and 46.64% respectively in MCF-7 and HeLa cells whereas without NAC protection the cell death was 66.98% and 79.34% respectively in MCF-7 and HeLa (Figure 9).

NAC known as ROS quencher it indicated NAC inhibited the ROS production by SA-AA, Sowe can assumed that SA-AA attacked the cancerous cell on its ETC chain and produced ROS which provoked the nuclear and membrane damage leads to cell death.

In case of pre treated with POF a potent TNF-α blocker, on POF protected SA-AA exposure in MCF-7 and HeLa cell. The cell viability was restored, it means it protected the cell death of MCF-7 &HeLa cells by 48.87% and 43.72% respectively whereas the only SA-AA treated group killed 66.98% and 79.34% respectively (Figure 9). So, it seemed that the SA-AA acts through TNF-α.

5. Discussion

Nowadays, cancer is considered as a human tragedy and one of the most prevalent diseases in the world, and its mortality is being increased and there are necessity to evolve a new strategy to treat cancer [30, 31] but much of them shows toxicity to the normal cell [32]. In this purpose to reduce the toxicity Plant products (secondary metabolites) are often considered as safe. Our study showed that the AA is a pentacycliterpinoids which was isolated from the bark of Terminalia arjuna which was self-assembled and successfully entered into the cancer cell due to self-assembled nano sized structure [33].

In the present study we have extracted AA from by extraction method [1]. The self-assembled property of AA was prepared when it was mixed with DMSO and water with a ratio of 16:4. The unique property of AA arised due to its molecular interaction between each other which make it vesicle structure SA-AA [34].

Here from the MTT/cell viability assay we observed that the SA-AA possesses a good anticancer effect with no or minimum toxicity on PBL (9.75%). HeLa cell killing activity (79.34%) was higher than the MCF-7 cells (66.98%) compared to control group after the 24 h treatment at 50 μg/ml dose.

GSH and GSSG level maintained the homeostatic level in our system, protects from free radicals and toxic substances. In pathological condition GSH converted to GSSG by oxidation. Present study shows that the SA-AA exhibited anti-proliferative effect on MCF-7 and HeLa cell line by reducing GSH level and increasing GSSG level by conversion GSH to GSSG due to free radical generation which damaged the different cellular organelles and hampers the ETC system which leads to cancer cell death [35, 36, 37].

Effect free radical generation after the treatment with AA also supported by DCFH2DA staining, a ROS generation marker [38]. It was observed that the in 50 μg/ml SA-AA caused the elevation of ROS generation significantly than the control; it may be the interaction of hydroxyl and keto group presence in the arjunolic [39] acid with ETC system.
Apoptotic study with the EtBr/AO seemed that thenano size arjunolic acid responsible for the cellular death by reddish-orange color stain and the live cells with greenish colour of the population [40]. In our study showed the presence of reddish orange is more than the greenish in SA-AA treated group it means the SA-AA activated the apoptotic pathway of arjunolic acid.

Nuclear degeneration or morphology change studies with DAPI stain revealed that the SA-AA directly interacted with the Nucleus of MCF-7

Figure 4. Estimation of GSSG level. a) Arjunolic acid against normal lymphocytes. b) Arjunolic acid against HeLa. c) Arjunolic acid against MCF-7. Values has been expressed as a Mean ± SEM where P value < 0.05. All the experiments have done in triplicate.

Figure 5. ROS estimation of MCF and HeLa cells (respectively upper and Lower groups) where A,C were control group and B,D were treated group.
HeLa cells and stop further proliferation of cell and caused cancer cell death [41].

Our results showed that both in MCF-7 and HeLa cells the SA-AA caused significantly elevation pre apoptotic cytokines TNF-α and IL12 and leads to apoptosis. It also noted that the SA-AA reduced the level of anti-apoptotic marker TGF-β and IL10 like other previous experiment on polyphenolic compounds [42].

The POF and NAC treatment showed that the SA-AA showed anticancer property through TNF-α and ROS generation because of the POF and NAC co-treated SA-AA did not showed that level of anticancer property like only SA-AA treated group, it inversely proved that SA-AA acts through TNF-α elevation and ROS production in cellular circumference of cancer cells [43]. It proved that the SA-AA causes the TNF-α elevation in normal cancer cell killing.

Figure 6. EtBr-AO double staining of MCF and HeLa cells (respectively upper and Lower groups) where A,C were control group and B,D were treated group.

Figure 7. Nuclear fragmentation of MCF-7 and HeLa cells (respectively upper and Lower groups) where A,C were control group and B,D were treated group.
So it can assumed that SA-AA a liposome like nano organic substance easily fused with cells and rapidly entered into the cell trigger c-Jun N-terminal Kinase pathway [44] which elevated the TNF-α makes ROS generation ultimately causing the nuclear damage and membrane bubbling and damage leads to cancer cell death [45].

6. Conclusion

From this study we can conclude that AA has self-assemble property with nano range size. Due to this interesting property it is easily permeable to cancer cells and makes it nontoxic to normal cells. So this organic compound has great chances to act as a potent anticancer drug. Further in vivo study is needed before clinical application.

Declarations

Author contribution statement

Subhankar Manna: Conceived and designed the experiments; Performed the experiments; Wrote the paper.
Aditi Dey: Performed the experiments; Wrote the paper.
Rakhi Majumdar: Contributed reagents, materials, analysis tools or data.
Braja Gopal Bag: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.
Chandradipa Ghosh: Analyzed and interpreted the data; Written the paper.

Somenath Roy: Conceived and designed the experiments; Wrote the paper.

Funding statement
This research did not receive any specific grant from funding agencies in the public, commercial, or non-profit sectors.

Competing interest statement
The authors declare no conflict of interest.

Additional information
No additional information is available for this paper.

References
[1] B.G. Bag, S.K. Dinla, P.P. Dey, V.A. Mallia, R.G. Weiss, Self-assembly of esters of arjunolic acid into fibrous networks and the properties of their organogels, Langmuir 25 (2009) 8663–8671.
[2] T. Hemalatha, S. Pulavendran, C. Balachandran, B.M. Manohar, R. Puvanakrishnan, Arjunolic acid: a novel phytomedicine with multifunctional therapeutic applications, Indian J. Exp. Biol. 48 (2010) 238–247.
[3] B.G. Bag, S.R. Pramanik, G.C. Maity, Arjunolic acid in molecular recognition: first synthesis and cation binding studies of a novel Arjuna-18-crown-6, Supramol. Chem. 17 (4) (2009) 297–302.
[4] E. Toppo, S.S. Darvin, S. Esakkkimuthu, K. Buvanesvaragurunathan, T.A. Krishna, S.A. Caesar, A. Stalin, K. Balakrishna, P. Pandikumar, S. Ignacimuthu, N.A. Al-Dhabi, Curative effect of arjunolic acid from Terminaliaarjuna in non-alcoholic fatty liver disease models, Biomed. Pharmacother. 107 (2018) 979–988.
[5] M. Sumitra, P. Manikandan, D.A. Kumar, N. Arutselvan, K. Balakrishna, B.M. Manohar, R. Puvanakrishnan, Experimental myocardial necrosis in rats: role of arjunolic acid on platelet aggregation, coagulation and antioxidant status, Mol. Cell. Biochem. 224 (1-2) (2001 Aug 1) 135–142.
[6] J.D. Djoukeng, E. Abou-Mansour, R. Tabacchi, A.L. Tapondjou, H. Boud, D. Lontsi, M.H. Park, E.M. Ahn, N.I. Baek, Triterpenoids from the roots of Syzygium guineense (Myrtaceae), J. Ethnopharmacol. Biochem. 224 (1-2) (2001 Aug 1) 135–142.
[7] B.G. Bag, S.R. Pramanik, Arjunolic acid in the ethanolic extract of Combretum leprosum root and its use as a potential multi-functional phytomedicine and drug for neurodegenerative diseases: anti-inflammatory and antioxidant activities, J. Braz. Chem. Soc. 22 (2011) 65–74.
[8] R.S. Bhukani, Y.N. Shukla, A.K. Tripathi, V. Prajapati, S. Kumar, Insect Growth inhibitor activity of arjunolic acid isolated from Combupicatta, Phytochemistry 42 (2002) 68.
[9] V.A. Facundo, K.A. Rios, C.M. Medeiros, J.S.L.T. Militao, A.L.P. Miranda, R.A. Epifanio, M.P. Carvalho, A.T. Andrade, A.C. Pinto, C.M. Rezende, Arjunolic acid in the ethanolic extract of Combretum leprosum root and its use as a potential multi-functional photomedicine and drug for neurodegenerative disorders: anti-inflammatory and anticlinohemisteric activities, J. Braz. Chem. Soc. 16 (2005) 1309.
[10] V.A. Facundo, K.A. Rios, C.M. Medeiros, J.S.L.T. Militao, A.L.P. Miranda, R.A. Epifanio, M.P. Carvalho, A.T. Andrade, A.C. Pinto, C.M. Rezende, Arjunolic acid in the ethanolic extract of Combretum leprosum root and its use as a potential multi-functional photomedicine and drug for neurodegenerative disorders: anti-inflammatory and anticlinohemisteric activities, J. Braz. Chem. Soc. 16 (2005) 1309.
[11] WHO Reports, September 2018, https://who.int/cancer/PRGlobocanfinal.pdf.
[12] M. Saxena, U. Faridi, R. Mishra, M.M. Gupta, M.P. Darokar, S.K. Srivastava, D. Singh, S. Luqman, S.P. Khanuja, Cytotoxic agents from Terminalia arjuna, Planta Med. 73 (14) (2007) 1486–1490.
[13] A. Sai Ramesh, J. Godwin Christopher, C.R. Setty, V. Thakankami, In-vitro study on antioxidant property of Arjunolic acid, J. Pharmacogn. Herbal Form. 1 (2010) 1–11.
[14] B.G. Bag, S.K. Dinla, P.P. Dey, V.A. Mallia, R.G. Weiss, Self-assembly of esters of arjunolic acid into fibrous networks and the properties of their organogels, Langmuir 25 (15) (2009 Apr 24) 8663–8671.