Original article

Molecular perspective and anticancer activity of medicinal plants

Anis Ahameda,a,⁎, A. Panneerselvamb, Abdullah Alaklabic, Ibrahim A. Arifa, V. Ambikapathyb, N. Thajuddind

a,⁎ Corresponding author.
E-mail address: nanisahamed@gmail.com (A. Ahamed).
Peer review under responsibility of King Saud University.

a Prince Sultan Research Chair for Environment and Wildlife, Department of Botany and Microbiology, College of Sciences, King Saud University (KSU), Riyadh, Saudi Arabia
b Faculty of Science, Department of Biological Science, University of Jeddah, Jeddah, Saudi Arabia
c Department of Botany and Microbiology, A.V.V.M. Sri Pushpam College (Autonomous), Poondi, Affiliated to Bharathidasan University, Thanjavur, India
d Department of Microbiology, Bharathidasan University, Tiruchirappalli, India

A R T I C L E   I N F O

Article info
Received 24 October 2019
Revised 28 November 2019
Accepted 28 November 2019
Available online 17 December 2019

Keywords:
Aloe castellorum
Aloe pseudorubroviolacea
Anticancer activity
Molecular docking

A B S T R A C T

To evaluate phytochemical constituents from the methanolic extracts of medicinal plants Aloe castellorum and Aloe pseudorubroviolacea. The cytotoxic activity of Aloe castellorum and Aloe pseudorubroviolacea leaf extracts against Human colon cancer cell line (HCT-116) was also assessed. The two medicinal plant extracts having significant cytotoxic activity, meanwhile the methanolic extract of Aloe castellorum shows higher cytotoxic activity than Aloe pseudorubroviolacea extract. The Aloe castellorum shows remarkable activity against respective cell line than control. The characteristic chemical constituents of Aloe castellorum and Aloe pseudorubroviolacea leaf extracts were recognized from Gas chromatography and Mass spectrometry (GC–MS) technique. The molecular docking studies also support the cytotoxic activity.

© 2019 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

The aloe species are commonly applied for ethnomedicine plus pharmacological potential for evaluate various ailments, injuries, digestive ailments, infection also anti plasmodial, antimicrobial, anthelmintic in addition anti-inflammatory, activities of particular of the species has been established provided that reason for their use in ancestral medicine (Watt and Breyer-Brandwijk, 1962, Amoo et al., 2014). The Aloe extracts are medically important and their application have been used to treatment of skin cancer, arthritis, eczema, heart attacks, burns, psoriasis, digestive problems, leukemia, high blood pressure and diabetes (Hossain et al., 2013, Maharjan and Laxmipriya, 2015). The Aloe emodin (AE), a naturally derived from plant anthraquinone, is described to have potential antiproliferative activity in different cancer cell lines (Suboj et al., 2012). As diverse of Aloe species would have different phytochemical compounds due to interspecies difference and changing soil conditions as well as climate, direct correlation of biological activity would be incorrect (Botes et al., 2008, Maharjan and Laxmipriya, 2015) therefore essential to concentrate on aloe species particularly Aloe pseudorubroviolacea and Aloe castellorum due to no previous studies recorded.

United States is recorded that second leading cause of death by cancer related (ACS, 2019). The Colorectal cancer (CRC) recorded that the third utmost common cancer in the worldwide. WCRF (2012) as well as the second most common cancer through CRC in Saudi Arabia (Mosli and Al-Ahwal, 2012; Zubaidi et al., 2015). In position first among men (10.6%) and woman among third (8.9%) (Al-Ahwal et al., 2013), the death rate from CRC is 8.3%. CFAG (2014) in Saudi Arabia stated that World Health Organization (WHO). Furthermore, retrieved data from the Saudi Cancer Registry (SCR; http://www.scr.org.sa/) showed rising in CRC occurrence between 2001 and 2006, and very nearly increase twofold between 1994 and 2003. Furthermore, Patients in Saudi are likely to present at a more advanced stage and at a younger age matched with Western countries (Aljebreen, 2007; Sibiani et al., 2011; Mosli and Al-Ahwal, 2012). Hence we attempted to evaluate phytochemical constituents from the methanolic extracts of medicinal plants Aloe castellorum and Aloe pseudorubroviolacea with cytotoxic activity of Human Cancer cell line HCT 116.
2. Materials and methods

2.1. Plant collection and processing

The selected plant leaves were dried at room temperature. Appropriate quantities (500 mg) of the leaves and in plant were using methanolic extraction with 5 times. The collected methanol extract was centrifuged at 5000 g for 10 min at room temperature, then the supernatant of methanol was prudently pipetted out transferred to sterilized eppendorf tubes without disturbing the inter-phase residues for further analysis. (Ahmed et al., 2013).

2.2. Cancer cell culture

The colon cancer cells HCT-116 were acquired from National Centre for Cell Science, Pune, India. Cells were inoculated in 10% fetal bovine serum and 1% antibiotics (penicillin, streptomycin and amphotericin) supplied Dulbecco's Altered Eagles Medium (DMEM), used incubated with humidifier at 37°C in a moisturized atmosphere provided with 95% and 5% Carbon dioxide and air incubation. The leaf extracts of Aloe castellorum and Aloe pseudorubroviolacea was completely dissolved in 1% DMSO prior treatment with cell.

2.3. Cytotoxicity of plant extracts Aloe castellorum and Aloe pseudorubroviolacea against HCT-116 cells

The cytotoxicity of extracts Aloe castellorum and Aloe pseudorubroviolacea was evaluated by employing the yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) MTT analyze based on the reduction of MTT through action of mitochondrial reductase enzyme which presents in viable cells. The amount of reduction of MTT to formazan in extracts Aloe castellorum and Aloe pseudorubroviolacea treated and untreated cells

### Table 1

| Peak# | R. Time | Area | Area% | Height | Height% | A/H | Name |
|-------|---------|------|-------|--------|---------|-----|------|
| 1     | 7.763   | 43.73| 0.70  | 13.096 | 0.73    | 3.34| 1-dodecanamine N,N-dimethyl-(CAS)DI-METHYLDODECYLAMINE |
| 2     | 13.315  | 375.13| 5.98  | 156.747| 8.73    | 2.39| METHYL 9-OCTADECANOATE |
| 3     | 13.410  | 108.75| 1.73  | 49.909 | 2.78    | 2.18| METHYL 9-OCTADECANOATE |
| 4     | 13.499  | 529.032| 8.43  | 228.824| 12.75   | 2.31| Hexadecanoic acid, methyl ester (CAS) Methyl palmitate |
| 5     | 13.706  | 907.056| 14.46 | 332.088| 18.50   | 2.73| 9-OCTADECANOIC ACID (Z) |
| 6     | 13.788  | 626.585| 9.99  | 190.983| 10.64   | 3.28| 9-octadecenoic acid (Z) - (CAS) Oleic acid |
| 7     | 13.869  | 440.036| 7.01  | 112.245| 6.25    | 3.92| Hexadecanoic acid (CAS) palmitic acid |
| 8     | 13.942  | 128.880| 2.05  | 51.184 | 2.85    | 2.52| 4, Nonenoic acid, methyl ester (CAS) methyl 4 nonenoate |
| 9     | 14.058  | 237.996| 3.79  | 32.635 | 1.82    | 7.29| Cyclohexanol, 3-methyl-(CAS) 3- Methylocyclohexanol |
| 10    | 14.336  | 53.981| 0.86  | 15.715 | 0.88    | 3.43| 1 heptanol (CAS) HEPTANOL |
| 11    | 14.439  | 114.669| 1.83  | 23.845 | 1.33    | 4.81| 1 heptanol (CAS) HEPTANOL |
| 12    | 14.532  | 57.020| 0.91  | 17.814 | 0.99    | 3.20| Hexanoic acid, methyl (CAS) 2-Methylhexanoic acid |
| 13    | 14.619  | 99.149| 1.58  | 41.141 | 2.29    | 2.41| 1,2-Cyclohexanediol (CAS) CYCLOHEXAN, 1,2-BIS(HYDROXYMETHYL) |
| 14    | 15.284  | 1,503.548| 23.97 | 285.684| 15.91   | 5.26| 9,12-Octadecadienoic acid methyl ester, (E,E)-(CAS) Methyl linoleolde |...
Fig. 1. Gas chromatography–mass spectrometry chromatogram of the methanolic extract of Aloe castellorum.

Fig. 2. Mass spectrum for the major constituent 9-octadecenoic acid from the methanolic extract of Aloe castellorum.

Fig. 3. Gas chromatography–mass spectrometry chromatogram of the methanolic extract of Aloe pseudorubroviolacea.
were directly proportional to the number of viable cells, it was assessed by taking absorbance values by dissolving the formazan with DMSO. In brief, cells (1 × 10⁵) were plated as well as incubated for 24 h at 37 °C in a humidified condition used 96 well plates. After complete incubation the old medium was aspirated without disturbing cells then the cells were tested with different concentrations (5, 10, 20, 30, 40, 50, 60, 70 and 80 μg per ml) of extracts *Aloe castellorum* and *Aloe pseudorubroviolacea* dissolved DMEM, the plate was kept in same incubation condition. After incubation, 100 μL of MTT reagent of 5 mg/mL in PBS was subsequently added into each individual well further, the plates were incubated for 24 h at 37 °C. The resulting formazan was dissolved by adding 100 μL of DMSO and the absorbance of the reactant solution was recorded at 595 nm wavelength using a multiwell plate reader (Tecan Multimode Reader, Austria). The concentrations of the test sample which showed 50% of cell death was then calculated (Gunaseelan et al., 2017).

2.4. Assessment of apoptotic morphological changes

The treated and untreated HCT-116 cells by staining with Ethidium Bromide (EtBr) plus Acridine Orange (AO) dye was used to determine the apoptotic cells morphological changes by extracts *Aloe castellorum* and *Aloe pseudorubroviolacea* (Kar thikeyan et al., 2011). The cultivated cells were in 6-well plate (3 × 10⁴ cells/well) and treated with extracts of *Aloe castellorum* and *Aloe pseudorubroviolacea* at various dosage ranges (25, 50 and 75 μg) for 24 h. The cells were fixed in (methanol: glacial acetic acid (3:1)) for 30 min at 4 °C. The cells were stained with

![Fig. 4. Mass spectrum for major constituent 3-dodecenol of the methanolic extract of *Aloe pseudorubroviolacea*.](image)

Table 3

| Cytotoxic activity of *Aloe castellorum* extract against HCT-116 cell line. |
|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                  | 20 μg/ml | 40 μg/ml | 60 μg/ml | 80 μg/ml | 100 μg/ml | 150 μg/ml | 200 μg/ml | 250 μg/ml | 300 μg/ml |
| Methanolic Extract 1 | 89.09616 | 78.19116 | 68.09824 | 50.11659 | 38.28351 | 30.97484 | 23.20213 | 18.21367 | 10.44096 |
| SD               | 5.12683  | 2.939281 | 3.83362 | 2.436223 | 2.436223 | 1.406554 | 1.063255 | 2.436223 |             |

![Fig. 5. Cytotoxic activity of *Aloe castellorum* extract against HCT-116 cell line.](image)

Table 4

| Cytotoxic activity of *Aloe pseudorubroviolacea* extract against HCT-116 cell line. |
|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                  | 20 μg/ml | 40 μg/ml | 60 μg/ml | 80 μg/ml | 100 μg/ml | 150 μg/ml | 200 μg/ml | 250 μg/ml | 300 μg/ml |
| Methanolic Extract 2 | 98.261  | 93.50457 | 82.48356 | 69.02632 | 56.61319 | 40.48771 | 23.52131 | 18.21367 | 10.44096 |
| SD               | 5.669111 | 1.569363 | 1.937759 | 4.351558 | 3.157962 | 4.542227 | 2.582198 | 4.195673 |             |

(Ethidium Bromide (EtBr) plus Acridine Orange (AO) 1:1 ratio) and left for 30 min after washing cells with PBS. Consequently, washed the stained cells with Phosphate buffered saline and a FLoid cell imaging station (Invitrogen, USA) for observation. The apoptosis was assessed by counting the cells showing apoptosis features in the total number of cells observed in the microscopic field.

### 2.5. Measurement of intracellular ROS generation

Intracellular ROS production in extracts of *Aloe castellorum* and *Aloe pseudorubroviolacea* treated and untreated HCT-116 cells were estimated through non-fluorescent probe DCFH-DA that can merely pass into the intracellular cell matrix, there it was oxidized into fluorescent dichlorofluorescin (DCF) by the action of produced ROS.

![Cytotoxicity of Methanolic extract 2 on HCT 116 cells](chart.png)

**Fig. 6.** Cytotoxic activity of *Aloe pseudorubroviolacea* extract against HCT-116 cell line.

![Ao/EtBr Staining](images.png)

**Fig. 7.** Ao/EtBr Staining explores the effect of Methanolic extracts on apoptotic morphological changes in HCT 116.
Fig. 8. Rhodamine 123 staining explores the effect of Methanolic extracts on Mitochondrial membrane potential of HCT 116 cells.

Fig. 9. DCF-DA staining explores the effect of Methanolic extracts on ROS production in HCT 116 cells.
Consequently, the fluorescence strength was comparatively proportional to the level of ROS production (Jesudason et al., 2008). The HCT-116 cells were inoculated (1 × 10^6 cells/well) into 6-well plate, tested with various concentrations (25, 50 and 75 μg) of extracts Aloe castellorum and Aloe pseudorubroviolacea and placed in CO2 (5%) incubator for 24 h. Then the cells were exposed to 100 μL of DCFH-DA for 10 min at 37 °C. Fluorescence depth was estimated through excitation and emission filters fixed at 485 and 530 nm, respectively (Shimadzu RF-5301 PC spectrofluorometer). The results showed an increased percentage of fluorescence depth.

2.6. Determination of mitochondrial membrane potential

The assessment of mitochondrial membrane potential of treated and untreated HCT-116 cells was estimated by staining the cells with lipophilic cationic, Rhodamine-123 (Rh-123) dye (Johnson et al., 1980). The cells (3 × 10^4 cells/well) were cultured in 6 wells plate and treated with various concentrations of extracts Aloe castellorum and Aloe pseudorubroviolacea (25, 50 and 75 μg) for 24 h. Then Rhodamine-123 fluorescent dye was added into treated and untreated cells and left for 30 min. The Mitochondrial membrane potential was qualitatively examined under a FLoid cell imaging station (Invitrogen, USA). Subsequently, the cells were harvested by Trypsinization of cells and the fluorescence strength was estimated at 485/530 nm wavelength under Spectrofluorometer (Shimadzu, USA). The positive control compared with results which maintained without treatment.

2.7. Assessment of level of reactive oxygen species (ROS)

The reactive oxygen species (ROS) level was quantitatively assessed through the H2DCFDA staining method. For this, the colon cancer cell lines tested with extracts Aloe castellorum and Aloe pseudorubroviolacea were set with 70% ice-cold methanol. After that, incubation was done consuming 10 μL of H2DCFDA for 30 min at room temperature. The cells was measured through the fluorescence intensity using excitation wavelength of 480 nm also the emission wavelength of 530 nm through used a flow cytometer. The acquired date were analyzed by cylogic software (Wang et al., 2019).

2.8. Caspase-8, 9 and 3 activity assay

The Caspase-8, 9 and 3 activities were reflected through caspase assay kit, as per company’s instruction the procedure was followed. Soon after that the colon cancer cells were loaded in (6 well plate) then treated with extracts of Aloe castellorum and Aloe pseudorubroviolacea then permitted to incubate for 24hr at CO2 incubator. Then, the cells were allowed to treat with appropriate caspase-8, 9 plus 3 reagents and incubated for 2 h in dark room followed by optical density for caspase-8, 9 and 3 were measured with a microplate reader at 400 or 405 nm.

2.9. Statistical analyses

The all experiments data were conducted in three independent replicated and the results were stated as the mean ± Standard Deviation (Mean ± SD) analyzing through one-way analysis of variance between the obtained values (ANOVA). Values of P < 0.05 indicated the significant differences in data.

2.10. Molecular docking

Docking studies are key component to inspect the interaction, binding mode between compounds 9-octadecenoic acid, 3-dodecanol and the Musashi-1 (MSI1) protein via Autodockvina 1.1.2 (Trott and Olson, 2010). The crystal structure of MSI1 protein

Fig. 10. Effect of Methanolic extracts on HCT 116 cell morphology.
(PDB ID: 2RS2) was reserved from Protein Data Bank (http://www.rcsb.org). The 3D assembly of the compounds 9-octadecenoic acid and 3-dodecanol were achieved via ChemDraw Ultra 12.0 and Chem3D Pro 12.0 software. The input files for AutodockVina were created by using Autodock Tools 1.5.6 program package. The search grid of 2RS2 protein was fixed at center_x: -1.568, center_y: 0.832, and center_z: -4.286 with dimensions size_x: 20, size_y: 20, and size_z: 20 with spacing of 1.0 Å. The exhaustiveness value was set to 8. The other parameters were set to default for Vina docking and not mentioned. The compound having least binding affinity value is the best-scoring compound and the results were visually analyzed using Discovery studio 2019 program.

![Docked complex](a), molecular surface (b), 3D (c), and 2D (d) interaction modes of compound 9-octadecenoic acid within the binding site of 2RS2 protein.

Fig. 11. Docked complex (a), molecular surface (b), 3D (c), and 2D (d) interaction modes of compound 9-octadecenoic acid within the binding site of 2RS2 protein.
3. Results and discussion

3.1. Spectral characterization and structural elucidation of major compound

After chromatographic separation, the methanolic extracts of *Aloe castellorum* and *Aloe pseudorubroviolacea* were subjected to GS-MS analysis. The methanolic extract of *Aloe castellorum* contains around 20 chemical constituents (Table 1) and the major compound is 9-octadecenoic acid with the retention time value of 13.706. The methanolic extract of *Aloe pseudorubroviolacea* contains around 20 chemical constituents (Table 2) and the major compound is 3-dodecanol with the retention time value of 15.731. The Gas chromatogram and respective mass spectrum for major constituent of *Aloe castellorum* were shown in Fig. 1 and Fig. 2. The Gas chromatogram and respective mass spectrum for major constituent of *Aloe pseudorubroviolacea* were shown in Fig. 3 and Fig. 4.

![Docked complex (a), molecular surface (b), 3D (c), and 2D (d) interaction modes of compound 3-dodecanol within the binding site of 2RS2 protein.](image)

Fig. 12. Docked complex (a), molecular surface (b), 3D (c), and 2D (d) interaction modes of compound 3-dodecanol within the binding site of 2RS2 protein.
### Table 5

| Compounds                      | Binding affinity (kcal/mol) | No. of H-bonds | H-bonding residues |
|--------------------------------|-----------------------------|----------------|-------------------|
| 9-octadecenoic acid            | -3.3                        | 1              | Arg61             |
| 3-dodecanol                    | -3.0                        | 1              | Arg59             |

#### 3.2. Cytotoxic activity

The plant extracts of *Aloe castellorum* and *Aloe pseudorubroviolacea* were evaluated for their cytotoxic activity examined against human colon cancer cell line (HCT-116). The methanol extracts of *Aloe castellorum* shows 89.09% of cell viability at 20 μg/mL and *Aloe pseudorubroviolacea* shows 98.26% of cell viability at 20 μg/mL individually. The values are abridged in Table 3, Fig. 5 and Table 4, Fig. 6. The effect of methanol extracts on apoptotic morphological changes in HCT 116 cell line were shown in Fig. 7. The effect of methanol extracts on Mitochondrial membrane potential of HCT 116 cell line were shown in Fig. 8. The effect of methanol extracts on ROS production in HCT 116 cells were shown in Fig. 9. The effect of methanolic extracts on HCT 116 cell morphology were shown in Fig. 10.

#### 3.3. Docked results with AutoDock vina

The compounds 9-octadecenoic acid and 3-dodecanol were studied for their docking behavior with 3OGN protein via AutoDock Vina program. The compound 9-octadecenoic acid shows good binding affinity (−3.3 kcal/mol) than 3-dodecanol with the binding affinity of (−3.0 kcal/mol) in 2RS2 protein respectively. Hydrogen bonding is one of the significant factor in the stability of protein-ligand binding, and the favorable bond distance amongst the H-donor and the H-acceptor atoms is less than 3.5 Å (Taha et al., 2015). The hydrogen bond distances of compounds 9-octadecenoic acid and 3-dodecanol were less than 3 Å in respective 2RS2 protein signifies strong hydrogen bonding. Compound 9-octadecenoic acid forms only one Hydrogen bond interaction with the receptor 2RS2. The amino acid residue Arg61 (bond length: 2.29 Å) was involved in hydrogen bonding contact. The amino acid residues Arg61 and A 106 were involved in hydrophobic interactions. The interactions of compound 9-octadecenoic acid with 2RS2 protein were shown in Fig. 11. The compound 3-dodecanol forms only one hydrogen bonds with the receptor 2RS2. The amino acid residue Arg59 (bond length: 2.36 Å) was involved in hydrogen bonding contact. The amino acid residues Arg59 and Arg61 were involved in hydrophobic interactions. The interactions of compound 3-dodecanol with 2RS2 protein were shown in Fig. 12. The results shows that compound 9-octadecenoic acid having remarkable inhibition ability than compound 3-dodecanol in anticancer protein 2RS2. The results were summarized in Table 5.

### 4. Conclusions

The conclusion of this study, which includes the extraction of phytochemical constituents from medicinal plants *Aloe castellorum* and *Aloe pseudorubroviolacea*. The methanolic extract of particular plants were further studied for their cytotoxic activity against human colon cancer cell line (HCT-116). The two plant extracts having significant cytotoxic activity, meanwhile the methanolic extract of *Aloe castellorum* shows higher cytotoxic activity than *Aloe pseudorubroviolacea* extract. The effect of apopotic morphological changes, Mitochondrial membrane potential, ROS production, and cell morphology on HCT-116 cell line are also assessed. Therefore, the methanolic extracts of medicinal plants *Aloe castellorum* and *Aloe pseudorubroviolacea* having potential cytotoxic chemical constituents itself for the development of new era cytotoxic drugs for colon cancer.

### Acknowledgement

The project was supported by King Saud University, Deanship of Scientific Research Chair. We are very grateful to Prince Sultan Research Chair for Environment and Wildlife & Saudi Biological Society. We thank the Department of Botany & Microbiology, College of Sciences, King Saud University (KSU), Riyadh, Saudi Arabia for encouragement and support for funding this work.

### References

Ahmed, A.B.A., Rao, A.S., Rao, M.V., Taha, R.M., 2013. HPTLC/HPLC and gravimetric methodology for the identification and quantification of gynemic acid from Gymnema sylvestre methanolic extracts. Acta Chromatogr., 2–10 https://doi.org/10.1556/ACchrom.25.

Al-Ahwal, M.S., Shaﬁk, Y.H., Al-Ahwal, H.M., 2013. First national survival data for colorectal cancer among Saudis between 1994 and 2004: What’s next?. BMC Public Health 13, 73.

Aljebreen, A.M., 2007. Clinico-pathological patterns of colorectal cancer in Saudi Arabia: Younger with an advanced stage presentation. Saudi J. Gastroenterol. 13 (2), 84.

American Cancer Society, 2019. Global Cancer Facts and Figures. American Cancer Society, Atlanta.

Amoo, S.O., Aremu, A.O., Van Staden, J., 2014. Unraveling the medicinal potential of South African Aloe species. J. Ethnopharmacol. 153 (1), 19–41.

Botes, L., Van Der Westhuizen, F.H., Loots, D.T., 2008. Phytochemical content and antioxidant capacities of two Aloe greatheadii var. davayana extracts. Molecules 13, 2169–2180. https://doi.org/10.3390/molecules13092169. PMid: 18830148.

CFAG – Colorectal Cancer Dubai. UAE: Centre for Arab Genomic Studies; [Last cited on 2014 Jul 11]. Studies CFAG. Available from: http://www.cags.org.ae/me2cancersenicolon.pdf.

Gunaseelan, S., Balupillai, A., Govindasamy, A., Ramsamy, K., Muthusamy, G., et al., 2017. Linalool prevents oxidative stress activated protein kinases in single UVB-exposed human skin cells. PLoS One 12, 5.

Hossain, M.S., Mamun-Or-Rashid, A.M.N., Towﬁque, N.M., Sen, M.K., 2013. A review on ethnopharmacological potential of Aloe vera l. Intercult Ethnopharmacol. 2 (2), 113. https://doi.org/10.5455/jiec.20130612035300.

Jesudason, E.P., Masilamoni, J.G., Jesudoss, K.S., 2005. The protective role of DL-alpha-lipoic acid in the oxidative vulnuria-bility triggered by Abeta-amylloid vaccination in mice. Mol. Cell Biochem. 270, 29–37.

Johnson, L.V., Walsh, M.L., Chen, L.B., 1980. Localization of mitochondria in living cells with rhodamine 123. Proc. Natl. Acad. Sci. 2, 990–994.

Karthekeyan, S., Kaninomizu, G., Prasad, N.R., 2011. Radiosensitizing effect of ferulic acid on human cervical carcinoma cells in vitro. Toxicol. In vitro 25 (11), 1366–1375.

Mahrjan, R., Laxmi, R., N. 2015. Evaluation of biological properties and clinical effectiveness of Aloe vera: a systematic review. J. Trad Complement Med. 5 (2015), 21–26.

Mosli, M.I., Al-Ahwal, M.S., 2012. Colorectal cancer in the Kingdom of Saudi Arabia: Need for screening. Asian Pac J. Cancer Prev. 13, 3809–3813.

Sibhani, A.R., Fallatah, H.L., Akbar, H.O., Qari, Y.A., Bazaarra, S., Merdad, A., et al., 2013. Colorectal cancer in Saudi Arabia king Abdul Aziz University Hospital: a five year experience. J. Med. Med. Sci. 2, 1126–1130.

Suboj, P., Babykutty, S., Valiyaparambil, G.D.R., Nair, R.S., Srinivas, P., Gopala, S., 2012. Aloe emodin inhibits colorectal cancer cell migration/angiogenesis by downregulating MMP-2/9, RhoB and VE-cad via reduced DNA binding activity of NF-KB. Eur. J. Pharm. Sci. 45, 581–591.

Taha, M., Ismail, N.H., Khan, A., Shah, S.A.A., Anwar, A., Halim, S.A., Fatmi, M.Q., Imran, S., Rahim, F., Khan, K.M., 2015. Synthesis of novel derivatives of oxindole, their urease inhibition and molecular docking studies. Bioorg. Med. Chem. Lett. 25 (16), 3285–3289.

Trott, O., Olson, A.J., 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efﬁcient optimization, and multithreading. J. Comput. Chem. 31 (20), 455–461.

Wang, L., Jia, X., Yang, Y., Yi, H., Liu, H., 2014. Green synthesis of gold nanoparticles from Scutellariabarba and its anticancer activity in pancreatic cancer cell (PANC-1). Artif Cell Nanomed. B 47, 1617–1627. https://doi.org/10.1111/1651-2214.12980.

Watt, J.M., Breyer-Brandwijk, M.G., 1962. The Medicinal and Poisonous Plants of the World. E. L. J. Intercult Ethnopharmacol. 2 (4), 1366–1375.