*Sys Rev Pharm 2020;11(12):409-417*
A multifaceted review journal in the field of pharmacy

# APOPTOSIS INDUCING POTENTIAL AND IMMUNOMODULATION OF COLOCASIA ESCULENTA AGAINST MYCOBACTERIAL SPECIES

Saeed Arrak Turkey [a],Mustafa M.Farhan[a*], Essa D Alhtheal[b], kamal jawad awni[d]
[a,c]Al Mamoun University College, Baghdad, Abu ghraib-10081, Iraq
[b,d]Materials University of Technology, Baghdad, Abu ghraib-10081, Iraq.

**ABSTRACT**
Colocasia esculenta (C. esculenta) is said to be widely cultivated plant species of Araceae family, used for consumption as food and also used in medicine. of both leaves and tubers. Commonly called as Taro, and contains a vast range of bioactive compounds like flavanoids, terpenoids and alkaloids. Corms of this plant is said to processes pharmacological activities to treat several diseases. The present study is aimed to study the possible immunomodulatory and apoptotic potential of this sample to treat and cure against the Mycobacteria species. The corms dried and extracted with methanol, hexane, acetone and dichloromethane and used to study the antimycobacterial activity by MIC activity and total activity. Its potent antiinflammatory activity was studied by the cytokine assay targeting the IL-6, TNF and Il-10 and apoptotic inducing potential by Annexin assay. The methanol extract showed maximum MIC values as compared to the positive control rifampicin. In accordance to the above result, methanol extract also showed an inducing apoptotic potential of 73±0.92 at 290µg/ml (Rifampicin 89±2.12). Methanol extract also significantly reduced the IL-6 and TNF levels to 11.23 ± 0.23pg/ml and 9.29± 0.41 respectively at 290µg/ml. It also enhanced the levels of IL-10 from 12.11± 0.71 (0.29µg/ml) to 48.76± 0.81 (290µg/ml); P < 0. 005.The present study confirmed the positive effect of the methanol extract as immunomodulatory and antiinflamatory by showing an inhibition in the expression of IL-6 and TNF. Thus in conclusion C.esculenta could be used as the best anti-inflammatory candidate and immunomodulatory supplement due to its containing many bioactive compounds.

**Keywords:** Colocasia esculenta, Antiinflammation, MIC, Mycobacteria tuberculosis, IL-6, IL-10, Apoptosis.

## INTRODUCTION

Globally, the ever-increasing outbreaks and cases of infectious diseases is driving the biologists to focus on immunomodulation rather than searching for candidate drugs. Both malnutrition and infectious diseases are always a piece of challenge within the developing nations as they greatly affect the individual's immune system [Joshua Nfambi, 2015]. Imbalanced nourishment especially that of proteins makes a person malnutritious and these could affect the immune system making him disease susceptible. Excess stress levels, diseases and acute infections of lungs and intestine, infectious tuberculosis, and HIV/AIDS are all the possible reasons of making a person immunocompromised [3]. However, proper diet filled with nutritious dietary intake could make a person immune system strong [Calder PC, 2000].

Mycobacterium tuberculosis, a facultative intracellular bacterium belonging to the M. tuberculosis complex, and is said to be the causative agent for tuberculosis (TB) in humans. In addition to M. tuberculosis, there are other strains like Mycobacterium bovis, My-cobacterium africanum, Mycobacterium microti and Mycobacterium canetti which were also found to cause TB in humans (Aro et al, 2015). TB though very old but still it is emerging rapidly making the highest spikes for the morbidity and mortality among the humans (Nguta et al, 2015b). As stated by WHO, more than nine million new cases of active tuberculosis globally get reported of TB (WHO, 2014) which could have led to one and half million deaths in the year 2013.

Chemotherapeutics of tuberculosis (Proksch et al, 2015) became more intricated due to increased prevalence of TB and moreover they are associated with viral infections such as HIV. The essential antitubercular drugs available in the market are facing alarming problems like multi-drug-resistant (MDR), extensively drug-resistant (XDR) and totally drug resistant (TDR). This leads to an emergency to discover new drugs with advanced mechanism of action against M. tuberculosis.

Many novel anti- mycobacterial plant products were studied in the recent years thus making the plant biodiversity a very hot topic which could provide more novel candidate drugs. Among those natural plant products, many of them were identified to be antitubercular in nature, which could be of utmost importance for TB drug discovery (Nguta et al., 2015b). Global health care needs were met by traditional medicine systems which played a pivotal role in the Indian medicine. Indian Systems of Medicine though oldest but largest, comprises of many medicines of Indian origin. Plants have always played a major role to sustain and improve the quality of human life and served humans with variety of products used in seasoning, beverages, cosmetics, and dyes along with medicine. As such, biologists round the world,

focussed on plant research to screen and trace out herbal combinations which could ail liver, CVD, digestive, metabolic and central nervous system (CNS) disorders. Many such medicinal plants or ethanobotanical herbs and their extracts were found to contain a wide spectrum of biological activities and one such plant is Colocasia esculenta.

Colocasia species is known as ancient crop of the world and found mainly in Africa, Asia, West Indies, and South America. It is widely grown along the humid tropics and its edible part is the underground corm and leaves, which are often used in healing liver disorders [Tuse TA, 2009]. Studies done so far also stated that the ethanolic extracts of the leaves of C. esculenta showed antiinflammatory activity. According to Shah et al, (2007), ethanolic extracts could show a reduction in the inflammation, on winstar rats induced with carrageenan [Shah BN, 2007]. The taro extract which was studied by kundu et al, (2012) also stated that, it could inhibit the prostaglandin E2 (PGE2) synthesis and also reported the downregulation of cyclooxygenase 1 and 2 expression. Taro extract was also said to inhibit the proliferation rate among the breast and prostate cancer cell lines and also very significantly blocked the tumor cell migration [Kundu N, 2012].

Many herbs are reported and are being searched for their capability to enhance the immune system or to act as an immunomodulator so as to treat and prevent various infections. Among such herbs are Morus alba, Triphala megaExtract (megaExt) which consists of phytochemicals from Terminalia chebula, Terminalia bellerica, and Emblica officinalis [Belapurkar P, 2014], Fiscus racemosa, and M. oleifera. M. oleifera was also studied for its medicinal values in treating diseases like asthma, bronchitis, mastitis, skin conditions and other worm infestations [Kasolo JN, 2010].

Even though majority of the molecular mechanisms are not well traced out, the poor documentation available confirms the role of M. tuberculosis (Mtb) in manipulating the host cells to their advantage [Afsal K, 2016]. Though ancient in nature, sacrificing own cells which are infected could be a first line of defence by the immune system cells what is called apoptosis. And in recent studies it was found that some of the viral pathogens tried to inhibit the apoptosis in the hosts. Same sort of anti-apoptotic capability is also well studied among protozoans and other bacterial pathogens [Aggarwal S, 2016].

Generally, apoptosis is studied by two pathways firstly the extrinsic pathway, which depends solely on the Fas/CD95 or the TNF-α receptor 1 (TNFR1) which activates Caspase-8/10; and secondly the intrinsic pathway, which is initiated by the intracellular stress and ultimately activation of Caspase-9. Both these pathways seem to unite at the Caspase-3/6/7 activation step, which further activates apoptosis and finally DNA fragmentation [Gan H, 2008]. Scientists also reported that Mtb could inhibit the intrinsic pathway of apoptosis within the host cell by regulating the pro- and anti-apoptotic proteins. Once Mtb infects the host, it induces the upregulation of genes like mcl-1 which are the anti-apoptotic in nature [Finkel T, 2003]. It was also reported that a gene called A1 which encodes for Bcl-2-like proteins also gets enhanced within the mitochondria. These results were supported by functional data analysis done using anti-sense oligonucleotides knocking down mcl-1 and A1. Even the studies done on A1 knock-out mice also demonstrated that they play a vital role in apoptosis inhibition within the hosts [41,43,44] [Prieto P, 2010].

Mtb infected macrophages also showed inhibition in the extrinsic apoptosis pathway by modifying the Fas (CD95) expression along with the TNF receptor 2 (sTNFR2). Cell surface levels of Fas were reduced vastly, on infection with Mtb [Green DR, 2004], protecting the infected cells from Fas-ligand induced apoptosis. Moreover, Mtb lipoglycan is found to activate the NF-κB proteins via TLR-2 [Al-Orainey IO, 2009]. This activation could lead to more upregulation of the antiapoptotic protein FLIP, which eventually inhibits the FasL-mediated apoptosis. On inhibiting the TNFα induced apoptosis, the macrophages which are infected are found to increase the TNFR2 (sTNFR2) secretion. The sTNFR2 binds to TNFα in the extracellular milieu and thus inhibiting its binding to the TNFR1 [Cho YS, 2009].

Tarin, compound of the Colocasia was found to be immunomodulatory by Kumar, Manjunath, Thaminzhmani, Kiran, and Brahmaiah (2012). It was found to act on the innate and adaptive immunity activating and promoting its suppression.

Tarin was also reported of its ability to stimulate the splenocyte proliferation to a maximum level, which was supported further by RNA expression of the pro-inflammatory cytokines like interleukin-1β (IL-1β), interleukin-2 (IL-2), interferon-γ (INF-γ), and tumor necrosis factor-α (TNF- α) [Pereira et al., **2014**]. Cytokines as known, are said to be very essential for the cancer treatments. As such many studies were driven towards the triggering or inhibiting the cytokines by tarin bringing health benefits [Pereira, P. R, 2017].

Natural products, especially those from the Colocasia were less intensively investigated in the past even though few studies reported of their role as immunomodulator. This has prompted us to screen and investigate the possible role of the selected plant for its anti-TB activity. However, few studies were done reporting the capability of Colocasia but with limited molecular validation. Hence our current study was designed to confirm the possible role of Colocasia as an immunomodulatory agent. The dried corms after extraction with solvents, was studied for its antimycobacterial activity by MIC activity and total activity. Its potent antiinflammatory activity was also confirmed by the cytokine assay targeting the IL-6, TNF and Il-10 molecules.

## MATERIALS AND METHODS:

### Extraction preparation

The Colocasia corms purchased from the local market were washed thoroughly and sun dried for about 7-10 days. The corms were then pulverized to a fine powder using a homogenizer and used for extraction with acetone, hexane, dichloromethane and methanol using the Soxhlet apparatus. About 20gm of the material was extracted with 300ml of the respective solvent at $45^0$C for 24–30 hours to efficiently extract the phytoconstituents. The extracts were then lyophilized and were reconstituted in dimethyl sulfoxide (DMSO; 20mg/mL).

**Determination of Minimum Inhibitory Concentration (MIC):** Minimum Inhibitory Concentration values are estimated by a slightly modified serial micro-dilution method described by Asal Mohseni et al (2016). MIC values are the lowest concentration of the sample at which the growth of the microorganisms is inhibited. Fresh colonies of mycobacteria were taken from solid medium and resuspended in 3ml of LJ liquid broth and homogenized on a Vortex mixer for 5min. The floating mycobacterial suspension was then added to 5ml of LJ broth and diluted. This suspension was then used in the study.

In brief, the extracts suspended in DMSO were used in the study. In a 96 well plate, about 180µl of Lowenstein-Jensen medium (LJ) was added along with 20µl of inoculum. In each well 50µl of extracts of varying concentration (2.9µg/ml, 29µg/ml and 290µg/ml) were added. DMSO blanks were included and rifampicin was used as positive control. The plate was incubated at 37°C for about 24-48hr and following incubation, 20µl of ρ-iodonitrotetrazolium

violet (HiMedia) was added to all the wells and incubated for 30min at 37°C. Purple- red colour depicts the positive growth and clear wells demonstrate the inhibition by the extracts and the positive. The plates were then read in plate reader (Genetix) at 540nm. Total activity of the given samples was calculated by dividing the amount of plant extract per 1gm of plant material with the MIC of the same extract and is usually expressed as ml/g [Eloff JN, 2004].

**Cell viability assay**

The effect of the obtained extracts on the viability of the cells were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. A549 Cell Lines were used in the study (donated by Stellixir). About 7500-9000 cells per well were incubated with 0.29-290µg/mL of the extracts for 24hr at 37°C in a humidified CO2 incubator. Dichloromethane fraction was excluded from the study. Wells containing DMSO as the solvent was considered as negative control. 10µL of MTT (5mg/mL) was added to all the wells and the plate was incubated for 4hr at 37°C. Following incubation, the plates were removed and added with lysis buffer (0.1N HCl in isopropanol) to dissolve the formazan produced from the treated cells. Absorbance was measured with a microplate reader (Genetix, Germany) at 570nm. Absorbance of the negative control wells was considered as 100% viability. The Results were expressed as percentages and was calculated using the following equation: [%viability $=(OD_{sample}-OD_{Blank})/(ODcontrol-OD_{Blank})$ *100]. The assay was done in triplicates and repeated twice.

**Intracellular Assay**

Intracellular activity of the plant extracts on the A549 cells was studied according to the protocol described by C. D. Carpenter (2012). In brief, A549 cells were grown as described in the previous section with 4µg/ml of gentamycin. About $10^6$ cells/well were seeded in a 96-well microtitre plate and the mycobacterial (Mtb) culture was inoculated into the wells for infection. The plate was then incubated for about 6hr and the excess Mtb cells were then washed off followed by treatment with amikacin (50µg/mL) for 2hr to kill the remaining extracellular bacteria. The plates were then incubated overnight with the respective plant extracts at varying concentrations (2.9, 29, 290µg/ml). Methanol and acetone only were used for the remaining study.

After the washing off the excess plant extracts on the next day, the cells were lysed with 01% sodium dodecyl sulphate (SDS) to release the bacteria from the cells on 0, $3^{rd}$, $5^{th}$, $7^{th}$ and $10^{th}$ day. The contents were centrifuged and the lysate after dilution with sterile PBS was then plated onto plates containing LJ medium. The plates were then incubated at $37^0$C for 3 weeks and the CFUs (Colony Forming Units) were counted using colony counter.

**Apoptosis by Flow Cytometry**

The A549 cells were cultured as described in the previous section supplemented with 10% FCS and 1% antibiotics (streptomycin). In brief, once the cells ($2 \times 10^6$cells/well) are seeded into 24-well plates, methanol extract at varying concentrations (0.29, 29 and 290µg/ml) was added after 24hr. DMSO served as negative control and Rifampicin was used as positive control. After 48 hr incubation, the cells were harvested and used for estimation of apoptotic activity using the Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) apoptosis detection kit (Genetic Bioscience). The percentage of apoptotic cells were estimated by using annexin V-FITC/PI assay.

**Cytokine assay**

The cytokines like IL-6, TNF, and IL-10 which would be released by the cell lines were measured using the ELISA kit (Everone Biosciences). The protocol was followed according to the manufacturer's instructions. The supernatant of cells cultured (control and treatment) was spinned down at 8000rpm, 10min and stored at -20°C until further use. The cells without stimulation with LPS was used as negative control. The day before the assay, the 96well plate was impregnated with the capture antibody against the specific molecules. The samples were added in their respective wells and done in triplicates. The plate was incubated for about 2hours and following washing with secondary antibody conjugated with HRP. $H_2O_2$-tetramethylbenzidine was sued as the substrate and after the incubation, the reaction was stopped using 2N $H_2SO_4$ and the absorbance was read at 405nm in a plate reader (Genetix). The concentration of the study proteins released into the media was estimated using the standard graph. The minimal detection for IL-6 and IL-10 was adjusted to 10pg/ml and for TNF it was adjusted to 100pg/ml.

## STATISTICAL ANALYSIS

All experiments were conducted in triplicate and values expressed as mean ± standard deviation. Statistical analysis was performed using SPSS software. Significant differences between treated and control were analysed at $P<0.05$.

## RESULTS:

**Extract preparation**

Extraction was done with various solvents to extract effectively the active constituents present within the experimental material. Methanol extract seems to have the highest total yield of about 14.34%, followed by hexane (9.67%) and acetone (8.92%).

**Minimum inhibitory concentration (MIC) and total activity values of plant extracts**

The MIC and total activity values of the selected plant extracts plants against M. tuberculosis was presented in Figure 1 &2. The methanol extract of C.esculenta showed good activity against the tested microbe at MIC value of 0.19mg/ml, followed by the acetone (.31mg/ml) and hexane extracts (0.54mg/ml). Dichloromethane did not show any potent activity at 1.08mg/ml. Rifampicin showed MIC values of 0.11mg/ml.

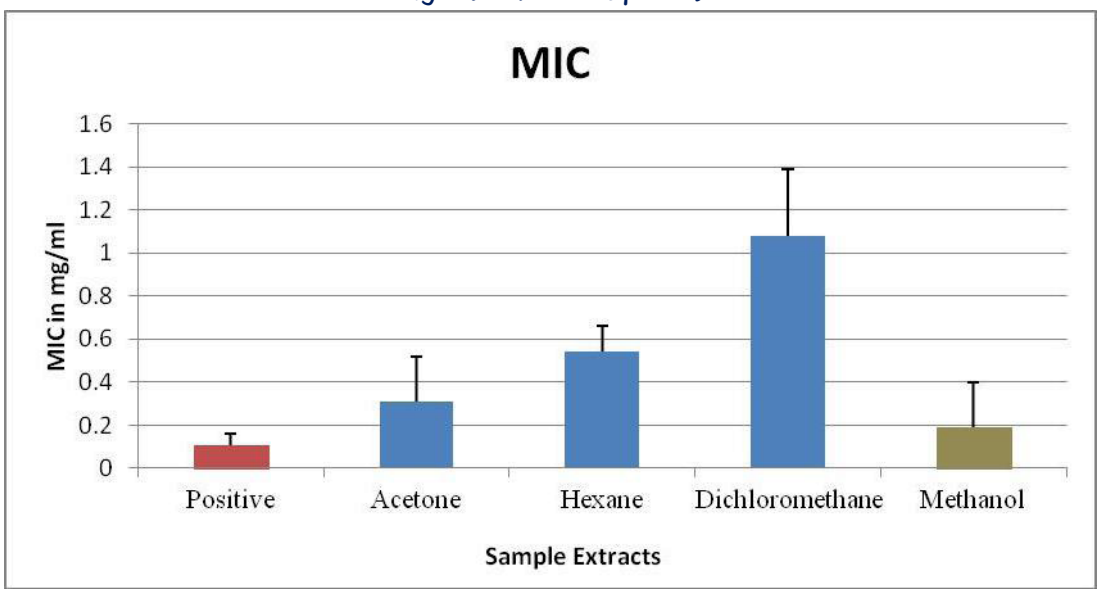


**Figure 1:** Graph showing the Minimum inhibitory concentration (MIC) in mg/ml. All the values are average of triplicates and expressed as value ±SD. Rifampicin is used as positive control.

Total activity value of any sample would actually indicate its efficiency to inhibit the growth of the microbes even at the lowest dilution. TA is shown to be highest for the methanol extract (1764.32mg/gm) followed by acetone (879.12) and hexane fractions (453.21). Moderate activity or minimal activity was shown by dichloromethane fraction (345.67) when compared to the positive control (1923.45). TA values are in accordance to the MIC values and confirms the efficiency of the methanol and acetone fractions when compared to the positive control. TA activity was again dose dependant in all the fractions. This shows that the plant fractions (methanol and acetone) shows a broad antimicrobial potential.

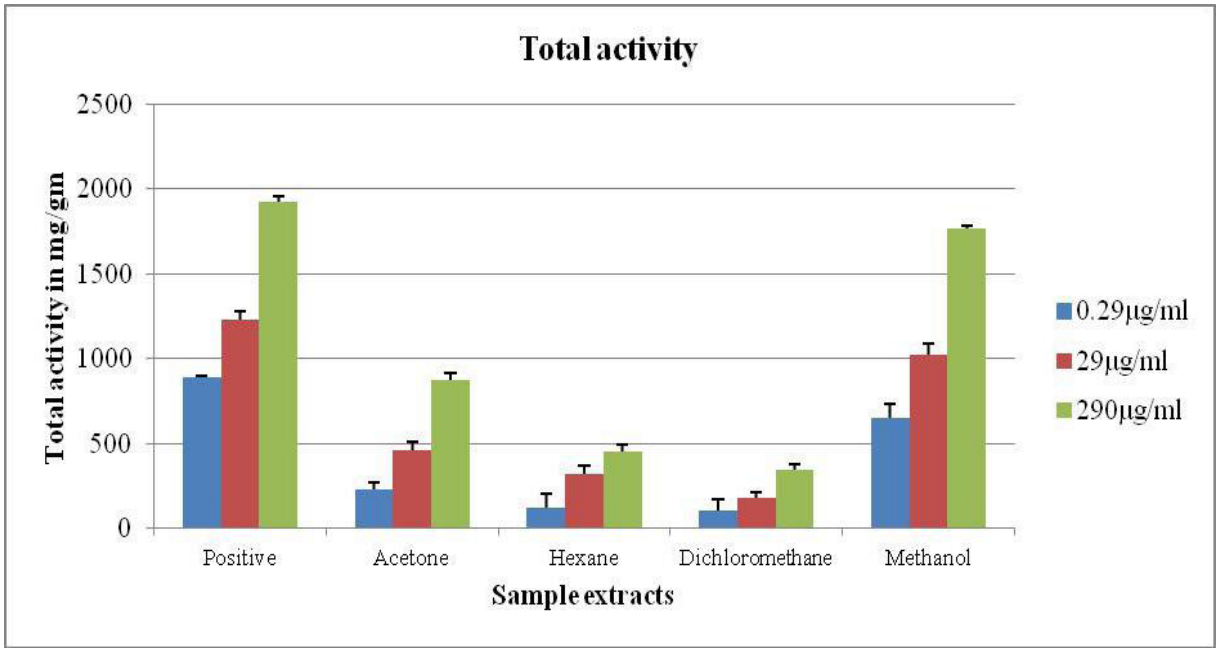


**Figure 2:** Graph showing the total activity (TA) in mg/gm. All the values are average of triplicates and expressed as value ±SD. Rifampicin is used as positive control.

**Cell viability assay**

MTT colorimetric assay was done with the extracts on the lung cancer lines to estimate their toxic effect of varying concentrations (0.29, 29, 290µg/mL). Almost all the fractions showed no toxicity towards the cell lines. Extracts of methanol, acetone and hexane showed no significant cytotoxicity on the cell lines even at a high concentration of 290µg/mL. This confirms the possible usage of the extracts on as medicinal compound.

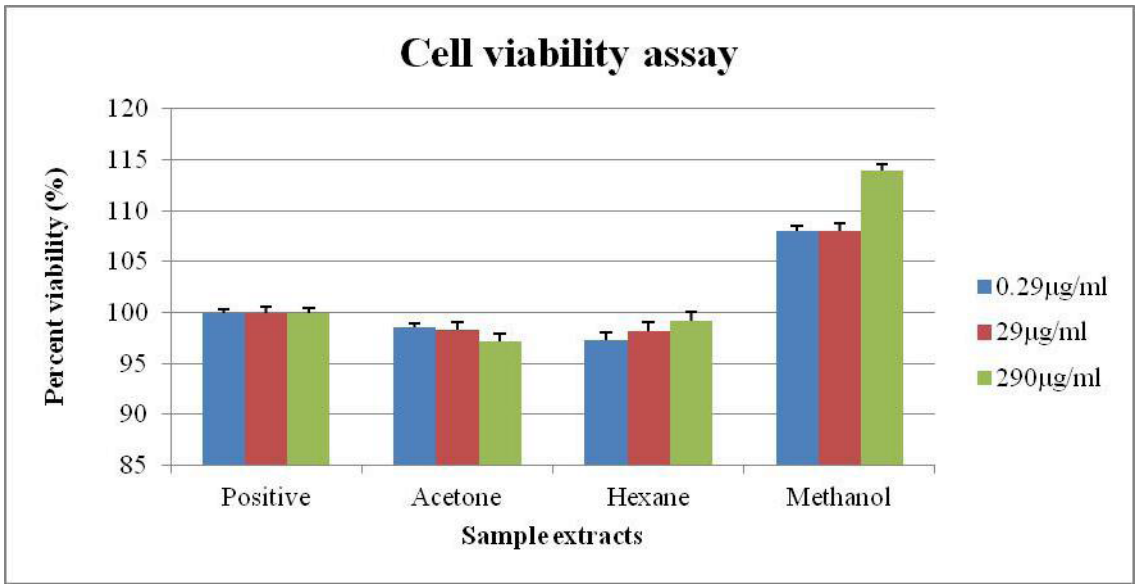


**Figure3:** Cytotoxicity assay of C.esculanta extracts on A549 cells. Cell viability was determined by MTT method. Positive control (Rifampicin) was considered 100% viable. The values were expressed as mean ±S. D and were performed in triplicates ($p<0.05$).

**Effect of Plant Extracts on Intracellular Growth of Mtb**

The plant extracts (methanol and acetone) were assessed at 290µg/ml for their toxicity in an in vitro test on A549 cells and was found to be nontoxic (Results not shown). Both methanol and acetone showed significant inhibition of intracellular bacterial growth and thus were considered active. Only the extracts which showed more than 65% inhibition were considered for further studies. Methanol extract showed greater than 65% of inhibition from the 3rd day (68%) and showed the maximum of 98% at 10th day. Acetone on the other hand showed 65% above inhibition on 5th day and showed maximum of 75% on 10th day. Positive control was considered as 100%.

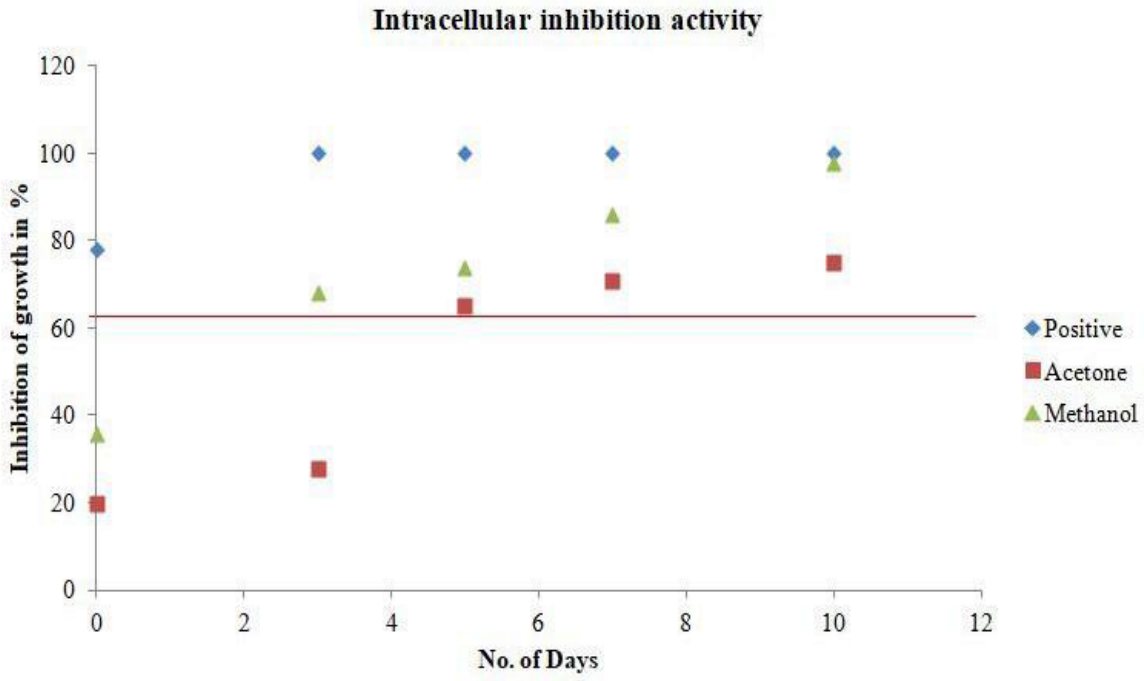


**Figure 4:** Intracellular inhibition of the growth of mycobacteria. Methanol and acetone extracts were used in the study on intracellular growth in A549 cells at 290 g/mL. The extracts only which shows inhibition of the bacteria at 65% and above were selected for further experiments. Saturation line was shown in red.

**Apoptosis Induction**

The A549 cells after exposing to varying concentrations of methanol extracts for 48hr was measured using the annexin V-FITC/PI assay. The methanol extract showed an increase in the percentage of annexin V positive apoptotic cells at 48hr when compared to positive control (Result not shown). The results were statistically significant and dose dependant. The percentage of apoptotic cells were found to be as low as 6±1.34 (2.9µg/ml) to 73±0.92 (290µg/ml). Positive control showed as low as 17±1.14 (2.9µg/ml) to 89±2.12 (290µg/ml). The percentage of apoptotic cells were found to be increasing with increasing concentration of the extract and this could

confirm the quantitative estimation of the rate of apoptotis induced within the cells infected with Mtb.

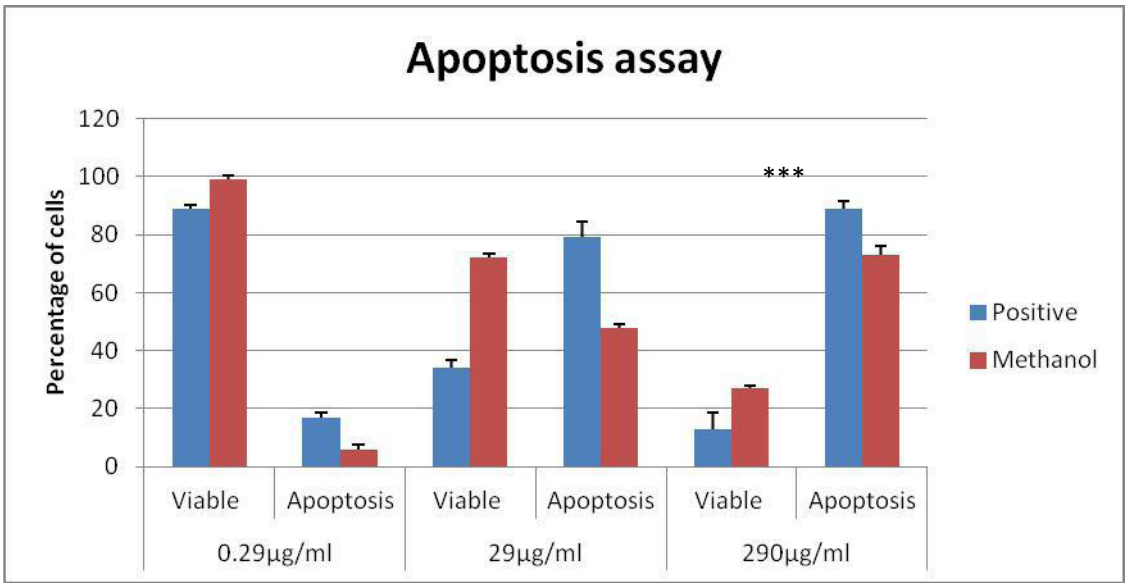


**Figure 5:** Graph showing the percentage of cells which are viable and apoptotic after the treatment with methanol extract. All the values are average of triplicates and expressed as value ± SD. (P<0.05).

**Cytokine assay**

This ELISA method was used in estimating the expression of the IL-6, TNF, and IL-10 cytokines on treatment with the methanol extract only (0.29, 29 and 290µg/ml). From the figure it was found that LPS stimulation has significantly reduced the levels of IL-6 and TNF and enhanced the levels of IL-10 when compared to the negative control values. The negative control values were found to be 38.54± 0.08pg/ml, 44.53± 0.11pg/ml and 11.56± 0.41pg/ml for IL-6, TNF and IL-10 respectively.

Methanol extract with varying concentrations (0.29-290µg/ml) significantly reduced IL-6 levels to 11.23 ± 0.23pg/ml (290µg/ml) from 37.89 ± 0.11pg/ml (0.29µg/ml) and TNF levels from 45.23± 0.28 (0.29µg/ml) to 9.29± 0.41(290µg/ml). On contrary it showed an enhancement in the production of IL-10 from 12.11± 0.71 (0.29µg/ml) to 48.76± 0.81 (290µg/ml); P < 0.005.

**Table 1:** table showing the concentration of IL-6, Il-10 and TNF in pg/ml. All the values are average of triplicates and expressed as value ± SD. Negative control values not shown in the table (IL-6 38.54± 0.08pg/ml; TNF 44.53± 0.11pg/ml; IL-10 11.56± 0.41pg/ml).

| | Methanol | | | Acetone | | |
|---|---|---|---|---|---|---|
| | 0.29µg/ml | 29µg/ml | 290µg/ml | 0.29µg/ml | 29µg/ml | 290µg/ml |
| **IL-6** | 37.89± 0.11 | 21.24± 0.26 | 11.23± 0.23 | 40.53± 0.21 | 36.53± 0.09 | 23.45± 0.11 |
| **TNF** | 45.23± 0.28 | 33.45± 0.45 | 9.29± 0.41 | 45.12± 0.45 | 41.28± 0.05 | 35.78± 0.39 |
| **IL-10** | 12.11± 0.71 | 23.56± 0.61 | 48.76± 0.81 | 14.32± 0.72 | 32.11± 0.18 | 34.21± 0.31 |

Acetone extract also showed significant results, but were not too significant when compared to methanol extract. Acetone extract also significantly reduced IL-6 levels to 23.45± 0.11pg/ml (290µg/ml) from 40.53± 0.21pg/ml (0.29µg/ml) and TNF levels from 45.12± 0.45 (0.29µg/ml) to 35.78± 0.39 (290µg/ml). in accordance to the above results, it also showed an enhancement in the production of IL-10 from 14.32± 0.72 (0.29µg/ml) to 34.21± 0.31 (290µg/ml); P < 0.005.

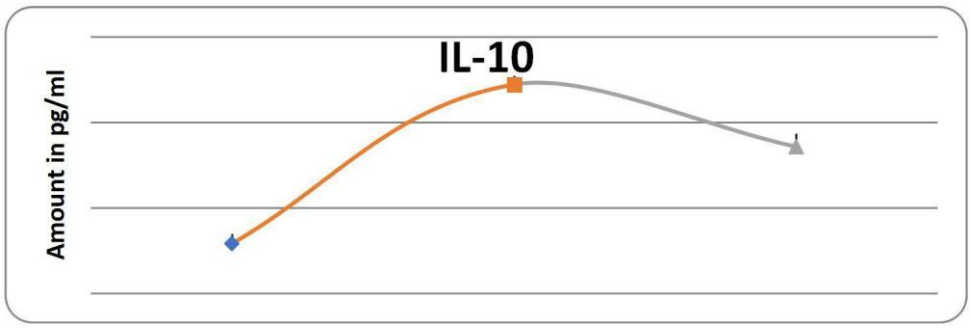


**Figure 6:** Graph showing the effects of the methanol and acetone extracts on LPS stimulated Il-6 and TNF levels. Cells are treated with 0.29-290µg/mL of the extracts and results at highest concentration was shown in the graph. Values are expressed as pg/ml ± SD. All the values were average of triplicates (P < 0.05)

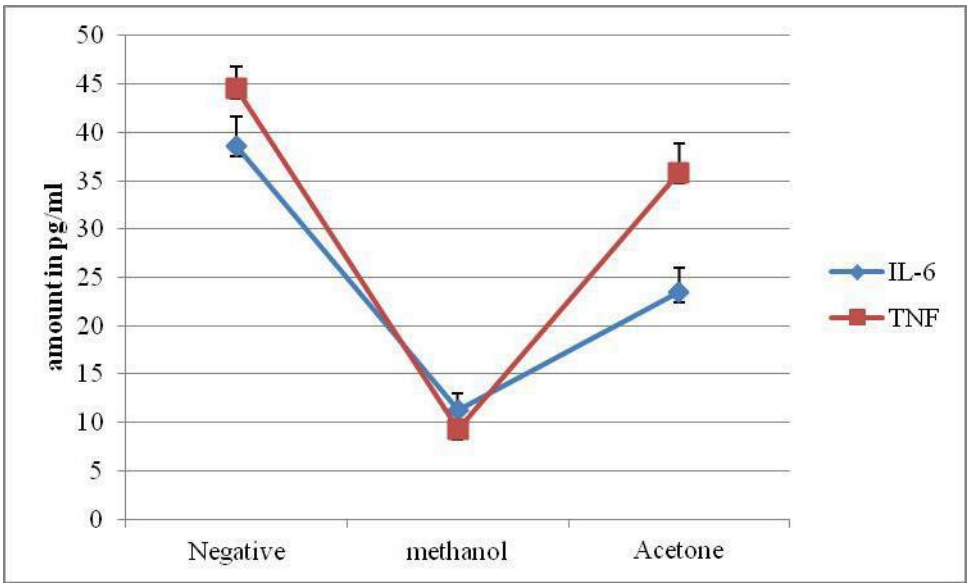


**Figure 7:** Graph showing the effects of the methanol and acetone extracts on LPS stimulated Il-10 levels. Cells are treated with 0.29-290µg/mL of the extracts and results at highest concentration was shown in the graph. Values are expressed as pg/ml ± SD. All the values were average of triplicates ($P < 0.05$)

## DISCUSSION

Tuberculosis is said to be the recurrent pathogenic disease which leads to high mortality and morbidity. In the recent decades, Mycobacterium spp. is thought to develop antibiotic resistance to many of the potent antibiotics making the current treatment scenario worst and as such biologists are now focussing to develop new anti-TB drugs from plants.

Plants of the Rubiaceae family were said to be potent in curing Malaria, hypertension, diabetis and were also good as antimicrobial and anti-inflammatory (Sirigiri, 2015). Aro et al (2015) have studied on the extracts on more than six African species belonging to Rubiaceae family and found that they were all very effective as antimycobacterial. These plants were used against the mycobacterial species and the MIC values of the tested extracts showed significant antimycobacterial activities along with the positive control; rifampicin. Might be the activity could be due to the presence of several bioactive phytochemicals.

Even the present study was targeted against the mycobacterium (Mtb) with the plant material Colocasia esculenta and the results were in accordance to the previous findings. Methanol extract followed by acetone and hexane was found to be more effective and showed promising MIC values against the microbe. Colocasia seems to be rich in terpenoids and alkaloids and might be this could be the possible explanation for the possible antimycobacterial activity (Keshav, 2019).

Our results are very much consistent with the report of Moraes et al. (2011) who also stated that the antimycobacterial activity seen in many of the Rubiaceae species was related to the presence of terpenoids and alkaloids.

Aro et al (2019), reported that the crude extracts of C. triflora and P. capensis could induce apoptosis in a dose-dependent manner at 100µg/mL. Pathogens are thought to evade the host defense mechanism by inhibiting its apoptotic activity and triggering necrotic activity among the macrophages which delays the adaptive immunity (Divangahi et al., 2010).

Hence therapeutic agents which could initiate apoptosis among the host macrophages could eventually be a potent chemotherapeutic agent to combat TB. Such apoptotic process could be detected by flow cytometric analayis. When the cells undergo apoptosis, they leak the phosphatidylserine from the inner side of the membrane to outside which could bind to annexin V and as such this is used as marker for apoptosis (Schutte et al., 1998). From the study mentioned before, the cell growth inhibition of the plant extracts (C. triflora and P. capensis) was due to induction of apoptosis induction when compared to negative control. The cells which are not treated showed less apoptotic activity when compared to the treated cells after 48hr.

Even our study suggested the same with an increase in apoptotic activity on treating with the extracts. The A549 cells when exposed to methanol extract showed an increase in the apoptotic activity when compared to the positive control (Rifampicin) at 48hr.

The results were in accordance to the previous mentioned work, and is dose dependant. The percentage of apoptotic cells were found to be 73±0.92 and 89±2.12 at 290µg/ml for methanol extract and positive control respectively. The percentage of apoptotic cells were found to be increasing with increasing concentration of the extract and this could confirm the quantitative estimation of the rate of apoptotis induced within the cells infected with Mtb.

Studies done on Bougainvillea xbuttiana (Bxb) also confirmed that the extracts could stimulate the production of cytokines and NO and were proven anti-inflammatory (Arteaga Figueroa L, 2015). TNF was found to be reduced at about 290 g/ml with Bxb extracts when compared to negative control ( < 0.001). Even the study confirmed that at 290 g/ml of same extract significantly reduced the levels of IL-6 when compared with negative control ( < 0.01). In contrary Bxb extract showed a drastic enhancement of IL-10 production among the macrophages which were treated with extracts at about 290 g/mL and is again dose dependant ( < 0.01). IL-10 is said to play the main lead as antinflammatory which is again initiated by the Th1 response (Gazzinelli et al., 1992).

The present study also reveals of the similar findings to those mentioned above. LPS stimulation could significantly reduce the levels of IL-6 and TNF and enhanced the levels of IL-10 when compared to the negative control values.

Methanol extract significantly reduced the IL-6 and TNF levels to 11.23 ± 0.23pg/ml and 9.29± 0.41 respectively at 290µg/ml. On contrary it showed an enhancement in the production of IL-10 from 12.11± 0.71 (0.29µg/ml) to 48.76± 0.81 (290µg/ml); $P < 0.005$. This could possibly suggest the plant extract especially methanol extract could be antiinflammtory in nature and could be a potent chemotherapeutic agent.

Rifampicin, is used as first-line anti-tubercular drug which is said to completely inhibit the production of pro-inflammatory cytokines TNF and IL-6 while stimulating the expression of anti-inflammatory IL-10. This antibiotic is thought to kill the pathogen by producing large quantity of pro-inflammatory cytokines which could also suppresses the production of the pro-inflammatory cytokines increasing the expression of anti-inflammatory cytokines (Nau and Tauber, 2008). Throughout the study our findings are in accordance to these results and the methanol extract which was found to be potent antimycobacterial was compared to this rifampicin. All our results are similar to this finding and confirms the possible role of the plant sample as antimycobacterial.

## CONCLUSION

C. esculenta is said to be largely cultivated plant used since ages as food and medicine. Presence of terpenoids and alkaloids in large amounts among them made them therapeutically active and might be the major reason for using it as a therapeutic agent. This plant was studied for its antimicrobial, antihepatotoxic, antidiabetic, and anti-inflammatory activity.

Findings from this study suggests that the methanol extract of the selected plant sample have the potential to act as antimycobacterial. This extract could potentially inhibit the growth of mycobacteria intracellularly as like the positive control among the in vivo models. Moreover, its effect in reducing the IL-6 and TNF, while enhancing the Il-10 states that this extract could act as anti-inflammatory. But the bioactive principles from these methanol extract need to be purified and studied which could aid in the standardization of herbal formulations to treat TB. This finding might facilitate in developing novel chemotherapeutic agents for treating the mycobacterial infections.

## CONFLICTS OF INTEREST

The authors declare that the study conducted do not process any conflicts of interest.

## REFERENCES


1. Afsal K, Selvaraj P. Effect of 1,25-dihydroxyvitamin D3 on the expression of mannose receptor, DC-SIGN and autophagy genes in pulmonary tuberculosis. Tuberculosis (Edinb) 99: 1–10, 2016. doi: 10.1016/j.tube.2016.03.010.
2. Aggarwal S, Mannam P, Zhang J. Differential regulation of autophagy and mitophagy in pulmonary diseases. Am J Physiol Lung Cell Mol Physiol 311: L433–L452, 2016. doi:10.1152/ajplung.00128.2016.
3. Al-Orainey IO. Diagnosis of latent tuberculosis: can we do better? Ann Thorac Med 4: 5–9, 2009. doi:10.4103/1817-1737.44778.
4. Aro, A. O., Dzoyem, J. P., Goddard, A., Fonteh, P., Kayoka-Kabongo, P. N., & McGaw, L. J. (2019). In vitro Antimycobacterial, Apoptosis-Inducing Potential, and Immunomodulatory Activity of Some Rubiaceae Species. Frontiers in pharmacology, 10, 185. https://doi.org/10.3389/fphar.2019.00185.
5. Aro, A.O., Dzoyem, J.P., Hlokwe, T.M., Eloff, J.N., McGaw, L.J., 2015.SomeSouth African rubiaceaetreeleafextractshaveantimycobacterialactivityagainst pathogenic andnon-pathogenic mycobacteriumspecies. Phytother.Res. http://dx.doi.org/10.1002/ptr.5338.
6. Arteaga Figueroa L, Barbosa Navarro L, Patiño Vera M, Petricevich VL. Preliminary Studies of the Immunomodulator Effect of the Bougainvillea xbuttiana Extract in a Mouse Model. Evidence-based Complementary and Alternative Medicine: Ecam. 2015; 2015:479412. DOI: 10.1155/2015/479412.
7. Asal Mohseni Sajadi Araghi, Sajjad Mohsin Irayyif and Sudhakar Malla. (2016). Biofilm & motility studies on food borne pathogens under the treatment of silver Nanoparticles, International Journal of Current Research, 8, (02), 25899-25906. https://www.journalcra.com/sites/default/files/issue-pdf/10669.pdf
8. Belapurkar P, Goyal P, Tiwari-Berua P. Immunomodulatory effects of Triphala and its individual constituents: A review. Indian J Pharm Sci. 2014; 76:467–75. [PubMed: 25593379]
9. C. D. Carpenter, T. O'Neill, N. Picot et al., "Anti-mycobacterial natural products from the Canadian medicinal plant Juniperus communis," Journal of Ethnopharmacology, vol. 143, no. 2, pp. 695–700, 2012.
10. Calder PC, Jackson AA. Undernutrition, infection and immune function. Nutr Res Rev. 2000; 13:3–29. [PubMed: 19087431]
11. Cho YS, et al. Phosphorylation-driven assembly of the RIP1-RIP3 complex regulates programmed necrosis and virus-induced inflammation. Cell. 2009; 137:1112–1123. [PubMed: 19524513]
12. Divangahi M., Desjardins D., Nunes-Alves C., Remold H. G., Behar S. M. (2010). Eicosanoid pathways regulate adaptive immunity to Mycobacterium tuberculosis. Nat. Immunol. 11 751–758. 10.1038/ni.1904
13. Eloff JN. Qualifying the bioactivity of plant extracts during screening and bioassay-guided fractionation. Phytomedicine 2004; 113:70-1
14. Finkel T. Oxidant signals and oxidative stress. Curr Opin Cell Biol. 2003; 15:247–254. [PubMed:12648682]
15. Gan H, et al. Mycobacterium tuberculosis blocks crosslinking of annexin-1 and apoptotic envelope formation on infected macrophages to maintain virulence. Nat Immunol. 2008; 9:1189–1197.
16. Gazzinelli R. T., Oswald I. P., James S. L., Sher A. (1992). IL-10 inhibits parasite killing and nitrogen oxide production by IFN-gamma-activated macrophages. J. Immunol. 148 1792–1796.
17. Green DR, Kroemer G. The pathophysiology of mitochondrial cell death. Science. 2004; 305:626– 629. [PubMed: 15286356]
18. Joshua Nfambi, Godfrey S. Bbosa*, Lawrence Fred Sembajwe, James Gakunga, and Josephine N. Kasolo. Immunomodulatory activity of methanolic leaf extract of Moringa oleifera in Wistar albino rats J Basic Clin Physiol Pharmacol. 2015 November 1; 26(6): 603–611. doi:10.1515/jbcpp-2014-0104.
19. Kasolo JN, Bimenya GS, Ojok L, Ochieng J, Ogwal-Okeng JW. Phytochemicals and uses of Moringa oleifera in Ugandan rural communities. J Med Plants Res. 2010; 4:753–7.
20. Keshav, Amit & Sharma, Alok & Mazumdar, Bidyut. (2019). Phytochemical Analysis and Antioxidant Activity of Colocasia esculenta (L.) Leaves. World Academy of Science, Engineering and Technology International Journal of Chemical and Molecular Engineering Vol:13, No:1, 2019; p20-23.
21. Kumar, U. A., Manjunath, C., Thaminzhmani, T., Kiran, Y. R., & Brahmaiah, Y. (2012). A review on

immunomodulatory activity of plants. Indian Journal of Novel Drug Delivery, 4(2), 93–103.
22. Kundu N, Campbell P, Hampton B, Lin CY, Ma X, et al. (2012) Antimetastatic activity isolated from Colocasia esculenta (taro). Anticancer Drugs 23: 200-211.
23. Nau R., Tauber S. C. (2008). Immunomodulatory properties of Antibiotics. Curr. Mol. Pharmacol. 1 68–79. 10.2174/1874467210801010068
24. Nguta, J.M., Regina,A.O., Nyarko,A.K., Manu,D.Y., Addo,P.G.A.,2015. Current perspectives in drug discovery against tuberculosis from natural products. Int.J. Mycobacteriol.4,165–183.
25. Pereira, P. R., Del Aguila, E. M., Vericimo, M. A., Zingali, R. B., Paschoalin, V. M., & Silva, J. T. (2014). Purification and characterization of the lectin from taro (Colocasia esculenta) and its effect on mouse splenocyte proliferation in vitro and in vivo. Protein Journal, 33(1), 92–99.
26. Pereira, P. R., Meagher, J. L., Winter, H. C., Goldstein, I. J., Paschoalin, V. M., Silva, J. T., & Stuckey, J. A. (2017). High-resolution crystal structures of Colocasia esculenta tarin lectin. Glycobiology, 27(1), 50–56.
27. Prieto P, et al. Lipoxin A4 impairment of apoptotic signaling in macrophages: implication of the PI3K/Akt and the ERK/Nrf-2 defense pathways. Cell Death Differ. 2010; 17:1179–1188. [PubMed: 20094061]
28. Proksch, P., Georgios, D., Rainer, K., Hendrik, K., Rudolf, H., Nicole, J., Victor,W., Wenhan,L., 2015. Callyaerins from the marine sponge Callyspongia aerizusa: cyclic peptides with antitubercular activity. J. Nat. Prod. http://dx.doi.org/ 10.1021/acs.jnatprod.5b00266
29. Schutte B., Nuydens R., Geerts H., Ramaekers F. (1998). Annexin V binding assay as a tool to measure apoptosis in differentiated neuronal cells. J. Neurosci. Methods 86 63–69. 10.1016/S0165-0270(98)00147-2.
30. Shah BN, Nayak BS, Bhatt SP, Jalalpure SS, Sheth AK (2007) The anti-inflammatory activity of the leaves of Calocasia esculenta. Sau Pharm J 15: 3-4.
31. Sirigiri Chandra Kala. (2015). Medicinal attributes of family Rubiaceae. International Journal of Pharmacy and Biological Sciences 5(2)**:** 179-181.
32. Tuse TA, Harle UN, Bore VV (2009) Hepatoprotective activity of Colocasia antiquorum against experimentally induced liver injury in rat. Malyasian J Pharma Sci 2: 99-112.