INVESTIGATION OF ANTICANCER AND ANTITUBERCULAR ACTIVITY OF SOME MEDICINAL PLANTS

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Abstract

Morinda angustifolia (MA), *Eclipta alba haska* (EA), *Callistemon macropanctatus* (CM) and *Chloroxylon swetienia* (CS) have been used as traditional medicines for various ailments. In the present work, the hexane, chloroform and ethanolic extracts of all the plants are being evaluated for their anticancer and antitubercular activities. MTT assay of EA extract exhibited significant anti-cancer activity with IC_{50} value of 17.03µg/ml against MG-63 (human osteosarcoma) and IC_{50} value of 300.7µg/ml against HepG2 (human liver) cancer cell line while MA was quite sensitive at 0.8µg/ml on H37RV strain using MABA. It could be concluded that MA and EA could be further investigated to isolate the active compound/s.

Keywords: Morinda angustifolia, Eclipta alba haska, Callistemon macropanctatus, Chloroxylon swetienia, anticancer, ant tubercular, osteosarcoma, MABA.

Introduction

Natural products have made a significant contribution to drug discovery and development research, as assessed by a broad regulatory agency in new small-molecule drug approvals from 1981 to 2014. During this time, natural products or synthetic compounds with a natural pharmacophore product represented a very high percentage of cancer chemotherapy agents (Newman, 2016; Newmann, 2014; Kaur, 2014; Singh *et al.*, 2015). From the literature, it could be argued that there were a number of reasons for the historical success of drug discovery research bases on natural products. One of these is the importance of the use of plant extracts in many traditional medicine systems that guide the selection of candidate species for study. (Gurib, 2006; Cordell, 2012) Another important feature is that natural products are ideally suitable for contact with biological targets as they are ecologically or evolutionarily adapted (Williams, 1989; Talniya, 2016). The area of research into natural products is being revitalized by the use and development of new techniques. Natural product work is being revitalized by the use and development of new techniques. (Kellogg, 2016; Yang, 2013; Ross, 2015; Kurita, 2015; Salvador, 2015)

Plants have been used for the health benefits of all cultures since ancient times, as well as the origin of medicines (Kaur *et al.*, 2019). Tuberculosis (TB) is a contagious infection mainly caused by Mycobacterium tuberculosis, an aerobic pathogenic bacterium. (Arya, 2011) It is estimated that about one third of the world's population, including 40% from India, is contaminated (Rawat, 2017; Khan, 2018). The current situation of multidrug-resistant tuberculosis (MDR) tuberculosis (TB) is of concern to health authorities around the world, especially in developing countries, where the situation is more severe. (Dwarampudi, 2014; Saha, 2015).

Callistemon is a genus of 34 medicinal plants, commonly known as bottlebrush. These are native of Australia. Mostly all the species have medicinal value but these are less studied with specific reference to *Callistemon*

macropunctatus. The plant's phytochemicals could be studied for its medicinal importance (Goyal, 2012).

The situation of multidrug resistant tuberculosis (MDR) tuberculosis (TB) today is of concern to health authorities around the world, especially in developing countries, where the situation is more severe.

The genus *Morinda*, classified in the Rubiaceae family, is native of tropical climate zones. Due to a number of biologically active and structurally interesting secondary metabolites of *Morinda* species has been widely studied. Almost all parts of these plants, have been used as traditional folk medicine having anti-microbial, anti-tumor, anthelminthic, anti-inflammatory, analgesic, and immune enhancing effects (Phakhodee, 2012). *Morinda angustifolia* has been not studied so much which gives a scope to discover new natural products with new biological activities and that might lead to new drugs.

Chloroxylon swietenia DC. (Rutaceae) is an aromatic tree with many medicinal properties. The bark is used as an astringent, and this is widely used as insect-repellant. The plant is less studied for anticancer and antitubercular potentials. (Kiran, 2007).

Eclipta alba Hassk [Asteraceae] commonly referred to as Bringaraja. The herb is widely used in Ayureveda to treat vitiated kapha and vata conditions. Traditionally, it is most widely used for the treatment of night-time blindness, headache and hair-related diseases and development. It is also known to be a rejuvenator. Therefore, *Eclipta alba* offers remarkable preventive and curative potential and further study of Eclipta alba as a health promoter is very much needed (Jadhav, 2009).

Based on the review, in the present study these medicinal plants are being investigated to evaluate the *in vitro* anti-cancer and anti-tubercular activity. The human liver (HepG2) and human osteosarcoma (MG-63) cancer cell lines were screened via MTT assay and H37VR strain for anti- TB via MABA.

In spite of the widespread use of these plants as herbal medicine these activities were not taken into consideration.

Due to their effectivity on various other diseases, two prominent activities are done to use them further for curing the cancer and TB on large scale.

Material and Methods

Four plants, viz. *Morinda angustifolia*, *Eclipta alba haska*, *Callistemon macropunctatus* and *Chloroxylon swetienia*

Plant material

All the plant samples were collected from the forests of Andhra Pradesh in 2018. The plants have voucher numbers assigned to them.

Preparation of extract

Dried leaves sample of plants are then subjected to extraction using hot extraction method with hexane, chloroform and ethanol. Soxhlet apparatus is used to prepare the various extracts. Then all the dried extracts were dissolved in 1 ml of DMSO for initial screening of anti-cancer and anti-tubercular activities.

Chemicals

Absolute ethanol from CYU, 85% extra pure hexane from Loba Chemie Pvt. Ltd., 99% pure chloroform from Loba Chemie Pvt. Ltd.

Cell culture

Two cell lines of human osteosarcoma (MG-63) cancer cell line and human liver (HepG2) cancer cell are used for anti-cancer activities and Mycobacteria tuberculosis (vaccine strain, H37 RV strain.

Purchase and maintenance of MG-63 cell lines

human osteosarcoma (MG-63) cell line was purchased from the National Cell Science Center (NCCS, Pune, India) and grown in T-25 flask, which was incubated in a humidified incubator at 37 ° C below 5 per cent CO2/95 per cent air mixture. The cell line was grown in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal Bovine Serum (FBS) and antibiotic antimycotic solution.

Procurement and maintenance of HepG2 cell line

Human liver (HepG2) cell line was purchased from NCCS, Pune, India and grown in round bottomed well incubated with 5% CO_2 at 37°C in class 2B bio cabinet suitable for drug experiments. Cell line was grown in Dulbecco Modified Eagle Medium (DMEM) with low glucose (Cat No-11965-092) containing 10% fetal Bovine Serum (FBS) (Gibco, Invitrogen) Cat No-10270106 and Antibiotic Antimycotic Solution (Thermofischer Scientific)-Cat No-15240062.

MTT assay

MTT assay (Mickisch, 1990) was used to evaluate ability of all the plant extracts to inhibit the proliferation of MG-63 cell line (Human osteosarcoma). The concentration of DMSO was 5% in 95% double distilled water (95: 5:: ddw:DMSO). 1×10^4 cells were seeded in a 96 well microplates and the plates were incubated for 24 hours. Cells were then treated with the different concentrations of the extracts using serial dilutions. After another 24 hours, 20 μl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well and further incubated for 4 hours to measure the color change due to the formation

of formazen. The supernatant containing MTT solution was then removed from each well and the intracellular MTT formazan was dissolved in 100 μl dimethyl sulfoxide (DMSO). The decrease in absorbance was recorded at 570 nm using multiwell plate reader (BioTek Synergy HT). The cell viability and IC_{50} values were calculated.

Following equation (1) and (2) is used for the calculation in case of MG-63 cancer cell lines.

$$\text{Cell viability} = \frac{\text{Absorbance}_{\text{test sample}}}{\text{Absorbance}_{\text{control}}} \times 100 \qquad ...(1)$$

% Growth inhibition (GI) = 100 – % cell viability   ...(2)

where,

Absorbance$_{\text{control}}$ = absorbance of untreated cells

Absorbance$_{\text{test sample}}$ = absorbance of cells treated with different concentrations of extracts/compounds

While for HepG2 cancer cell line equation used is (3).

$$\text{Surviving cells (\%)} = \frac{\text{Mean OD (test compound)}}{\text{Mean OD (Negative control)}} \times 100 \quad (3)$$

Camptothecin was used as positive control.

Procedure for anti-TB activity

The anti-mycobacterial activity of the compounds was tested against *M. Tuberculosis* with Microplate Alamar Blue Assay (MABA) (Lourenco, 2007). This approach showed a good correlation with the proportional and the radiometric BACTEC system. Briefly, 200μl of sterile deionized water was applied to the 96-well sterile plate well and then 100μl of the 7H9-broth middle brook. The sequential dilution of the compounds was done directly on the sheet. The final concentrations of the drug measured were between 100 and 0.2μg / ml. Plates have been incubated at 37°C for five days. After this time, 25 μl of freshly prepared 1:1 mixture of Almar Blue reagent and 10 percent tween 80 was added to the plate and incubated for 24 hours. The blue color of the well was perceived as not having bacterial growth, and the pink color was labeled as growth of bacteria. The MIC was defined as the minimum concentration of drugs that is able to prevent the change in color from blue to pink.

Standard value for anti-TB

Standard values for the Anti-Tb test performed. Pyrazinamide - 3.125μg/ml, Ciprofloxacin - 3.125μg/ml Streptomycin- 6.25μg/ml (Lourenco, 2007).

Statistical Analysis

Data was analysed with GraphPad Prism Software.

For osteosarcoma cancer cell line

All the experimental values were represented as Mean ± standard errors and the regression equation was obtained using Microsoft Excel. The statistically significant values were calculated using one-way analysis of variance ANOVA (F-test). Tukey's test was used to analyse the honestly significant difference (HSD) values between the means. IC_{50} value (concentration of extract/compound required for 50% growth inhibition of cancer cells) was also calculated. At 5% level of significance, all the values were statistically significant at $p \leq 0.05$. All the plant extracts were evaluated for their activity against these cell line.

For human liver cancer cell line

All the experimental values were represented as Mean ± standard deviation. While $p < 0.05$ and $p < 0.01$, it was considered statistically significant for analysis of percent inhibition of cell growth.

Results and Discussion

For osteosarcoma (MG-63) cancer cell line

From Figure 1 it is clear that for all the extracts, the percentage inhibition of cancer cells is increasing with the increase in the concentration of extract per ml which clearly shows it is dose dependent. At 500 µg/ml the percentage inhibition is highest in all the extracts. It is well known that efficiency of a drug/compound/extract depends on the concentration at which it inhibits the growth of cell. The extract showing more percentage inhibition at lower concentration is said to be more active against that cell line. The hexane extract of *Eclipta alba haska* (EAH) is showing about 56.7% inhibition at 31.25 µg/ml (Table-1) and hence it could be considered to be most effective. The IC_{50} value (Figure 2) for this extract was also found to be minimum amongst all the test extract that was 17.03. The ethanolic extract of *Morinda augustifolia* (EAH) showed 43% inhibition at 62.5 µg/ml concentration whereas EAE and CMC exhibited more than 60% inhibition with 125 µg/ml concentration of extract. The IC_{50} value has been tabulated in Table-2.
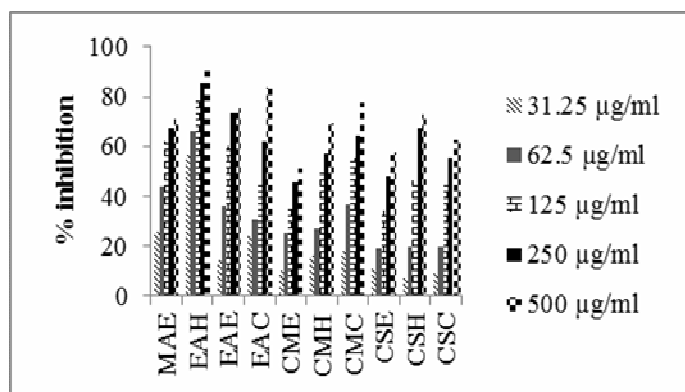

Fig. 1: Antiproliferative activity different extracts against MG-63 cancer cell line

At the lowest concentration of 31.25 µg/ml MAE and EAC showed almost half of the activity as compared to EAH therefore those were not as effective as the later. From the values of percentage inhibition, it became quite evident that CSH, EAC and CMH showed moderate activity while CME, CSC and CSE are least active against MG-63 cancer cell line
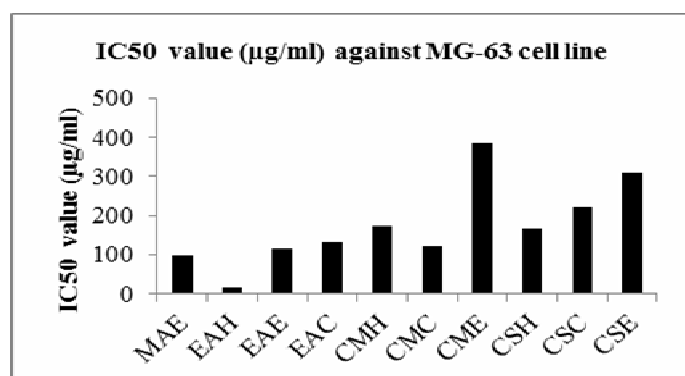

Fig. 2: IC_{50} values of different extracts against MG-63 cancer cell line

For human liver (HepG2) cancer cell lines

From the Figure 3 it is quite clear that extracts are not so effective against HepG2 cell lines up to 100 µg/ml since the mean cell viability of extracts were found to be much higher and those were unable to resist the growth of cancer cells.
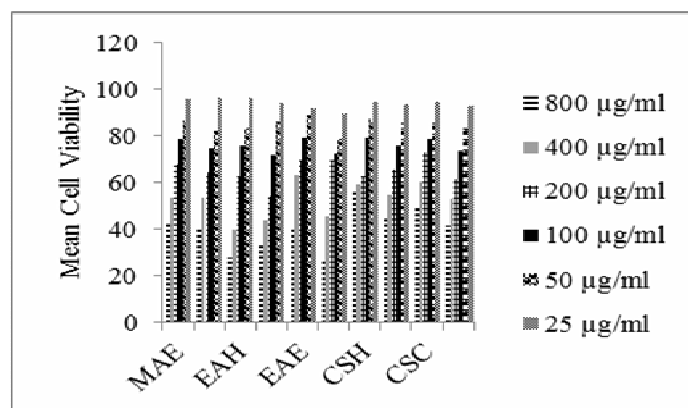

Fig. 3: Mean cell viability of different extracts at different concentrations against hepG2 cancer cell line

Nevertheless, three extracts EAH, EAC and CMC showed better activity than the rest of the extracts as is quite evident from the data given in Table 3.

The efficiency of these extracts was also evident from their IC_{50} values as given in Table 4 and Figure 4. Further work could be undertaken to get the lead compound for these extracts.
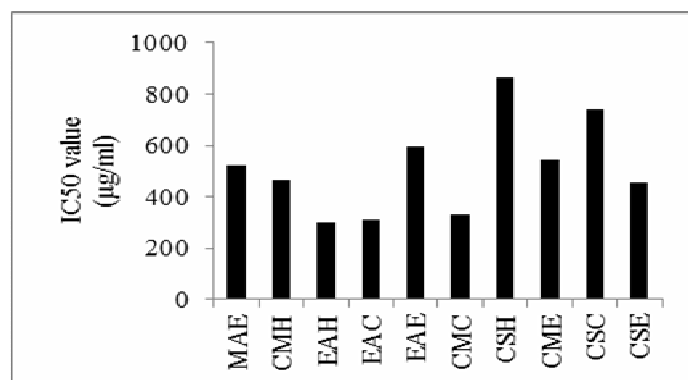

Fig. 4: Graph representing the IC_{50} value of all the extracts against HepG2 cancer cell line

For anti-tubercular activity

All the extracts were then evaluated for the anti-tubercular activity against *Mycobacteria tuberculosis* (vaccine strain, H37 RV strain) using MABA as tabulated in Table 5. It was found that ethanolic extract of *Morinda angustifolia* (MAE) was most effective and inhibited the growth of bacteria at even at least concentration of 0.8 µg/ml. The extracts CMC, EAE, EAC showed bacterial inhibition at a concentration of 1.6 µg/ml. EAH, CSH, CME and CSE showed moderate activity and are effective at a concentration of 12.5 µg/ml. CMH is found to be least active and exhibited bacterial inhibition at concentration of 25 µg/ml. It could be concluded that further work on these extracts could be undertaken to get few antitubercular agents.

Conclusion

From the above discussion it is clear that hexane extract of *Eclipta alba haska* (EAH) was most effective against MG-63 cancer cell line with IC_{50} value of 17.03 µg/ml, and

ethanolic extract of *Morinda angustifolia* (MAE) followed it with IC$_{50}$ value of 97.458 µg/ml. All the tested extracts were not effective against HepG2 cancer cell line as the IC$_{50}$ values are quite high. The preliminary evaluation of antitubercular activity revealed that ethanolic extract of *Morinda angustifolia* (MAE) was most potent even at concentration of 0.8 µg/ml followed by chloroform extract of *Callistemon macropunctatus* (CMC), chloroform extract of *Eclipta alba haska* (EAC), ethanolic extract of *Eclipta alba haska* (EAE) exhibiting inhibitory activity at a concentration of 1.6 µg/ml. From the above discussion, it became clear that extensive work is required on *Eclipta alba haska* as this plant could be a source of anticancer and antitubercular agents.

Table 1: Percentage inhibition of extracts at different concentrations

Conc-> Extract	31.25µg/ml	62.5µg/ml	125µg/ml	250µg/ml	500µg/ml
MAE*	25.81±1.79	43.81±3.80	62.26±3.51	67.68±2.93	70.82±1.27
EAH*	56.70±1.81	66.48±2.97	79.75±1.77	85.58±0.68	92.75±0.63
EAE*	14.19±5.20	36.23±0.95	60.25±0.86	73.22±0.77	75.13±1.73
EAC*	24.51±2.16	30.44±3.88	44.73±3.16	62.10±3.89	83.67±1.46
CME*	10.29±2.07	25.05±2.01	35.84±4.26	45.68±1.58	50.89±0.72
CMH*	16.11±1.67	27.02±3.15	49.24±2.10	57.36±3.47	9.25±2.60
CMC*	17.96±2.29	36.72±3.55	55.41±3.86	64.19±1.79	77.57±2.63
CSE*	10.82±3.03	19.42±2.47	34.33±1.66	48.19±1.88	57.56±2.71
CSH*	7.07±1.22	19.79±3.28	46.08±2.35	67.14±2.54	73.09±1.10
CSC*	9.88±1.17	19.92±2.34	44.62±2.47	55.50±1.47	62.72±3.07

*(Ethanolic extract of *Morinda angustifolia* (MAE), hexane extract of *Eclipta alba haska* (EAH), ethanolic extract of *Eclipta alba haska* (EAE), chloroform extract of *Eclipta alba haska* (EAC), ethanolic extract of *Callistemon macropunctatus* (CME), hexane extract of *Callistemon macropunctatus* (CMH), chloroform extract of *Callistemon macropunctatus* (CMC), ethanolic extract of *Chloroxylon swetienia* (CSE), hexane extract of *Chloroxylon swetienia* (CSH), chloroform extract of *Chloroxylon swetienia* (CSC))

Table 2: Inhibition of all the extracts against MG-63 cancer cell line

Extract	MAE	EAH	EAE	EAC	CMH	CMC	CME	CSH	CSC	CSE
IC$_{50}$ value (µg/ml)	97.46	17.03	115.53	130.45	171.2	122.9	383.08	166.1	219.4	309.1

Table 3: Mean cell viability of different extracts at different concentrations against HepG2 cancer cell line

Conc./extract	MAE	CMH	EAH	EAC	EAE	CMC	CSH	CME	CSC	CSE
					Mean Cell viability					
800 µg/ml	43.32	40.55	27.76	34.35	40.55	27.4	56.61	45.29	49.01	41.4
400 µg/ml	53.87	53.52	40.14	43.79	63.57	45.48	59.11	54.55	60.28	52.98
200 µg/ml	68.02	64.7	62.79	54.35	69.56	69.42	62.79	65.43	73.38	61.66
100 µg/ml	78.96	74.85	75.73	71.9	79.09	72.51	79.37	75.73	78.66	73.42
50 µg/ml	86.76	82.2	83.38	86.46	89.39	78.99	87.58	85.87	86.05	85.04
25 µg/ml	96.17	96.29	96.32	94.35	91.86	89.44	94.85	93.82	94.85	93.16
Negative Control	100									

Table 4: Data for IC$_{50}$ value of all the extracts against HepG2 cancer cell line

Extracts	MAE	CMH	EAH	EAC	EAE	CMC	CSH	CME	CSC	CSE
IC$_{50}$ (µg/ml)	523.2	465.3	300.7	309.9	593.2	330.8	862.3	543.9	741.5	454.4

Table 5: Anti-tubercular activity against H37VR strain

Sl. No.	Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
01	MAE	S	S	S	S	S	S	S	S
02	CMH	S	S	R	R	R	R	R	R
03	EAH	S	S	S	S	R	R	R	R
04	EAC	S	S	S	S	S	S	S	R
05	EAE	S	S	S	S	S	S	S	R
06	CMC	S	S	S	S	S	S	S	R
07	CSH	S	S	S	S	R	R	R	R
08	CME	S	S	S	S	R	R	R	R
09	CSE	S	S	S	S	R	R	R	R
10	CSC	S	S	S	R	R	R	R	R

S= sensitivity   R= resistance

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