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Triterpenoid Saponins Discovery Research 2013-2016

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Abstract
Triterpenoid saponins isolated and characterized from various sources are reviewed. The recent techniques used in their isolation and structure elucidation are discussed. A compilation of the triterpenoid saponins isolated during the period 2013-2016 along with their occurrence, available physical data and spectroscopy used for their characterization is included. The biological activities and corrosion inhibition of the triterpenoid saponins are also discussed.

1. Introduction

Saponins are the plant secondary metabolites and occurs as glycosides of steroids, triterpenoids or steroidal glycosides of diverse chemical structures and various biological activities for commercial applications in the pharmaceutical, ingredients in cosmetics, fine chemicals, neutraceuticals industries, alleolochemicals in agriculture and green corrosion inhibitor in metal industry. Triterpenoid saponins are predominating constituents of this class and widely distributed throughout the plant kingdom and certain marine organisms and also marine flora and fauna. Previous review on triterpenoid saponins[1] covering the literature up to 2012 recently reported. This review will focus on isolation and structure determination of novel triterpenoid saponins, new triterpenoid saponins isolated and biological activities and corrosion inhibition of these products reported during 2013-2016.

2. Extraction and Isolation

The isolation of triterpenoid saponins are challenging due to their occurrence as complex mixture. The recent techniques HPLC and SPE for isolation of triterpenoid saponins in complex mixture have been applied.

Ulososides and urabosides, new triterpenoid saponins from the carribbean Marine Sponge *Ectyoplasia ferox* were isolated. The sponge specimen was lyophilized and extracted with dichloromethane-methanol (1:1) mixture. The dried CH_2Cl_2-MeOH extract was chromatographed by RP-C_{18} column and eluted with H_2O/MeOH (7:3) and MeOH/CH_2Cl_2 (1:1) mixture. The methanol fraction thus obtained was further purified by HPLC (Phenomenex, Gemini C_6-phenylhexyl $110A^0$, 250 X 10 mm, 5µm) with H_2O/Acetonitrile/TFA (Flow rate 3.0Ml/min, 60:40:0.1-45:55:0.1) to give ulososides F and Urabosides A & B[2].

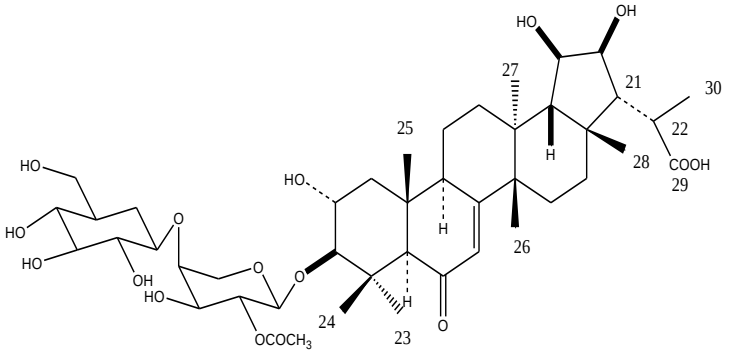
The aerial parts of *Spergula fallax* were macerated and extracted with 80% MeOH under reflux. The crude saponin extract obtained was dissolved in H_2O and purified by flash chromatography (C_{18} column 6x10cm, Lichroprep, RP-18, 40-60µm, Merck) on silica gel (particle size 40-60µm) under a pressure of 2 bar with H_2O, MeOH/H_2O, 20%, 40%, 60%, 80% and MeOH respectively. The fractions thus obtained were further purified by HPLC on C_{18} µ Bondapack column to yield four glycosides[3].

3. Structure Elucidation

The structure elucidation of pure saponins were generally investigated by a combination of chemical and spectroscopic methods. The newer spectroscopic techniques has been applied for complete structure determination of small amount of the intact saponin.

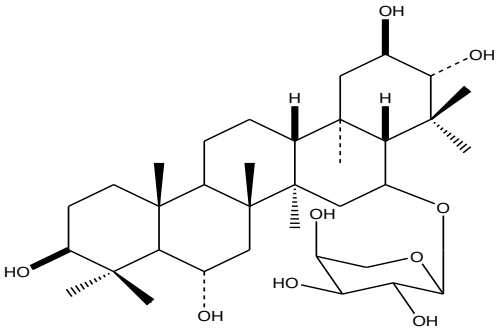
3.1. NMR spectrometry

The structures of four glycosides with novel aglycones isolated from *Spergula fallex* were determined by ^{1}H, ^{13}C, TOCSY and 2D NMR (DQF-COSY, HSQC, HMBC and ROESY experiments).



Compound **1**

The COSY correlation between H-3/H-2 was observed indicating the presence of two hydroxyl groups at C-2 and C-3 and also their configurations as α and β respectively by the coupling constant H-3 and by the ROESY correlations between H-2/Me-25 and H-3/H-5/Me-23. The HMBC correlations between H-7/C-5 and C-7/C-6 were observed suggesting the presence of double bond at C-7 and the carbonyl group at C-6 of the aglycone moiety. The ROESY spectrum correlations was observed between H-9/M-27 and H-18/Me-26/H-21. The ROESY correlations were not observed between H-9/Me-25, Me-28/H-18/H-21. Thus compound **1** is a fernane-type triterpenoid. The long range correlation between Me-30/H21 revealed that one of the methyl groups of the isopropyl function connected by a carboxylic group. ^{1}H & ^{13}C NMR indicated the presence of one arabinose and one glucose units. The linkage site of the two sugars arabinose and glucose were confirmed by HMBC spectrum. The configurations of the arabinose and glucose units were established from $^{2}J_{H\text{-}1,\ H\text{-}2}$ which were found to be 5.2 Hz and 8.0 Hz[3].



Compound **2 (2)**

The structure of a new triterpenoid glycoside, compound **2** with new aglycone was determined. The ^{1}H & ^{13}C, HSQC, HMBC and ROESY spectra identified the hydroxylated derivative of tetrahymanol type skeleton of the aglycone moiety attached with one sugar. It showed eight methyl groups, seven methine carbons and and one anomeric signal. The attachment of the arabinose unit to the C-16 of the aglycone was confirmed by HSQC and HMBC correlations. The L configurations of the arabinose unit was established by GC analysis[3].

3.2. Mass spectrometry

The molecular masses of saponins are conveniently determined by Fast atom mass spectrometry (FABMS) and electrospray ionization mass spectrometry (ESIMS) in the positive and / or negative mode.

The molecular ion peak at *m/z* 877 $[M+Na]^+$ and other fragments ions at *m/z* 835 $[M+Na-42]^+$, *m/z* 673 $[M+Na-42-162]^+$ and *m/z* 541 [M+Na-42-162-132] were obtained in positive ESIMS for the monodesmoside fernane saponin [3]. In the ESIMS/MS spectrum the $[M+H]^+$ ion at *m/z* 881 of oleanane saponin provided fragments by loss of two units at *m/z* 705 and *m/z* 529 indicating the presence of two glucuronic acid or galactouronic acid in the glycone moiety[4].

4. Biological activities

4.1. Antimicrobial activity

Triterpenoid saponins exhibit divergent antimicrobial activities. Saponins are generally good antifungal and antibacterial agent. The antifungal activity is found to be more effective with saponins than the sapogenins and the acetylated saponins, the activity being highly influenced by the number of component monosaccharides and their sequence.

Antibacterial activity of the methanolic extract containing saponins of the leaves of *Pavetta indica* was investigated against *Bacillus subtilus, Escherichia coli* and *Saccharomyces cerevisiae* using disc diffusion assay. The antimicrobial studies was performed using 250mg/ml of the crude extract in DMSO and serially diluted (1:1) with media to concentrations of 125, 62.5, 31.3, 15.6, 7.8, 3.9, 1.95 and 0.98mg/ml. It showed bactericidal activity against *Bacillus subtilus* (**Table 1**). Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) were found to be between 1.95-7.89mg/mL after incubation at 30^0C for 18-24hrs. The inhibition zones formed around the discs was measured. Ampicillin (250µg/ml) and commercial Fluconazole (10mg/ml) were used as positive controls (**Table 2**). The leaf extracts in 1.5ml microfuge tubes at a 250mg/ml concentration in DMSO were treated at 40^0, 60^0, 80^0 and 100^0C and autoclaved at 15psi for 15min separately to determine the effect of temperature on the stability. The samples were cooled to room temperature and the residual antibacterial activities were determined against the bacteria by disc diffusion method. The isolated constituents of *Pavetta indica* may have application as preservative in food processing industry to inhibit the microbial growth in processed food products and human health care[5].

The antimicrobial effect of *Silene vulgaris* extract was studied by using broth microdilution assay for determination of minimum inhibitory concentrations in attempt to evaluate its preservative efficacy. *S.vulgaris* extract was found to be active against Gram-negative bacteria *Pseudomonas aeruginosa* and the yeast *Candida albicans* at the concentrations 3mg/ml and 6mg/ml whereas the extract was less active against *Escherichia coli* ATCC2522 and *Staphylococcus aureus* ATCC29213 and *Aspergilus brassiliensis* ATCC16404 (**Table 3**). The microbial challenges test results performed by the European Pharmacopocia using the tested strains demonstrated that *S.vulgaris* extract at 10% and 20% (w/w) reduced the bacteria and fungi inocula with a significant conservation during a period of 28 days compared to synthetic preservative, phenoxyethanol. The silene extract could be considered as an natural preservative for cosmetic formulation[6].

The in-vitro fungicide activity of hydroalcoholic extract of saponin mixture from *Sapindus saponaria* against 125 vaginal yeasts including *Candida* and *Saccharomyces* genus was evaluated by broth microdilution method. A time-kill assay was performed to determine the growth profile curves for *C.albicans* ATCC90028 in YPD broth that was exposed to 1560µg/ml HE (i.e the highest MIC value found) for 240 min (**Figure 1**). A substantial reduction of colony forming units (CFU) was observed compared with the control groups fluconazole and Nystatin indicating the fungicide activity of HE in the first 60 min of exposure. General morphological changes in *Candida albicans* ATCC90028 cells using SEM and TEM. Hydroalcoholic extract of *S.saponaria* was further studied. It suggests that saponins bind components of the cytoplasmic membrane in yeast cells and cause celllysis which occurred within minutes after contact (**Figure 2A**). After 30 min a sharp drop in CFU which persisted up to 120 min and reached a plateau unit until 240 min (**Figure 2B**) was observed. A significant reduction of the amount of yeast was observed after

30 min exposure to HE. **Figure 3B** show rupture of the cell wall indicating celllysis. The interaction between saponins and the membrane constituents of yeast cells caused the loss of intracellular contents and cell wall disorganization causing the presence of irregularities in the cell wall (**Figure 3C**) and consequently cell death[7].

The antibacterial activity of the methanol extract containing saponins of *Gymnema sylvestre* was investigated by agar well diffusion method against four Gram-negative (*Escherichia coli, Klebsialla pneumonia, Pseudomonas aerruginosa, Proteus vulgaris*) and five Gram-positive bacteria (*Bacillus subtilus, Enterococcus faicalis, Micrococococcum luteus, Staphylococcus aureus, Steptococcus pseumeniae*). Methanol extract exhibited higher inhibition zones against all the tested bacteria. The low MIC values of methanol extract were 15.6µg/ml against *B.subtilis, S.aureus,* 31.2µg/ml against *E.facelis, M.luteus, S.pneumoniae. G.sylvestre* may possess promising therapeutic action in the treatment of infectious diseases caused by the species like *E.coli* and *S.aureus.* The antimicrobial potential is due to the lytic action of the extracts causing cell death. The methanolic extract reacts with peptidoglycan layer of the outer lipolysaccharide layer to form protease inhibitors of the gram negative strains by damaging its cell membrane. Further studies needed to identification of saponins responsible for antibacterial activity[8].

The highest antifungal activity was observed in methanolic extract of *Bacopa monniera* at 10mg/ml, 5mg/ml, 2.5mg/ml and 1.25mg/ml concentrations, and maximum zone of inhibition was observed against *Aspergilus niger and Candida albicans* at 2.5mg/ml and 1.25mg/ml concentration where as in aqueous extract no antifungal activity was observed by the agar well diffusion method.. No antibacterial activity was observed against *S.aureus, B.subtilis, E.coli, Pseudomonas aeruginosa* in aqueous and methanolic extracts of *B.monniera* (L.)[9]. The antimicrobial activity studies of the ethanolic and water extract of the garlic (*Allium sativum*) by the agar well diffusion method against the test organisms *Staphylococcus aureus, Salmonella typhimurium* and *Pseudomonas aeruginosa* in comparison with gentamycin and chloromphenicolas positive controls showed that both the extract have higher inhibitory activity. Inhibitory test was carried by 1.5ml of the test organism from the 48h-old culture of the ethanol and water extract. 20ml of sterile media was aseptically poured in each dish. A clear zone around the disc of the ethanol and water extract after incubation at 37^0C for 24hrs indicative of inhibition was observed. The garlic extract may be used in foods and pharmaceutical products[10].

The invitro antimicrobial activity of methanolic fractions obtained from rhizome of *Curcuma longa* was investigated against standard strain and clinical isolated from *Staphylococcus aureus*. The antibacterial spectra showing zone of inhibition in millimeters and calculated as percentage by taking gentamycin as positive control with 100% inhibition. Scanning electron microscopic observations revealed that test pathogen treated with methanolic extracts showed morphological deformity with partial lack of the cytoplasmic membrane which leads to cell disruption. The ability of rhizome of *C.longa* methanolic extracts to inhibit the growth of test pathogen is an indication of its broad spectrum antimicrobial potential which may be employed in the management of microbial infection. The methanolic fraction of *C.longa* rhizome showed high potential to inhibit some pathogenic bacteria of *Staphylococcus aureus*[11].

4.2. Antitumor/Anticancer and Antioxidant activities

Jujuboside B isolated from the seeds of *Zizyphus jujuba* used as a traditional medicine for the treatment of insomnia and anxiety was investigated for the studies of antitumor mechanism in vivo and in vitro of AGS and HCT 116 human cancer cells in a tumor xenograft model. Cell viability was observed with IC_{50} values of 107 and 114µM in AGS human gastric cancer cells and HCT 116 human colon cancer cells. The IC_{50} value of 182µM in chang hepatic normal cells indicated a more potent cytotoxicity effect of Jujuboside B against cancer cells (**Figure 4A, 4B, 4C, 4D**). The annexin V positive apoptotic cell population increase in AGS cells and the sub G_1 phase increase in HCT 116 cells indicated apoptotic cell death (**Figure 5C, 15D**). Jujuboside B inhibited tumor growth 60% compared with the control group (**Figure 15A**) and reduced the

expression of the proliferation biomarker K_i-67 in the tumor tissues (**Figure 5C**). No toxicity and body weight change in each mouse were observed (**Figure 5B**).

Jujuboside B induced extrinsic pathway-mediated apoptosis through Caspase-8 activation and the increase in FasL and Caspase 3 activation and PARP-L cleavage detection (**Figure 6A, 6B, 6C**). The autophagy inhibitor bafilomycin A_1 (B_aF) decreased jujuboside-induced-cell-viability and increased pp38, pJNK, FasL, caspase-8-activation and caspase-3-activation. These results demonstrated jujuboside B induced protective autophagy to retard extrinsic pathway-mediated apoptosis and indicated by the formation of cytoplasmic vacuoles and microtubule-associated protein 1 light chain-3I (LC3-I) (**Figure 7, Figure 7A, 7B, 7C**)[12].

Cytotoxicity evaluation of crude extract and total saponin fraction of *Chlorophytum borivilianum* against MCF-7 and HCT-116 cancer cell lines using MTT cell viability assay indicated a higher cytotoxicity activity of the crude extract than the total saponin fraction on all cell lines being most effective and selective on MCF-7 human breast cancer cell line. Total saponin has higher IC_{50} than crude extract indicating highly quenching capacity of crude extract (**Table 4**)[13].

The effects of 100µg/ml of both water and ethanol extracts on pancreatic cancer cells derived from both water and ethanol extracts of *Papaya carica* on pancreatic cancer cells derived from both primary (MiaPaCa-2) and metastatic (ASPC-1) sites was assessed with control gemcitabine[14].

Human pancreatic cancer cells (MiaPaCa-2 and ASPC-1) were cultured at 37^0C, 5% CO_2, Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% foetal bovine serum (FBS), horse serum and L-glutamine (100µg/mL) was used for MiaPaCa-2, while 10% FBS in RPM1 media was used for ASPC-1. Cell viability was determined using the Dojindo Cell Counting Kit-8. Cells were seeded into a 96 well plate at 5 x 10^3 cells per well and allowed to adhere for 24h. The cells were then treated with 100µg mL^{-1} of crude papaya ethanolic extract, crude papaya water extract or gemcitabine (IC_{50} – 50nM). After 72h, 10µL of CCK-8 solution was added before incubating at 37^0C for 90 min. The absorbance was recorded (450nm), and cell viability was determined as a percentage of control. The ethanol extract decreased cell viability of MiaPaCa-2 and ASPC-1 pancreatic cancer cells by 81% and 54% at 100µg/ml. Ethanol extracts were more effective in inhibiting the proliferation of two pancreatic cells MiaPaCa-2 ($P=0.09$) and ASPC-1 cells ($P=0.04$) (**Table 5**) and were at least as effective as the chemotherapeutic agent

The rate of discoloration by extracts or antioxidant compounds indicates the potential of their scavenging in terms of hydrogen donating ability. The lowest absorbance at 517nm of reaction between DPPH and serial dilution of crude and total saponin extracts of mother plant tubers of *C.borivilianum* indicated higher free radical scavenging activity. Total saponin has higher IC_{50} than crude extract indicating highly quenching capacity of crude extract. The inhibition of peroxidation of macromolecules by extracts of mother plant tubers of *C.borivilianum* was investigated by ferrous ions (Fe^{2+}) chelating activity. The chelating abilities of the crude and total saponin extracts were 2.4% and 36.5% at 0.5 mg mL^{-1} and 30% and 72.2% at 2.5mg mL^{-1}, respectively. Total saponin in all concentrations (0.5, 1, 1.5 and 2.5mg mL^{-1}) showed high FIC values ($p<0.05$) indicating higher antioxidant activity. In this study FC_{50} value of total saponin was 1mg mL^{-1} but crude extract showed >2.5mg/mL.

The antioxidant activity of crude and total saponin extracts of *C.borivilianum* was also evaluated by the BCB assay. In this study the crude extract displayed stronger inhibition effect (80.6±0,8%) at 2mg compared to total saponin which showed 61±1.14% at the same quantity. IC_{50} of the crude and total saponin extracts showed 0.7 and 1.3 mg/mL indicating higher antioxidant activity of crude extract[13].

The ethanol extract of *Papaya carica* used as folk medicine showed higher antioxidant capacity than the water extract above 100µg/ml concentrations compared to the one-fifth of tocopherol using SSA and ABTS assay. The ethanol and water extract at concentrations 200µg/ml showed higher free radical scavenging capacity (**Table 6**)[14].

The antioxidant activities of the isolated saponin was assessed on the basis of the radical scavenging effect of DPPH from the leaves of *Tridox procumbens*. The IC_{50} value of saponin fractions was found to be 0.13mg/ml. It showed moderate to good antioxidant activity[15].
The antioxidant activities of saponin extracted from the root of *Garcinia kola* was studied in terms of the free radical theory of oxidation. The saponin extract at the dose of 100, 200 and 400mg/kg body weight daily for 7 days given to the hyperglycemic rats treated with 200mg/kg of control metformin. The in vivo antioxidant assay results showed that saponin at different concentrations significantly decrease the MDA level compared to metformin and the control group ($p<0.05$), the activities of antioxidant enzymes, superoxide dismutase (SOD) and catalase of albino rats given saponin increases compared with the control group. The in vitro antioxidant assay results suggest that the saponin extract has effective radical scavenging activity against DPPH, ferric induced, hydrogen peroxide, hydroxyl, nitric oxide, superoxide radicals in a dose dependent manner. The saponin extract have significant antioxidant and free radical scavenging activities and could be a potential source of natural antioxidant for antiaging factor[16].
The n-butanol extract of *Radix trichosanthis* has lowest IC_{50} compared with the EtOAc indicating the potential antioxidant reagent in vitro. The EtOAc extract and n-butanol extract have higher scavenging ability than that of control group in a time dependent manner. In vivo assay, an increase of SOD and T-AOC and decrease of MDA and LDH levels were only observed in n-butanol (2mg/kg/d of crude drug) extracts pretreatment group. An increase in MDA and decrease in SOD and T-AOC levels was observed in EtOAc extracts. n-butanol fraction has the antioxidant potency both in vitro and in vivo[17].

5. Green Corrosion Inhibition

Achyranthes aspera (AA) extracts were studied as corrosion inhibitor for mild steel (MS) in industrial water medium using gravimetric and electrochemical measurements. The inhibition efficiency was determined by hanging the steel coupon measuring 1.0 X 1.0 X 0.1cm^3 into the solution (100cm^3) containing the extract solution at 30^0-60^0C for 10 to 50h. The electrochemical experiments were performed using a CH-analyzer model CH1660D. Saturated calomel electrode, platinum counter electrode and mild steel as working electrode were used respectively as reference auxiliary electrode. Electrochemical impedence spectroscopy (EIS) studies were performed by the potential range from -350 to -800mV with a scan rate of 0.4mV_s^{-1} and in the frequency range from 10KHz to 0.05Hz with signal amplitude of ± 10mV. The surface morphology of the mild steel samples in the absence and presence of AA methanolic extracts (1200ppm in industrial water) at 30^0C was investigated by scanning electron microscopy (SEM) technique[18].
The inhibition efficiency of AA extracts increases with the inhibitor concentration and reaches a maximum at 1200ppm of AA extracts. IE% decreases with the increasing temperature in industrial water medium and remains constant at higher temperature. The adsorption of AA extracts in industrial water medium on MS surface obeys Langmuir adsorption isotherm. The adsorption process involved is both physorption and chemisorptions. Polarization curves indicated that AA extracts acted as mixed type of inhibitor.
The *Polisota hirsute* extract was found to be effective green inhibitor of aluminium alloys corrosion in 0.25M KOH environment at 303K. The extract inhibited the corrosion of aluminium alloys media by means of hindering both cathodic and anodic electrode processes because the greater the number of bonds in the extracts, the higher the inhibition efficiency. The inhibitive action was basically controlled by the concentration of the inhibiting extract in the medium. The inhibitor obeys both the Langmuir adsorption isotherm and the Temkin isotherm in the medium[19].
Thymus Vulgarize extract (TVE) was reported to be good corrosion inhibitor for copper and brass in acid media. Experimental study was investigated the efficiency of Thyme leaves extract as corrosion inhibitor for concrete reinforcing steel samples exposed to alkaline solution consisting of 2% KOH and 3% NaCl which is a simulation to the chloride contaminated concrete pore solution (SCP) using open circuit potential and

potentiodynamic polarization technique. Various concentration (100 ml/L, 150ml/L, 250ml/L) of TVE were used in this experiment[20].

The anticorrosion behaviours of agarwood leaves extracts in 1M HCl solution on mild steel were studied using weight loss, potentiodynamic measurement, electrochemical impedance spectroscopy and scanning electrol microscopy techniques. The extracts showed good inhibition efficiencies for the gravimetric electrochemical methods. EIS analysis revealed that inhibition efficiency increases proportionately with the concentration and the charge transfer resistance. The potentiodynamic polarisation measurements showed the extracts acted as mixed-type inhibitors with predominantly cathodic effectiveness. SEM techniques supported the success of corrosion inhibition with the presence of inhibitors and the methanol extract best fitted the Temkin adsorption isotherm while the aqueous extract best fitted the Langmuir and Temkin adsorption isotherms. The adsorption mechanisms for both extracts were mainly physisorption[21].

Emila sonchifolia extracts were investigated as green corrosion inhibitor of mild steel in 1.0M H_2SO_4 by weight loss method. This method showed that inhibition efficiency increased with an increase in ES extract concentration, but decreased with rise in temperature. Adsorption on mild steel was found to obey Langmuir adsorption isotherm and kinetic thermodynamic model of EI-Anady et al from the fit of experimental data. The presence of ES increased the corrosion activation energy. The free energy and heat of adsorption gave negative values. The values obtained support the physical adsorption mechanism[22].

Corrsion inhibitors from natural products have been considered preferential due to the environment friendly effect. Black tea extract containing triterpenoid saponins was tested as corrosion inhibitors for carbon steel in 1M HCl solution using electrochemical frequency modulation (EFM), Potentiodynamic polarization and electrochemical impedance spectroscopy (EIS) techniques.

Potentiodynamic polarization measurement indicated that black tea acted as a mixed type inhibitors. The adsorption of the extract on carbon steel is found to obey Temkin adsorption isotherm. SEM study confirmed the adsorption of the extract molecules on the carbon steel surface[23].

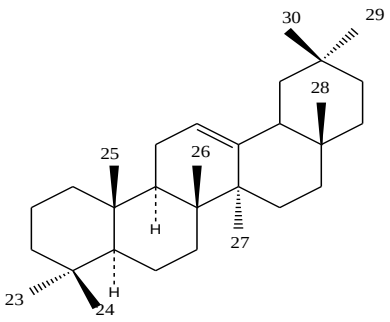
6. Conclusions

The clarification of structure-activity relationships on the basis of medicinal importance, agriculture, commercial value and potential for further research and development has been increasing among the novel and potential bioactive triterpenoid saponins. The recent trend has been for use of herbal and marine drugs or medicines among the people due to its low cost, natural abundance and least side effects. The applications of triterpenoid saponins in food processing industry and cosmetics have been increasing in recent years to explore alternative sources of safe, effective and acceptable natural preservatives. The study of saponins has by now provided enough material for scientists to extract structural information that can be used to make designed compounds. The study of corrosion inhibition of crude extract of plant saponins in metal industry has been developed as low cost and less environmental impact.

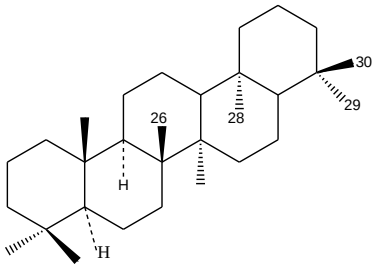
Reports of New Triterpenoid Saponins during 2013-2016

New triterpenoid saponins isolated during the period 2013-2016 together with their natural distribution, available physical data

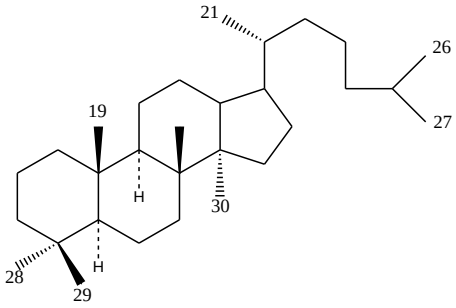
and various spectra recorded for their characterization and listed in **Table 7**. The structures **3-38** are of the aglycones of various saponins listed.



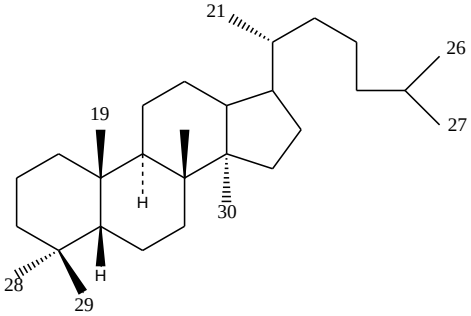
(3) HO-3β, 16α, 21α, CO_2H-28, acacic acid
(4) HO-3β, 16α, CO_2H-28, echinocystic acid
(5) HO-3β, 23, CO_2H-28, hederagenin
(6) HO-3β, 16α, CHO-23, CO_2H-28, gypsogenin
(7) HO-3β, 22β, 24, 29
(8) HO-3β, 22β, 24, CO_2H-29
(9) HO-3β, 21β, 22α, 24
(10) HO-3β, 22β, 24
(11) HO-3β, 24, oxo-22, CO_2H-29
(12) HO-3β, 21β, 22α, 24, 29
(13) HO-3β, CO_2H-28, 15:16-ene
(14) HO-3β, OAc-22β, oxo-11, CO_2H-30
(15) HO-3β, 24, oxo-11, CO_2H-30
(16) HO-3β, 24, oxo-11, CO_2H-29
(17) HO-3β, oxo-11, CHO-30
(18) HO-3β, oxo-11, 22β→30 lactone
(19) HO-3β, 21α, oxo-11, CO_2H-30
(20) HO-3β, 22β, oxo-11



(21) HO-3β, 6α, 16α, 20β, 21α



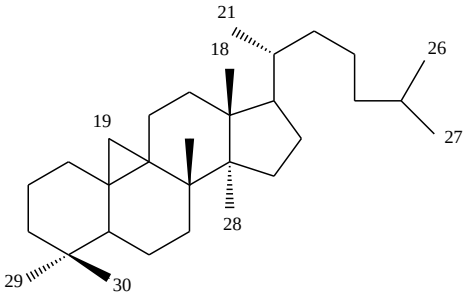
(22) HO-3β, 6α, 20(S), oxo-12, 24:25-ene
(23) HO-2α, 11α, 20(R), 25, OAc-3α
(24) HO-2α, 11α, 25, OAc-3α, 20:21-ene
(25) HO-2α, 11α, 20(S), OAc-3α, 24:25-ene
(26) HO-2α, 11α, 20(R), 25, OAc-3α, 24:25-ene



(27) HO-3β, 28, oxo-23, CO_2H-29, 8:9-ene

(28) HO-3β, oxo-23, CO_2H-28, 29, 8:9-ene

(29) HO-3β, 22(R), 23(S), CO_2H-29, 8:9-ene



(30) HO-3β,10(S), 24(R), 25, oxo-16, 23, 9, 10 seco, 7:8, 9:11-ene

(31) HO-3β,10(R), 24(R), 25, oxo-16, 23, 9, 10 seco, 7:8, 9:11-ene

(32) HO-3β,10(S), oxo-16, 23, 23:24(R)-epoxy, 7:8, 9:11-ene

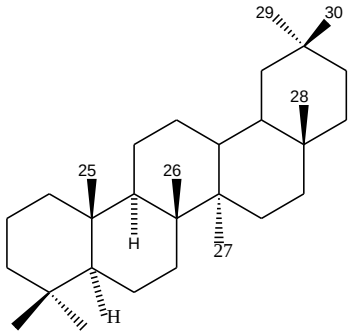
(33) HO-3β,11β, 23α, oxo-16, 23, 9, 10 seco, 7:8, 24:25-ene

(34) HO-3β, 23α, OAc-12β, oxo-24, 16β:23-epoxy

(35) HO-3β, 24£, 25, OAc-12β, 16β:23-epoxy, 22:23-ene

(36) HO-3β, 23(S), 24(S), OAc-12β, 16β:23, 22:25-epoxy

(37) HO-3β, 10(R), oxo-16, 23, 23:24-epoxy, 7:8, 8:9-ene



(38) OH-3β, CO_2H-29, 11:12, 13:18-ene

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Conflict of Interest

Financial support from CSIR, New Delhi.

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Table 1. Antimicrobial activity of *P.indica* leaf extracts against test microorganisms by disc diffusion method.

Extracts/controls	**Zone of inhibition (mm)**		
	B.subtilis	***E.coli***	***S.cerevisiae***
Methanol	7.5	No	No
Aqueous	7.5	No	No
DMSO	No	No	No
Ampicillion	28.0	13.0	No
Fluconazole			18.5

All the extracts used at 750 µg/disc. Inhibition zone diameter including disc diameter of 6 mm. No = No detectable inhibition. (-) = Not tested. DMSO was used as negative control. Ampicillion (250 µg/ml) and Fluconazole (10 mg/ml) were used as positive controls at 3 µl/disc.

Table 2. MIC and MBC values of aqueous and methanolic extracts of *P.indica* leaf extracts against *B.subtilis*.

Extracts	**MIC(mg/mL)**	**MBC (mg/mL)**
Aqueous	3.91-7.81	3.91-7.81
Methanol	1.95-3.91	1.95-3.91

Table 3. Minimum inhibitory concentration (MIC) and Minimum microbicidal concentration (MMC) of *S.vulgaris* extract

	***Silene vulgaris* extract**		**Streptomycin**	
	MIC[a]	**MBC[b]**	**MIC[a]**	**MMC[b]**
Staphylococcus aureus	25	50	0.004	0.004
Escherichia coli	25	50	0.008	0.064
Psedomonas aeruginosa	3	12.25	0.032	0.032
Candida albicans	6	6	-	
Aspergillus brasiliensis	100	100		

MIC[a]: minimum inhibitory concentration as mg/ml, MMC[b]: minimum microbicidal concentration (as mg/ml), - : not determined

Table 4: Free radical scavenging activity of crude and total saponin extract from mother plant tubers of *C.borivilianum*

Sample	Antioxidant activity	
	IC_{50} (µg ml^{-1})	AEAC (mg AA/100g)[12]
Total saponin	440+49[b]	1062+31
Crude extract	181±34	2578±111[a]

Results are expressed as means ± SD (n=3). For each column, values followed by different letter (a-b) are statistically significant ($p< 0.05$) as determined using ANOVA

[1]IC_{50} of AA = 4.5µg mL^{-1}

[2]100g fresh plant materials.

Table 5: Cell viability (%) of pancreatic cancer cell lines exposed to papaya leaf ethanol and water extracts, compared with gemcitabine

	Water extract	Ethanol extract	Gemcitabine
MiaPaCa-2	95.96±5.15[a]	18.96±1.52[b]	23.28±2.97
ASPC-1	107.68±4.67[cd]	45.94±3.51[e]	66.45±4.60

[a]$p<0.0001$ of ethanol extract and gemcitabine

[b]$p=0.09$ of gemcitabine

[c]$p<0.0001$ of ethanol extract

[d]$p<0.0004$ of gemcitabine

[e]$p=0.0036$ of gemcitabine

Table 6: Correlation of saponins with antioxidant capacity of the extracts

Antioxidant capacity	**R^2 value**
Saponins	
Total antioxidant capacity	0.7305
ABTS antioxidant capacity	0.6593
DPPH free radical scavenging capacity	0.5866
H_2O_2 radical scavenging capacity	0.3275
CUPRAC	0.4830
FRAP	0.5508

CUPRAC. Cupric ion-reducing antioxidant capacity

FRAP. ferric-reducing antioxidant power

Table 7. Triterpenoid Saponins Isolated during 2013-2016

Source (1)	Saponin, mp, $[\alpha]_D$, spectra recorded (2)	Structure (3)	Reference (4)
Albizia lebbeck (Leguminosae)	Lebbeckanin I 200-202^0, IR, ^{1}H, ^{13}C, 2D, ESIMS	Echinocystic acid (**4**) Xyl-^{2}Ara-^{6}(2'-acetylamino-2'-deoxy) Glc(OH-3β) Ara-^{4}Rha-^{2}Glc(CO_2H-28) |^3 (2'-acetylamino-2'-deoxy) Glc	(Abdel Ghani et al., 2016)
	Lebbeckanin II 233-235^0, IR, ^{1}H, ^{13}C, 2D, ESIMS	Acacic acid (**3**) Xyl-^{2}Ara-^{6}(2'-acetylamino-2'-deoxy) Glc(OH-3β) Glc(OH-21β) Xyl-^{2}Ara-^{6}(2'-acetylamino-2'-deoxy) Glc(OH-3β) Ara-^{4}Rha-^{2}Glc(CO_2H-28) |^3 (2'-acetylamino-2'-deoxy) Glc	(Abdel Ghani et al., 2016)
Aralia elata (Araliaceae)	Compound **1** -66^0, IR, ^{1}H, ^{13}C, ESIMS	Aglycone (**22**) Glc-^{3}Glc(OH-3β)	(Wu et al., 2014)
Astragalus tauricolus (Leguminosae)	Compound **2** +13.1^0, , IR, ^{1}H, ^{13}C, 2D, MALDITOFMS	Aglycone (**8**) Rha-^{2}Xyl-^{2}GlcA(OH-3β) Glc(CO_2H-29)	(Gulcemal et al., 2013)
	Compound **3** +9.3^0, , IR, ^{1}H, ^{13}C, 2D, MALDITOFMS	Aglycone (**7**) Rha-^{2}Glc-^{2}GlcA(OH-3β) Glc(CO_2H-29)	(Gulcemal et al., 2013)
	Compound **4** +22.4^0, , IR, ^{1}H, ^{13}C, 2D, MALDITOFMS	Aglycone (**9**) Rha-^{2}Xyl-^{2}GlcA(OH-3β) Rha(OH-21β)	(Gulcemal et al., 2013)
	Compound **5** +21.1^0, , IR, ^{1}H, ^{13}C, 2D, MALDITOFMS	Aglycone (**9**) Rha-^{2}Glc-^{2}GlcA(OH-3β) Rha(OH-21β)	(Gulcemal et al., 2013)
	Compound **6** +15.6^0, , IR, ^{1}H, ^{13}C, 2D, MALDITOFMS	Aglycone (**8**) Rha-^{2}Glc-^{2}GlcA(OH-3β) Glc(CO_2H-29)	(Gulcemal et al., 2013)
	Compound **7** +19.6^0, , IR, ^{1}H, ^{13}C, 2D, MALDITOFMS	Aglycone (**10**) Rha-^{2}Xyl-^{2}GlcA(OH-3β) Rha(OH-22β)	(Gulcemal et al., 2013)
	Compound **9** +9.8^0, , IR, ^{1}H, ^{13}C, 2D,	Aglycone (**11**) Rha-^{2}Glc-^{2}GlcA(OH-3β)	(Gulcemal et al., 2013)

	MALDITOFMS		
	Compound **11**	Aglycone (**12**)	(Gulcemal et al., 2013)
	$+16.8^0$, , IR, ^1H, ^{13}C, 2D, MALDITOFMS	Rha-^{2}Glc-^{2}GlcA(OH-3β)	
	Compound **12**	Aglycone (**8**)	(Gulcemal et al., 2013)
	$+16.8^0$, , IR, ^1H, ^{13}C 2D, MALDITOFMS	Glc-^{2}GlcA(OH-3β) Glc(OH-29α)	
	Compound **14**	Aglycone (**8**)	(Gulcemal et al., 2013)
	$+11.5^0$, , IR, ^1H, ^{13}C, 2D, MALDITOFMS	Xyl-^{2}GlcA(OH-3β) Glc(OH-29α)	
Chiococca alba (Rubiaceae)	Chiococasaponin III	Aglycone (**13**)	(Borges et al., 2013)
	^1H, ^{13}C, 2D	GlcA(OH-3β) Xyl-^{4}Rha-^{2}Ara(CO_2H-28)	
	Chiococasaponin IV	Aglycone (**13**)	(Borges et al., 2013)
	^1H, ^{13}C, 2D	GlcA(OH-3β) Rha-^{2}Ara(CO_2H-28)	
Cimicifuga foetida (Ranunculaceae)	Cimifoetidanoside A	Aglycone (**30**)	(Chen et al., 2014)
	+73.6, IR, ^1H, ^{13}C, 2D, ESIMS	Xyl(OH-3β)	
	Cimifoetidanoside B	Aglycone (**31**)	(Chen et al., 2014)
	-29.7, IR, ^1H, ^{13}C, 2D, ESIMS	Xyl(OH-3β)	
	Cimifoetidanoside C	Aglycone (**32**)	(Chen et al., 2014)
	+95.2, IR, ^1H, ^{13}C, 2D, ESIMS	Xyl(OH-3β)	
	Cimifoetidanoside D	Aglycone (**37**)	(Chen et al., 2014)
	-24.1, IR, ^1H, ^{13}C, 2D, ESIMS	Xyl(OH-3β)	
	Cimifoetidanoside E	Aglycone (**33**)	(Chen et al., 2014)
	-51.4, IR, ^1H, ^{13}C, 2D, ESIMS	Xyl(OH-3β)	
	Cimifoetidanoside F	Aglycone (**34**)	(Chen et al., 2014)
	-44.0, IR, ^1H, ^{13}C, 2D, ESIMS	Xyl(OH-3β)	
	Cimifoetidanoside G	Aglycone (**35**)	(Chen et al., 2014)
	-60.2, IR, ^1H, ^{13}C, 2D, ESIMS	Xyl(OH-3β)	
	Cimifoetidanoside H	Aglycone (**36**)	(Chen et al., 2014)
	-27.0, IR, ^1H, ^{13}C, 2D, ESIMS	Xyl(OH-3β)	
Combretum inflatum (Combretaceae)	Combretaside A	Aglycone (**23**)	(Williams et al., 2013)
	^1H, ^{13}C, ESIMS	Fuc(OH-2α)	

	Fuc(OH-11α)		
	Combretaside B	Aglycone (**23**)	(Williams et al., 2013)
	^{1}H, ^{13}C, ESIMS	Quin(OH-2α)	
	Fuc(OH-11α)		
	Combretaside C	Aglycone (**24**)	(Williams et al., 2013)
	^{1}H, ^{13}C, ESIMS	Fuc(OH-2α)	
	Fuc(OH-11α)		
	Combretaside D	Aglycone (**24**)	(Williams et al., 2013)
	^{1}H, ^{13}C, ESIMS	Quin(OH-2α)	
	Fuc(OH-11α)		
	Combretaside E	Aglycone (**25**)	(Williams et al., 2013)
	^{1}H, ^{13}C, ESIMS	Fuc(OH-2α)	
	Fuc(OH-11α)		
	Combretaside F	Aglycone (**25**)	(Williams et al., 2013)
	^{1}H, ^{13}C, ESIMS	Quin(OH-2α)	
	Ara(OH-11α)		
	Combretaside G	Aglycone (**26**)	(Williams et al., 2013)
	^{1}H, ^{13}C, ESIMS	Fuc(OH-2α)	
	Fuc(OH-11α)		
Ectyoplasia ferox (Raspailiidae)	Uraboside A -33.0, IR, ^{1}H, ^{13}C, 2D, ESIMS	Aglycone (**27**) Ara-^{2}Gal-^{3}Gal(OH-3β)	(Colardo et al., 2013)
	Uraboside B	Aglycone (**28**)	(Colardo et al., 2013)
	-130, IR, ^{1}H, ^{13}C, 2D, ESIMS	Gal-^{2}Glc(OH-3β)	
	Ulososide F	Aglycone (**29**)	(Colardo et al., 2013)
	-4.0, IR, ^{1}H, ^{13}C, 2D, ESIMS	GlcA-^{6}(NHAc-2')Glc(OH-3β)	
Glycyrrhiza uralensis (Fabaceae)	Uralsaponin M +204, UV, IR, ^{1}H, ^{13}C, 2D, ESIMS	Aglycone (**14**) Gal-^{2}GlcA(OH-3β)	(Song et al., 2014)
	Uralsaponin N	Aglycone (**15**)	(Song et al., 2014)
	+193, UV, IR, ^{1}H, ^{13}C, 2D, ESIMS	Gal-^{2}GlcA(OH-3β)	
	Uralsaponin O	Aglycone (**18**)	(Song et al., 2014)
	+188, UV, IR, ^{1}H, ^{13}C, 2D, ESIMS	Gal-^{2}GlcA(OH-3β)	
	Uralsaponin P	Aglycone (**19**)	(Song et al., 2014)
	+190, UV, IR, ^{1}H, ^{13}C, 2D, ESIMS	Gal-^{2}GlcA(OH-3β)	
	Uralsaponin Q	Aglycone (**19**)	(Song et al., 2014)
	+190, UV, IR, ^{i}H, ^{13}C, 2D, ESIMS	GlcA-^{2}Xyl-^{2}Rha(OH-3β)	
	Uralsaponin R	Aglycone (**19**)	(Song et al., 2014)
	+189, UV, IR, ^{1}H, ^{13}C, 2D, ESIMS	GalA-^{2}Glc-^{2}Rha(OH-3β)	

	Uralsaponin S +189, UV, IR, ^{1}H, ^{13}C, 2D, ESIMS	Aglycone (**19**) GlcA-^{2}Glc-^{2}Rha(OH-3β)	(Song et al., 2014)
	Uralsaponin T +199, UV, IR, ^{1}H, ^{13}C, 2D, ESIMS	Aglycone (**20**) GlcA-^{2}GlcA(OH-3β)	(Song et al., 2014)
	Uralsaponin U +192, UV, IR, ^{1}H, ^{13}C, 2D, ESIMS	Aglycone (**16**) GlcA-^{2}GlcA(OH-3β)	(Song et al., 2014)
	Uralsaponin V +187, UV, IR, ^{1}H, ^{13}C, 2D, ESIMS	Aglycone (**38**) GlcA-^{2}GlcA(OH-3β)	(Song et al., 2014)
	Uralsaponin W +170, UV, IR, ^{1}H, ^{13}C, 2D, ESIMS	Aglycone (**17**) GlcA-^{2}GlcA(OH-3β)	(Song et al., 2014)
	Uralsaponin X +193, UV, IR, ^{1}H, ^{13}C, 2D, ESIMS	Aglycone (**14**) GlcA-^{2} GlcA-^{2}Rha(OH-3β)	(Song et al., 2014)
	Uralsaponin Y +185, UV, IR, ^{1}H, ^{13}C, 2D, ESIMS	Aglycone (**18**) GlcA-^{2}GlcA-^{2}Rha (OH-3β)	(Song et al., 2014)
Momordica charantia (Cucurbitaceae)	Compound C_1 ^{1}H, ^{13}C, 2D, ESIMS	Gypsogenin (**6**) Glc-^{2}GlcA(OH-3β) Rha-^{3}Fuc(CO_2H-28) | ^{2} Xyl-^{3}Xyl- ^{4}Rha	(Ma et al., 2014)
	Compound C_2 ^{1}H, ^{13}C, 2D, ESIMS	Gypsogenin (**6**) Glc-^{2}GlcA(OH-3β) Rha-^{3}Fuc(CO_2H-28) | ^{2} Rha- ^{4}Xyl	(Ma et al., 2014)
Momordica charantia (Cucurbitaceae)	Compound C_1 ^{1}H, ^{13}C, 2D, ESIMS	Gypsogenin (**6**) Glc-^{2}GlcA(OH-3β) Rha-^{3}Fuc(CO_2H-28) | ^{2} Xyl-^{3}Xyl- ^{4}Rha	(Ma et al., 2014)
	Compound C_2 ^{1}H, ^{13}C, 2D, ESIMS	Gypsogenin (**6**) Glc-^{2}GlcA(OH-3β) Rha-^{3}Fuc(CO_2H-28) | ^{2} Rha- ^{4}Xyl	(Ma et al., 2014)
Polyscias fulva (Araliaceae)	Compound 11 213-215, -38.9, IR,	Hederagenin (**5**) Rha-^{2}Ara(OH-3β) (OAc-4')Rha-^{4}Glc-^{6}Glc(CO_2H-28)	(Njateng et al., 2015)
Sapindus	Compound 1	Hederagenin (**5**)	(Sharma et al., 2013)

Mukorossi (Sapindaceae)	^{1}H, ^{13}C, ESIMS	(OAc-3')Ara-^{2}Xyl(OH-3β) Rha(CO_2H-28)	
Spergula fallax Caryophyllaceae)	Compound 1 -36.0, IR, ^{1}H, ^{13}C, MALDITOFMS	(**1**)	(Hamed et al., 2014)
	Compound 2 +20.7, IR, ^{1}H, ^{13}C, MALDITOFMS	Aglycone (**21**) Ara(OH-16α)	(Hamed et al., 2014)
	Compound 3 +32.0, IR, ^{1}H, ^{13}C, MALDITOFMS	(**2**)	(Hamed et al., 2014)
	Compound 4 +5.23, IR, ^{1}H, ^{13}C, MALDITOFMS	Aglycone (**21**) (OAc-4')Ara(OH-16α) Glc(OH-20β)	(Hamed et al., 2014)

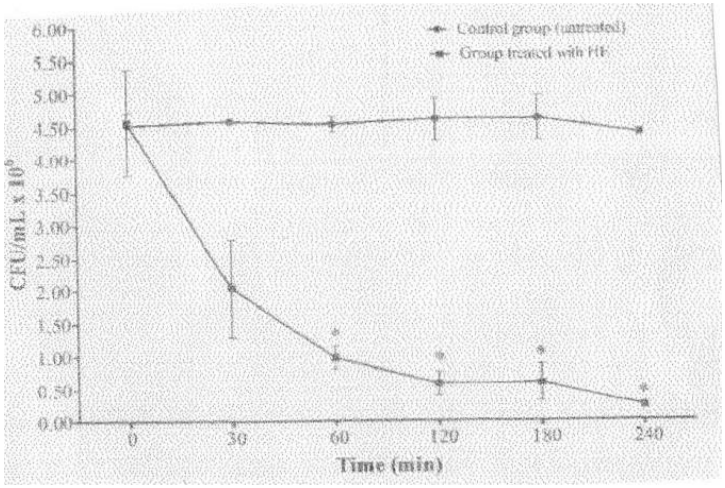

Figure 1. Time kill profile of *C.albicans* ATCC 90028 during exposition to HE (1560 µg/mL). CFU/mL: Colony forming unit per mililitre *Indicates significant reduction in CFU

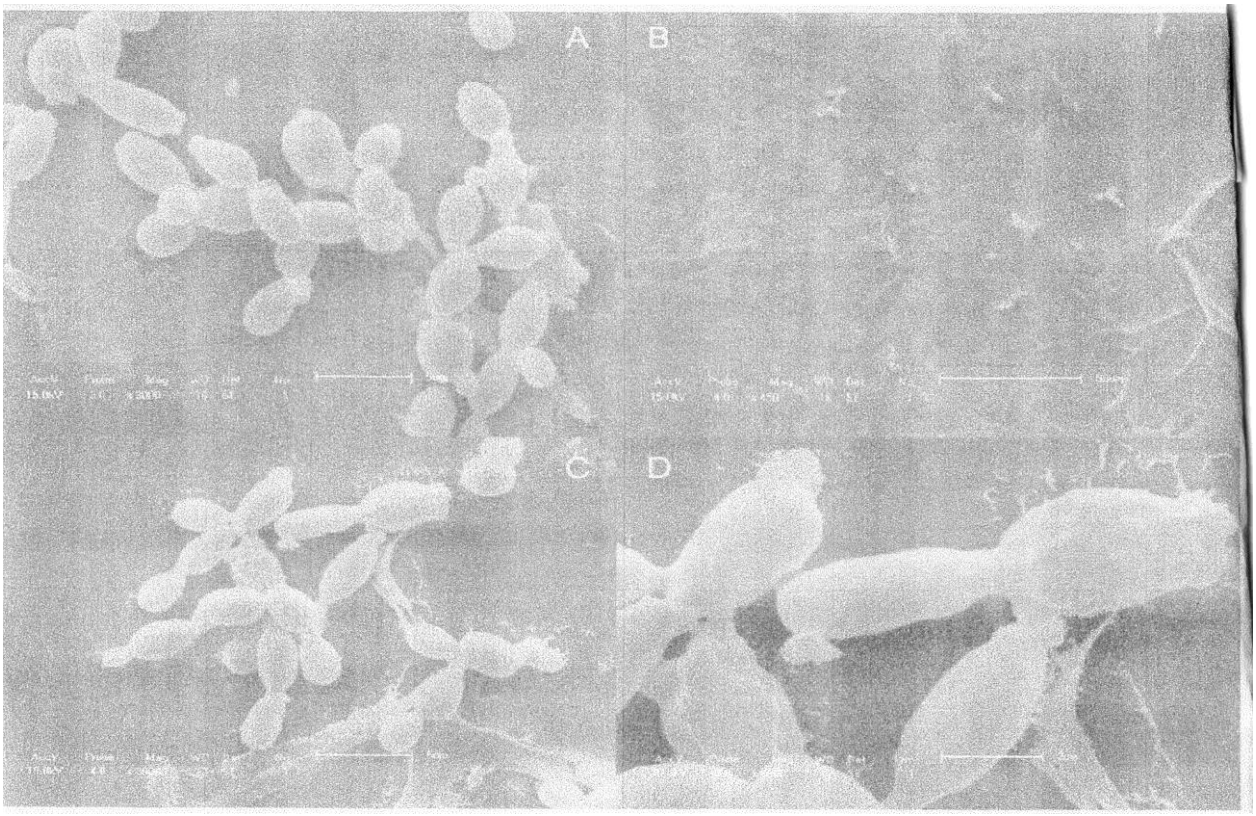

Figure 2. SEM micrographs of the untreated (**A**), 30 min (**B**) and 120 min (**C, D**) HE (1560µg/mL) treated *C.albicans* ATCC 90028 cells.

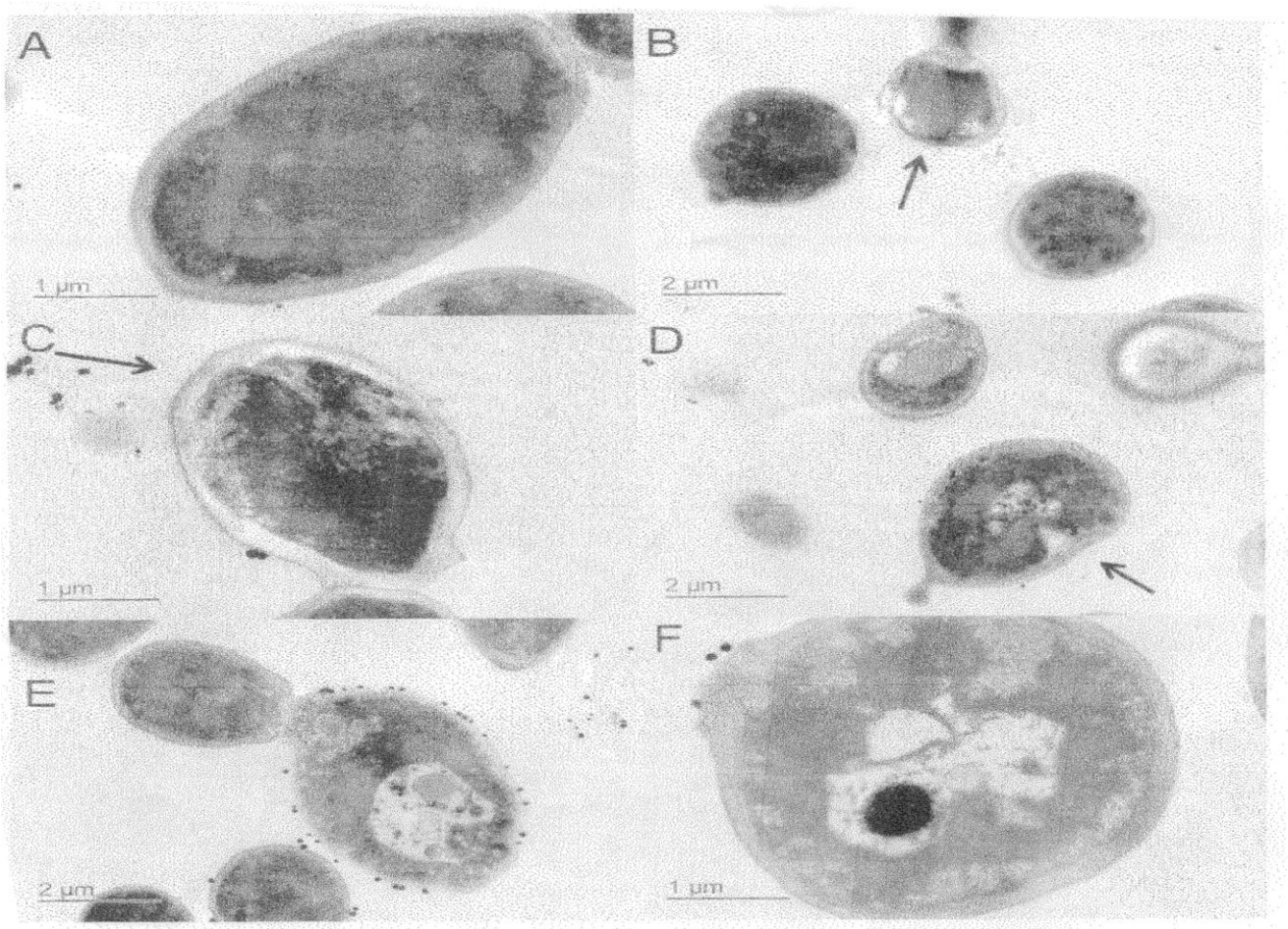

Figure 3. TEM micrographs of the untreated (**A**), 30 min (**B** and **C**) and 120 min (**D-F**) treated cells of *C.albicans* ATCC 90028 with HE with concentration of 1560µg/mL.

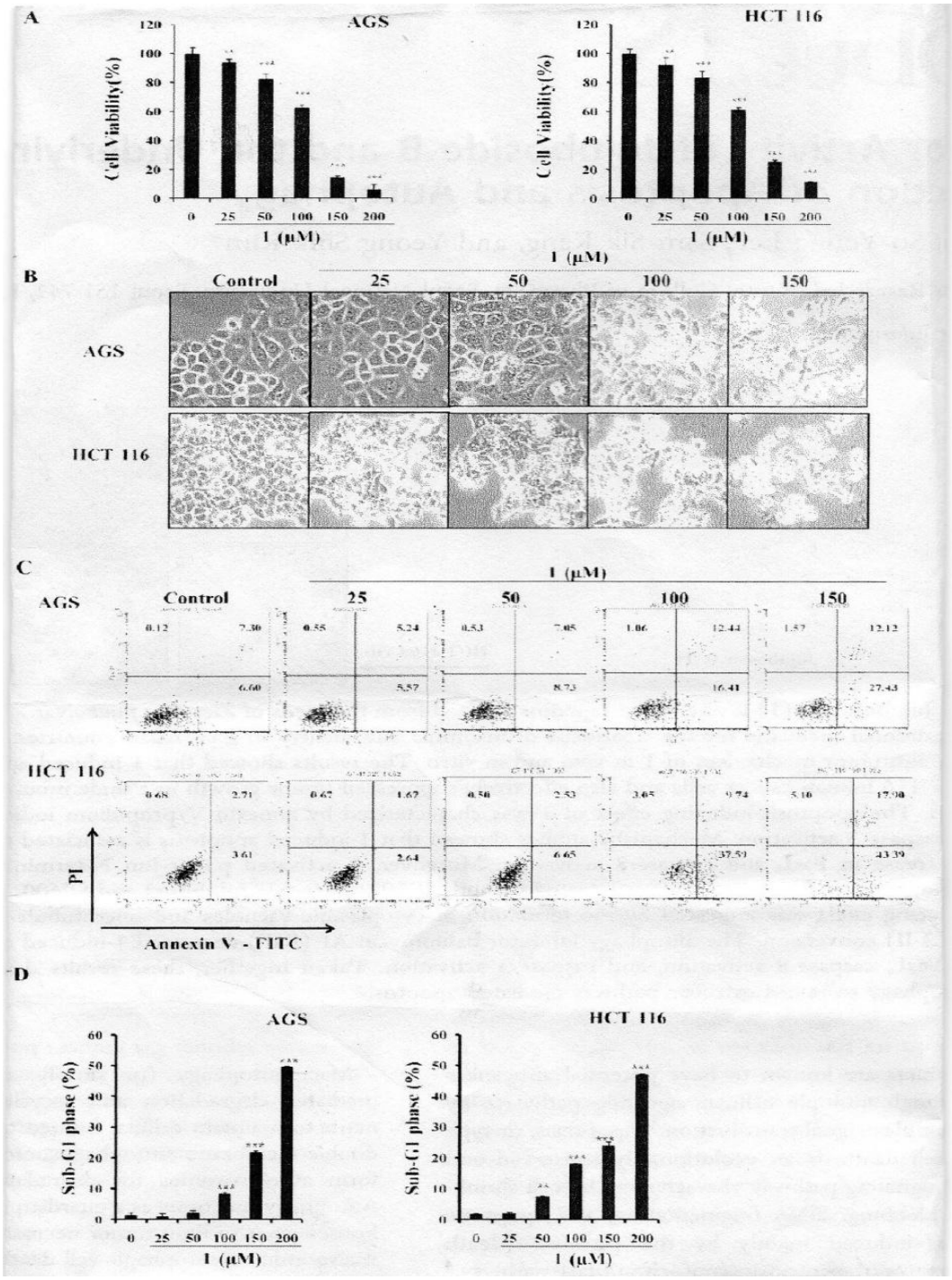

Figure 4. Jujuboside B induces apoptosis in AGS and HCT 116 cells. Cells were treated with the indicated concentrations of Jujboside B for 24 h, and (**A**) the cell viabilities were determined using the MTT assay.The values are expressed as the means ± SD of three individual experiments (* p< 0.05; **p < 0.01; ***p <0.001 vs the untreated control group). (**B**) Morphological changes were visualized at 100X magnification under CKX41 microscopy. (**C**) Flow cytometry analysis of annexin V/PI double staining was performed to measure the apoptotic and necrotic cells. A representative result from three separate experiments is shown. (**D**) The ratio of sub-G1 phase was measured by Flow cytometry. The values are expressed as the means ± SD of three individual experiments (* p< 0.05; **p < 0.01; ***p <0.001 vs the untreated control group).

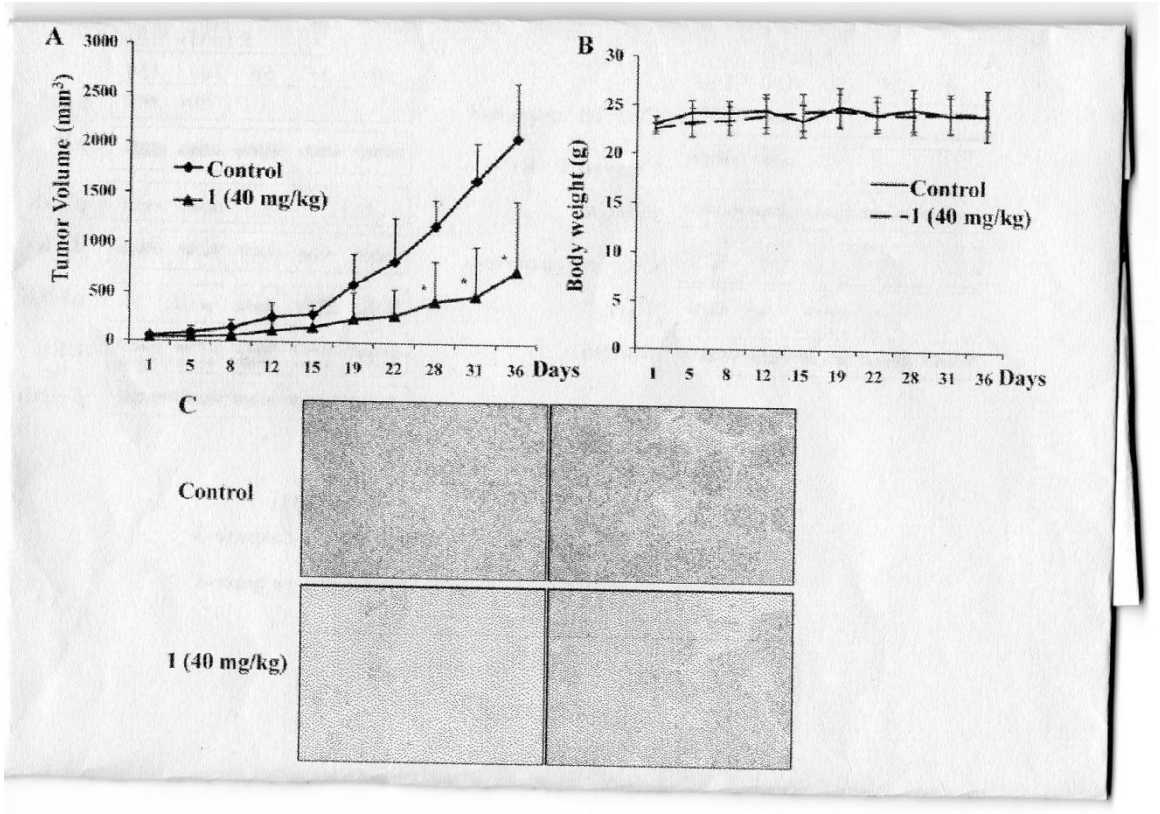

Figure 5. Antitumor effect of Jujuboside B in a tumor xenograft model beating HCT 116 cells were implanted subcutaneously into the right flank of each mouse. When the tumor size reached 60 mm^3, the mice were treated intraperitoneally with Jujuboside B (40mg/kg) three times a week for 5 weeks. **(A)** The tumor sizes were measured twice a week ($^*p<0.05$ vs the untreated ontrol group). (**B**) The body weight of each mouse was monitored for toxicity. (**C**) Immunohistochemical analysis of the cell proliferation marker K_i-67 from the tumor tissues, visualized at 200x magnification under CKX41 fluorescence microscopy. A representative result from two different tumor tissues is shown.

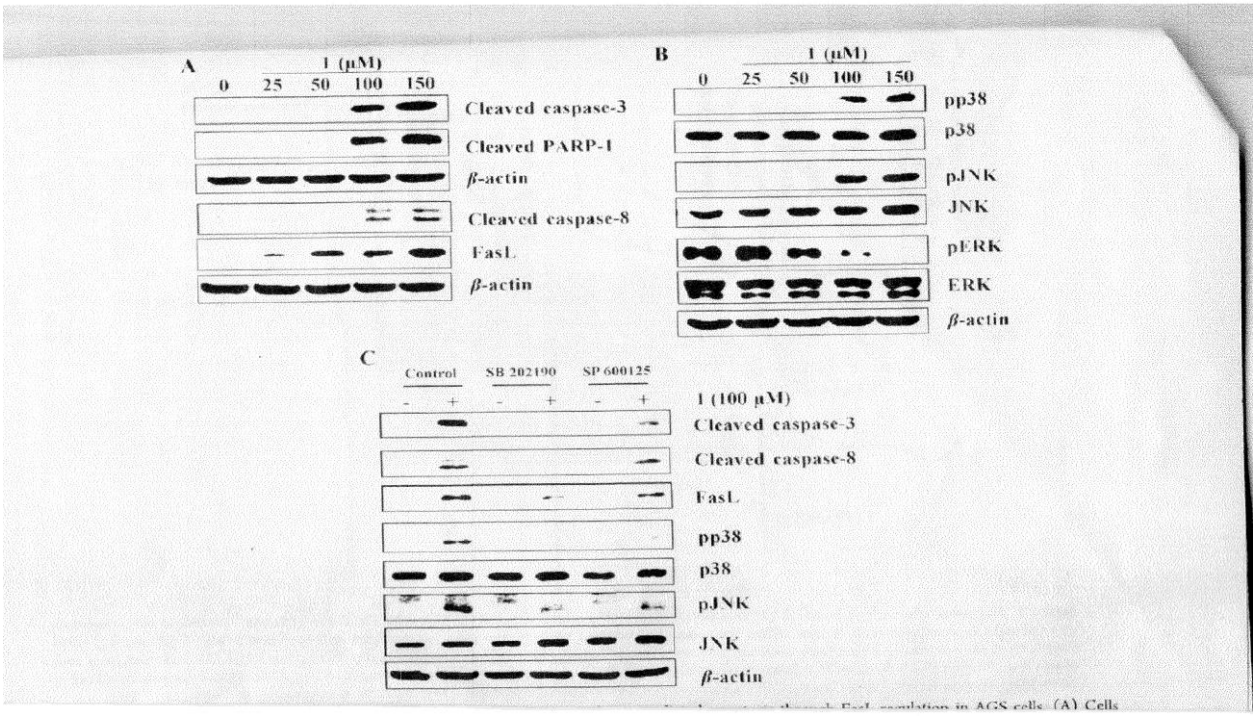

Figure 6. Jujuboside B activates p38/JNK to promote extrinsic pathway-mediated apoptosis through FasL regulation in AGS cells. (**A**) Cells were treated with the indicated concentrations of Jujuboside B for 24h, and western blotting was performed for cleaved caspase-3, cleaved caspase-8, cleaved PARP-1, Fasl. β-

Actin was used as a loading control (B) Cells were treated with the indicated concentrations of Jujuboside B for 24h, and Western blotting was performed for pp38, p38, pJNK, JNK, pERK, and ERK, β-Actin was used as a loading control (C) Cells were pretreated with 5μM SB202190 and 5μM SP600125 for 30 min, and then 100μM for 24h. Western blotting was performed for cleaved caspase-3, cleaved caspase-8, FasL, pp38, p38, pJNK and JNK. β-Actin was used as a loading control. A representative result from three separate experiments is shown.

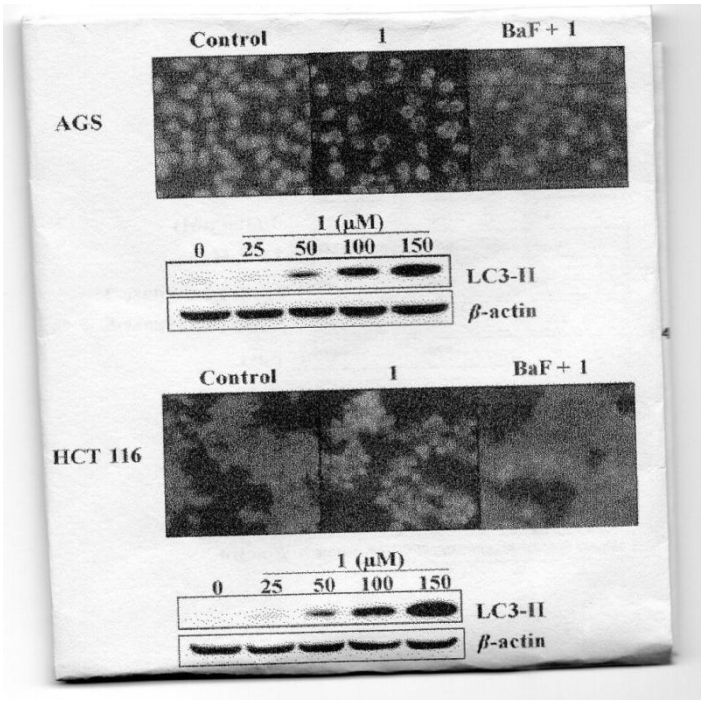

Figure 7. Jujuboside B induces autophagy in AGS and HCT 116 cells. Cells were treated with 50μM Jujuboside B in the presence or absence of 2.5 nM bafilomycin A1 (BaF) for 24 h, stained by acridine orange (AO), and visualized at 200x magnification under CKX41 fluorescence microscopy. Cells were treated with the indicated concentrations of Jujuboside B for 24 h, and Western blotting was performed for LC3-II. β-Actin was used as a loading control. A representative result from three separate experiments is shown.

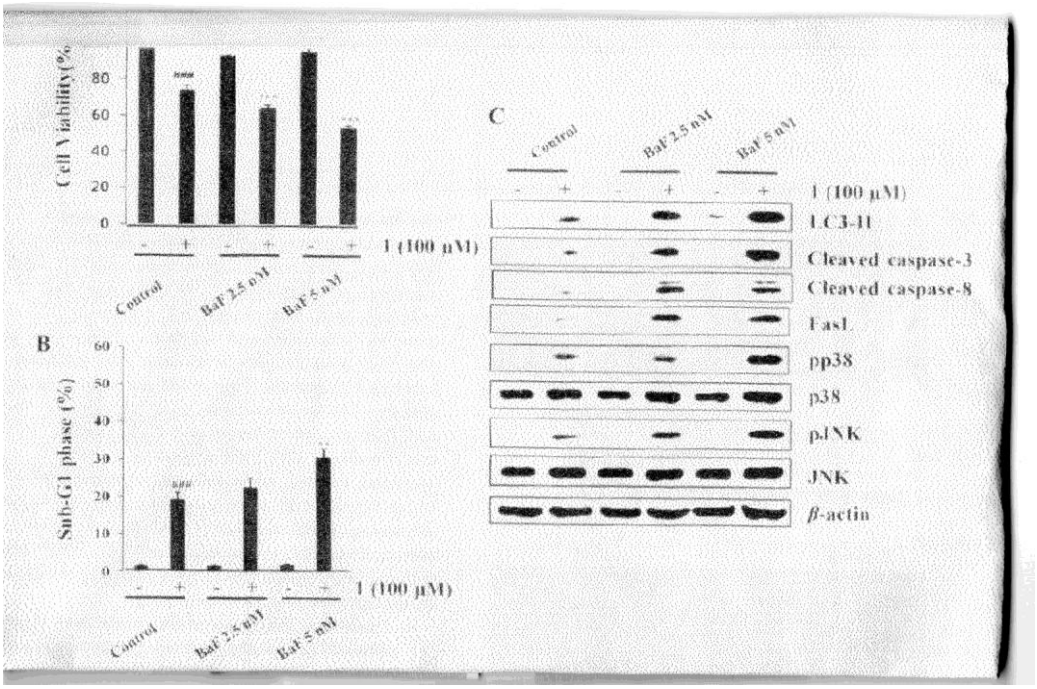

Figure 8. Jujuboside B induces protective autophagy to retard apoptosis in AGS cells were pretreated with BaF for 30 min, then 100μM Jujuboside B was added for 24h and (A) the cell viabilities were determined

using the MTT assay. The values are expressed as the means ± SD of three individual experiments ($^{***}p < 0.001$ vs the untreated control group, $^{***}p < 0.001$ vs the Jujuboside B treated group). (B) The ratio of sub-G1 phase was measured by flow cytometry. The values are expressed as the means ± SD of three individual experiments ($^{***}p < 0.001$ vs the untreated control group, $^{**}p < 0.001$ vs the Jujuboside B treated group). (C) Western blotting was performed for cleaved caspase-3, cleaved caspase-8, FasL, pp38, p38, pJNK and JNK. β-Actin was used as a loading control. A representative result from three separate experiments is shown.