

Anticancer Activity of *Grewia obtusa* Fruit on HCT- 116, MCF-7, and HeLa Tumour Cells Besides Antitubercular Activity

Mazin Aboobaida Abdalla Abdelaziz¹, Vijayan N Azger Dusthacker², Ramalingam Peraman^{3,4,*},

¹Faculty of Pharmaceutical Sciences, Jawaharlal Nehru Technological University Anantapur (JNTUA), Anantapur, Andhra Pradesh, INDIA.

²ICMR-National institute for tuberculosis and Research (ICMR-NIRT), Chennai, Tamil Nadu, INDIA

³RERDS-Centre for Pharmaceutical Research, Raghavendra Institute of Pharmaceutical Education and Research (RIPER)-Autonomous, Anantapur, Andhra Pradesh, INDIA.

⁴National Institute of Pharmaceutical Education and Research (NIPER)-Hajipur, Bihar, INDIA

ABSTRACT

Background and Objectives: Considering the unexplored therapeutic potential of a plant, *Grewia obtusa* (*G. obtusa*), and the study evaluated the biological potential of fruit extract for antitubercular and anticancer activities. **Materials and Methods:** *G. obtusa* fruit extracts were obtained by a process of cold maceration with the solvents, which includes methanol, ethyl acetate, and n-hexane. The phytochemical constituents were identified in the obtained three extracts by various qualitative tests and further screened for the activity of anticancer on HeLa, HCT- 116 and MCF-7 tumour cells, along with anti-tubercular activity against *H37Rv Mycobacterium tuberculosis H37Rv*. **Results:** The results revealed that the presence of alkaloids, terpenes, lignans, carbohydrates, steroids, glycosides and flavonoids. Anticancer screening revealed the 3-4-fold efficacy of ethyl acetate extract (32.94 µg/ml) in inhibiting HCT-116 cells comparing to n-hexane (89.69 µg/ml) and methanol (128.86 µg/ml) extracts. Same result was notice on HeLa, for ethyl acetate extract with 4-5-fold potency (45.83 µg/ml) as compared to other test extracts of methanol (164.58 µg/ml) and n-hexane (194.58 µg/ml). In case of MCF-7 cell line also the GFE showed 2 to 3-fold potency (73.04 µg/ml) as compared to other test extracts of n-hexane (140.15 µg/ml) and methanol (251.79 µg/ml). The antitubercular activity showed the MIC of > 200 µg/ml for all extracts. **Conclusion:** Ethyl acetate extract exhibited more potent activity of inhibition on HCT- 116, MCF-7, and HeLa tumour cell lines than methanol and n-hexane extracts. In specifically *G. obtusa* fruit ethyl acetate extract was more active on HCT-116 tumour cell lines. But the antitubercular activity was not appreciable in all three extracts.

Keywords: *Grewia obtusa* fruit, Extraction, Anticancer activity, Antitubercular activity, HeLa, HCT 116, MCF-7.

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Correspondence:

Dr. Ramalingam Peraman
RERDS-Centre for
Pharmaceutical
Research, Raghavendra
Institute of Pharmaceutical
Education and Research
(RIPER)-Autonomous,
Anantapur-515721,
Andhra Pradesh, INDIA.
E-mail: drramalingamp@
gmail.com

INTRODUCTION

In this 21st century, the use of plant extracts as medication for various chronic ailments and life-threatening diseases has begun in public health. The most vital usages of ancient system of medicine is by crude drug and natural extracts are suitability for long term treatment, multiple mechanism, synergism, low toxic, and multi-targeting.¹ Worldwide, cancer and multi-drug resistant tuberculosis (MDR-TB) have emerged as the most life-threatening diseases in public

health and they associated with human immune system.² Despite the existing treatments and newer treatment approaches, yet the mortality rate is increasing for every year, it's absolutely due to treatment failure. Thus, finding new drugs with new process of mechanism and less toxicity is continuing interest among researchers.³ Indeed, the natural product research is the most attracted area in discovery of new drugs, especially for cancer. In fact, natural



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molecules are structurally diverged, mostly biologically well accessible, multi-targeting and less toxic.⁴ Thus, most of the medicinal plants has to be investigated for tuberculosis. An additional advantage of plants in cancer treatment is the immunomodulant property many constituents that may stimulates cell mediated and humoral immune retorts to the tumour and also may induces the apoptosis through several mechanisms in tumour cell.⁵ Hence from few years, immunomodulators (naturally available from plants) are prescribed to treat various cancers.⁶ A very less data of plants/herbs usage is available to treat tuberculosis when compared with other diseases. Therefore, its worthy to unravel the antitubercular potency of uninvestigated plants.

In finding suitable plant for our research, we found that *G. obtuse* has not been investigated for antitubercular and anticancer activities. It was decided based on the observations that the genus, *Grewia* (Tiliaceae) found as small trees and shrubs, approximately 150 species found in subtropical and tropical locations such as Arabia, China, Madagascar, tropical Africa, the Himalayas, India, Pakistan, Bangladesh, Thailand, Myanmar, Malaysia, the Pacific Islands, and northern Australia. This genus consists of chemical constituents like triterpenoids, alkaloids, sterols, flavones, anthocyanins, lignans, lactones, vitamins, organic acids etc. with diverged biological activities such as antimalarial, antimicrobial, antiemetic, hepatoprotective, radioprotective, antioxidant, anti-proliferative, analgesic and antipyretic, anti-inflammatory.⁷ In particular, aqueous extract of leaves and fruit of *G. asiatica* showed promising anticancer activity against epidermal cervical (HELA), kidney (HEK-293), lung (NCI-H522), breast (MCF-7), and laryngeal (Hep-2) cell lines.⁸⁻⁹ Reported cytotoxic activity (IC_{50} values) of 50% hydro-methanolic extract of *G. asiatica* against MCF-7 (34.9 μ g/mL), NCI-H522 (73 μ g/mL) and Hep-2 (80.4 μ g/mL) cancer cell lines.¹⁰ Kakotia BB *et al.* reported significant cytotoxic effect (IC_{50} values) of the *G. asiatica* methanolic extract of leaves by MTT and found 54.9 μ g/ml in K-562, 53.70 μ g/mL in HL-60, 199.5 μ g/mL in MCF-7 and 177.8 μ g/ml in HeLa Cell lines respectively.¹¹ *G. subinaequalis* DC fruit aqueous extract showed significant cytotoxic activity (IC_{50} values) on lung (58.65 μ g/mL) and breast (59.03 μ g/mL) cancer cell lines. Marya B *et al.* reported *G. asiatica* aqueous fruit extract reported cytotoxicity of against lung cancer cell line with 58.65 μ g/mL in MCF-7 and IC_{50} of 59.03 μ g/mL cell line.¹² While *G. asiatica* leaf aqueous extract was active on breast cancer cell line (IC_{50} of 50.37 μ g/Ml) and Hep – 2 cell lines (61.23 μ g/Ml). Vincenzo DF *et al.* reported *G. asiatica* methanol fruit extract reported *in vitro*

cytotoxicity (IC_{50} values) on lung (59.03 μ g/mL) and breast (58.65 μ g/mL) cancer cell lines, whereas the extract of leaves exhibited cytotoxicity on breast (50.37 μ g/mL) and Hep-2 (61.23 μ g/mL) cell lines.⁸ Administration of the extract 500 and 250 mg/kg intraperitoneal (*In vivo*) to Swiss albino mice (male) bearing Ehrlich's ascites carcinoma (EAC) tumor, the life span was increased in mice by 61.06% and 41.22% respectively.

G. obtusa documented as synonym *G. bracteata*, *G. nightiana*.¹³ The preclinical and clinical investigation on this plant and plant parts has not yet reported in any other studies. At this juncture the need to explore the biological potential of *G. obtusa* for it potential towards cancer and tuberculosis is prioritized. In this study, the fruit of *G. obtusa* has been cold macerated to obtain extracts using n-hexane, ethyl acetate and methanol. These extracts were evaluated for their cytotoxic effect on different cell lines (HCT- 116, HeLa and MCF-7), along with antitubercular activity by broth dilution method against *Mycobacterium tuberculosis* H37Rn.

MATERIALS AND METHODS

Collection and Authentication of Plant Part

Fruits of *G. obtusa* were collected from Tirupati, Andhra Pradesh, India. A Voucher specimen of the plant has been placed at the biodiversity conservation division, Sri Venkateshwara University, Tirupati Voucher No. 0331. *G. obtusa* (fruits) were washed with distilled water further removal of mud, dust and contaminants, dried in shade for about 30 days (at room temperature) and blended into fine powder. Then air tight container was used for storage and future use.

Preparation of Plant Material

A quantity of 100g of *G. obtusa* fruit powder was subjected to extraction by a cold-maceration technique. The extraction was done with different three solvents (ethyl acetate, n- hexane and methanol). Whatmann No.1 filter paper used for extraction filtration and the filtrates were concentrated by using rotary evaporator (Aditya Scientific, RE-2 at 400 mmHg). All the three extracts, namely methanol extract (GFM), ethyl acetate extract (GFE), n-hexane extract (GFN) was concentrated into semisolid mass and packed in airtight container and stored under refrigeration until further use.

Chemicals, Reagents and Cell Lines

For cytotoxic study HeLa (Human Cervical Cancer), HCT 116 (Human Colorectal Cancer), and MCF7 (Human Breast Cancer) Cell lines were obtained from

NCCS, Pune. Cell culture medium: DMEM media with high glucose (#AL111, Himedia), Fetal Bovine Serum (#RM10432, Himedia), Antibiotic-Antimycotic solution-100x (Cat No. A002-50ML, HiMedia), MTT Reagent (5 mg/ml) (# 4060 Himedia), DMSO (#PHR1309, Sigma), Camptothecin (#C9911, Sigma), D-PBS (#TL1006, Himedia). 7H9 medium, Rifampicin (Sigma).

Phytochemical Tests of the Extracts

One hundred milligram of fruit extracts of *G. obtusa* dissolved in 100 mL mother solvent to get the concentration of 1mg/mL. The diluted solution was subjected for various preliminary qualitative phytochemical tests as per standard procedures. The chemical tests performed were Dragendorff's and Mayer's test to detect alkaloids; Shinoda test, FeCl_3 test and lead acetate test to detect flavonoids; Molisch's test to identify carbohydrates; Biuret test to detect proteins; Salkowski test for steroids/terpenoids; Borntrager's test for anthraquinone glycosides; Sodium picrate test to identify cyanogenic glycosides; Foam test for saponins; FeCl_3 test, Iodine test, HNO_3 tests for tannins and phenols. The chemical constituents present or absent were reported in the Table 1.

Finger prints the extract by Thin Layer Chromatography

The *G. obtusa* fruit n-hexane, ethyl acetate and methanol extracts were spotted by using pre-coated silica gel TLC plates. The mobile phase n-hexane and ethyl acetate (85:15%) was used to develop the TLC plates in a pre-saturated TLC chamber. After optimum development in TLC chamber, plates were removed, and air dried for 5-10 min. After visual examination, the appearance of colour of the spots under iodine vapour has been considered presence of chemical constituents. The retardation factor (R_f) values for various spots were calculated and reported in Table 1. Further, the respective reagent for phytochemicals like alkaloids, glycosides, flavonoids, phenols, terpenoids, carbohydrates, was sprayed to confirm the chemical constituents. The developed fingerprints are available as supplementary file.

In vitro Antitubercular Activity

All the three extracts of *G. obtusa* were screened for their MIC at 200, 100, 50, 25, and 10 $\mu\text{g}/\text{ml}$ against Mtb H₃₇Rv using broth dilution method. The 7H9 medium was used for dilution without any alteration of the composition. The culture suspension was adjusted to 1 McFarland standard and then diluted to a 1:10 ratio (well contains 1×10^5 cells). The Rifampicin drug considered as a standard at 1 $\mu\text{g}/\text{mL}$ (critical

Table 1: Results of phytochemical screening of *Grewia obtusa* fruit Extracts.

Sl. No.	Parameters	GFE	GFM	GFN
1.	Extraction process	Maceration	Maceration	Maceration
2.	Extract Color	Brownish green	Brown	Light brown
3.	Extract Texture	Semi-solid	Semi-solid	Semi-solid
4.	% Yield	10.3%	8.4%	6.3%
5.	Qualitative test			
	Alkaloids	+	+	+
	Steroids	+	+	+
	Glycosides	-	+	-
	Amino acids	+	+	-
	Carbohydrates	+	+	+
	Flavonoids	+	+	-
	Tannins	-	-	-
	Saponins	+	+	+
	Diterpenes	-	+	-
6.	TLC profile			
	Major spots	4	5	3
	Retardation factor	0.42 (Light yellow) 0.55 (Yellow) 0.79 (Light brown) 0.85 (Light blue)	0.3 (Yellow) 0.43 (Light Yellow) 0.86 (Light brown) 0.9 (Yellowish brown) 0.93 (Brown)	0.45 (light yellow) 0.76 (yellow) 0.87 (Light brown)

+ Present, - Absent

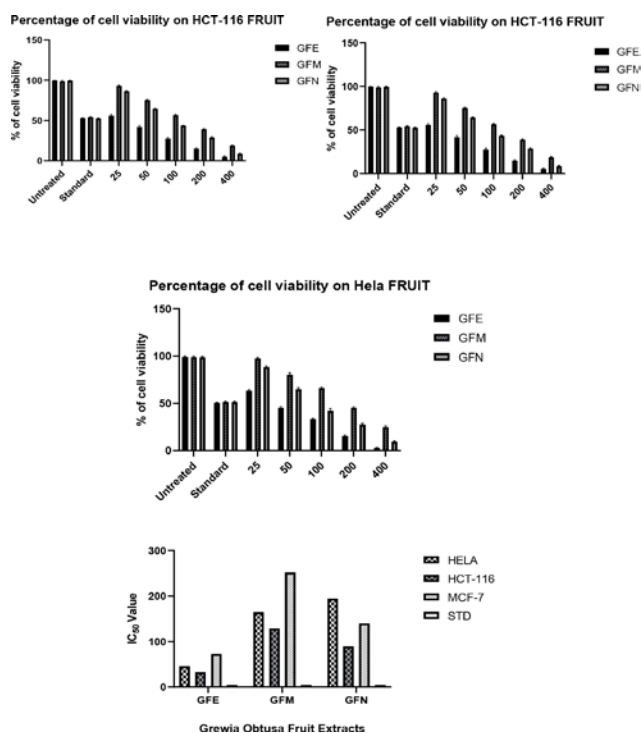
concentration). The both solvent (DMSO) and culture control were also included in the study. The well plates were incubated for five days at 37°C, and determining gradation (based on the serpentine chord formation of Mtb culture growth) in the plates was done by observing under the microscope.¹⁴ All the extracts were screened in triplicate and the MIC of the extracts considered by inhibiting the growth (>90%) of the Mtb culture showed in Table 2.

Anticancer Activity on Cell Lines of HeLa, MCF-7 and HCT- 116

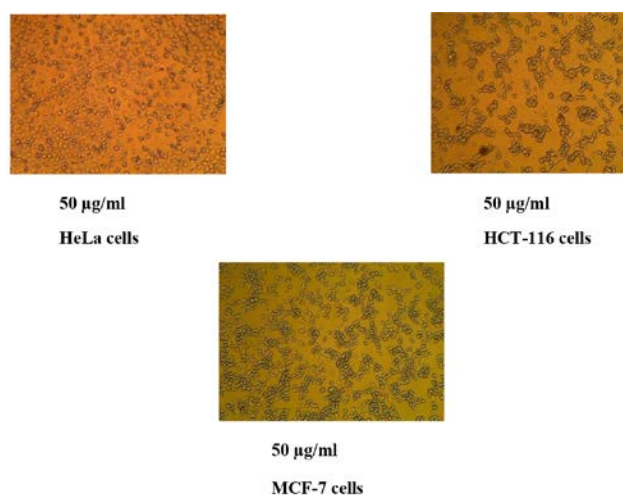
MTT assay (colorimetric assay) used in determining cytotoxicity and cell proliferation, based on reduction water-soluble tetrazolium dye MTT which is yellow-colored to formazan crystals.¹⁵⁻²⁰ A 96 well plate, seeded with 200 μL cell suspension (20,000 cells in each well) without using the test agent. All the cells were permitted for 24 hr to grow, then the test drug (exact concentration) was added, and incubated at 5% CO_2 atmosphere for about 48 hr at 37°C. After 48 hr the plates were taken out from the incubator and removed spent media. MTT

Table 2: *In vitro* anticancer and antitubercular activity screening of *G. obtusa* fruit extracts.

Sl. No	Extracts / Compound	Anticancer activity by MTT Assay (IC ₅₀ value)			Antitubercular activity by broth dilution method on <i>M. tuberculosis</i> H37RV
		HeLa	HCT-116	MCF-7	MIC in µg/ml
1	GFE	45.83±1.25	32.94±1.25	73.04±2.36	> 200
2	GFM	164.58±1.07	128.86±0.95	251.79±1.2	> 200
3	GFN	194.58±2.31	89.69±0.64	140.15± 1.85	> 200
4	Camptothecin	4.4±02	4.4±04	4.4±03	--
5	Rifampicin	---	---	---	1.0

**Figure 1: Percentage of cell viability of GFE, GFM, and GFN on HCT-116, MCF-7, and HeLa cell lines. Bar graph of IC₅₀ concentrations of the extracts treated with MCF-7, HCT-116 and HeLa cells after the treatment period of 24 hr.**

reagent was added to a final concentration of 0.5mg/mL of total volume, then the plates were wrapped by using aluminum foil in order to avoid exposure to light and the plates were again incubated for about 3 hr. (The incubation time depends on cell lines used). Further MTT reagent was removed and then 100 µL DMSO (solubilization solution) was added and gyratory shaker used for stirring in order to enhance dissolution. Finally, by using spectrophotometer or an ELISA reader absorbance was recorded at 570nm and 630nm. The linear regression equation used for the determination of IC₅₀ value and the results were shown in Table 2, Figure 1, and Figure 2.

**Figure 2: Images of cell viability and death due to *G. obtusa* fruit ethyl acetate extracts on HeLa, HCT-116 and MCF-7 at 50 µg/ml concentration.**

Statistical Analysis

The results data was represented as mean ± SD and the statistical evaluation was carried by two-way ANOVA in Graph pad prism version 9.

RESULTS AND DISCUSSION

TLC, Phytochemical Analysis and Extraction

The traditional knowledge of the *G. obtusa* and existing literature of the plant concerned our focus to investigate the fruit part for *in vitro* antitubercular and anticancer activities. Totally 3 extracts were obtained by a process of cold maceration by using methanol, n-hexane, and ethyl acetate as solvents. The nature of crude extracts was resinous and percentage of yield was found to be 8.4, 10.3, and 6.3%, respectively for the methanol, ethyl acetate, and n-hexane solvents. This indicated that ethyl acetate yielded high extract value. The preliminary qualitative tests were performed to identify the various phytochemical constituents such as alkaloids, steroids, glycosides, amino acids, Carbohydrate, Flavonoids,

Tannins, saponins and diterpenoids. (Table 1). The thin layer chromatography (TLC) analysis with the solvent ratio n-hexane:ethyl acetate (85:15) gave five major spots were shown for methanolic extract (Retardation factor: 0.3, 0.43, 0.86, 0.9 and 0.93), four spots were shown for ethyl acetate extracts (0.42, 0.55, 0.79 and 0.85) and three spots for n-hexane (0.45, 0.76 and 0.87) respectively.

Anti-tubercular Activity (*In vitro*) against *M. tuberculosis* H₃₇Rv

Anti-tubercular *in vitro* screening of the fruit extracts of *G. obtusa* on *Mycobacterium tuberculosis* H₃₇Rv revealed that all the three extracts exhibited mild antitubercular activity at >200 µg/ml and the results were showed in Table 2. This result clearly indicates all the three extracts *G. obtusa* fruit very less potent activity towards tuberculosis when compared with the standard rifampicin MIC at 1 µg/mL.

Anticancer Activity on Cell Lines (HCT-116, MCF-7 and HeLa)

The activity of anticancer was performed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay on cell lines like MCF-7 (breast cancer), HCT-116 (colon cancer), and HeLa (cervical cancer). The three extracts of *G. obtusa* were tested for their cytotoxic property on all three cell lines with a serial concentration of 25, 50, 100, 200 and 400 µg/mL in triplicate manner. By using standard Camptothecin at a concentration of 12.5 µg/mL, showed significant inhibition on (MCF-7, HCT-116 and HeLa) cell lines. The IC₅₀ values were tabulated in Table 2.

Overall, the promising effect was shown by extracts *in vitro* growth inhibition on HCT-116 and HeLa cells, while the effect on MCF-7 cells of these extracts was moderate on inhibition. Amongst, the extract of ethyl acetate (GFE) showed promising inhibition effect on HCT-116, HeLa, and MCF-7 cell lines with respective IC₅₀ values of 32.94 µg/ml, 45.83 µg/ml, and 73.04 µg/ml. The n-hexane extract (GFN) showed the inhibition on HCT-116, MCF-7, and HeLa cell lines with IC₅₀ values of 89.69 µg/ml, 140.15 µg/ml, and 194.58 µg/ml. Similarly, the methanol extract (GFM) showed moderate inhibition (IC₅₀ values) on HCT-116, HeLa, and MCF-7 cell lines with 128.86, 164.58, and 251.79 µg/mL respectively.

The comparison of IC₅₀ value from anticancer activity, it revealed that the GFE demonstrated 3 to 4-fold potency on the inhibition of HCT-116 cell line (32.94 µg/ml) as compared to other test extracts from n-hexane (89.69 µg/ml) and methanol (128.86 µg/ml). In case of

HeLa cell line, the GFE showed 3 to 4-fold potency (45.83 µg/ml) as compared to other test extracts of methanol (164.58 µg/ml) and n-hexane (194.58 µg/ml). On MCF-7 cell line also the GFE showed demonstrated better activity with IC₅₀ of 73.04 µg/ml whereas other test extracts of n-hexane (140.15 µg/ml) and methanol (251.79 µg/ml) were less active. Overall, the GFE exhibited significant cytotoxic activity on all screened cell lines than methanol and n-hexane extracts. The potency IC₅₀ values (cell death) represented in Figure 1 and Figure 2.

The report clearly reliable to state the anticancer potency of plant fruit ethyl acetate extract by its high potency against inhibition of tumour growth when compared with remaining two extracts of n-hexane and methanol. In case of *in vitro* antitubercular activity evaluation the MIC results (>200 µg/mL) were revealed that there is no extract has good antitubercular activity than standard rifampicin.

CONCLUSION

In anticancer screening, the ethyl acetate extract of *G. obtusa* fruit showed excellent anticancer activity by inhibition of MCF-7, HeLa and HCT-116 cell lines with IC₅₀ value of 73.04, 45.83 and 32.94 µg/ml respectively when compared with the methanol and n-hexane extracts. Overall, the GFE demonstrated 3-fold potency as compared with the remaining two other test extracts and HCT-116 showed better results compared with the two other cell lines (HeLa, MCF-7) in two different extracts (methanol and n-hexane). But all the three extracts of the *G. obtusa* fruit demonstrated less potency (>200 µg/ml) on tuberculosis compared with the standard rifampicin (1 µg/ml).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

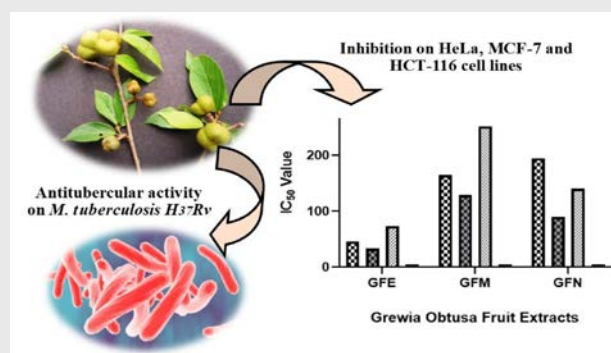
GFE: *Grewia obtusa* Fruit Ethyl acetate extract; **GFN:** *Grewia obtusa* Fruit N-hexane extract; **GFM:** *Grewia obtusa*

Fruit Methanol extract; **MTT**: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide; **TLC**: Thin Layer Chromatography; **MIC**: Minimum inhibitory concentration; **DMSO**: Dimethyl sulfoxide; **ELISA**: enzyme-linked immunosorbent assay; **IC₅₀**: Half maximal inhibitory concentration; **SD**: standard deviation; **ANOVA**: Analysis of variance.

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PICTORIAL ABSTRACT



SUMMARY

Ethyl acetate extract of *G. obtusa* fruit significantly inhibited the MCF-7, HeLa and HCT-116 tumour cells with IC₅₀ value of 73.04, 45.83 and 32.94 μ g/ml respectively, whereas the methanol and n-hexane extracts were relatively less potent. Overall, ethyl acetate extract showed 3-fold potency than two other test extracts. Further, ethyl acetate extract predominantly inhibits the HCT-116 cells than other cell lines (HeLa, MCF-7). Besides, the antitubercular screening demonstrated inactive result on *M. tuberculosis* at the test concentration of 200 μ g/ml.

About Authors



Mr. Mazin Aboobaida Abdalla Abdelaziz, research scholar, JNTUA, Ananthapuramu has pursued his master's from Dr. MGR medical university in the year 2015. He also completed diploma courses in Marketing Management, Hospital management, and the English language. He attended more than 10 national and international conferences or seminars.



Dr. Azger Dusthacker, Scientist at National Institute for Research in Tuberculosis, Chennai. He was awarded a Ph.D. degree in microbiology by the University of Madras in the year 2011. He published 2 patents, 3 book chapters, 35 papers in SCI/SCIE/Scopus indexed journals, and presented more than 25 papers at conferences across the globe.



Dr. Peraman Ramalingam was awarded a Ph.D. degree in pharmaceutical sciences by Andhra University, India. He has a total of 18 years of experience, he received funding for 3 research grants from AICTE, DST-SERB, and BIRAC-GYTI (worth 65 lakhs). He published 2 patents, 2 books, 4 book chapters, 140 papers in SCI/SCIE/Scopus indexed journals, and presented more than 45 papers at conferences across the globe in medicinal chemistry and analytical method.

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