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Appraisal of anti-mycobacterial potential against MDR-MTB in pediatric patients, cytotoxicity and mutagenicity of *Aloe vera* and *Allium sativum*

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Abstract: The purpose of the studies was to evaluate an *in-vitro* anti-mycobacterial activity of *Aloe vera* and *Allium sativum* against MDR-MTB, their cytotoxicity and mutagenicity. Four extracts of *Aloe vera* and *Allium sativum* were prepared by Soxhlet apparatus and their minimum inhibitory concentrations (MIC's) were determined by BACTEC MGIT960 system against multi drug resistant *Mycobacterium tuberculosis* (MDR-MTB) isolates, collected from pediatric patients. Fractions of *Aloe vera* and *Allium sativum* extracts were separated using glass column chromatography, followed by evaluation of cytotoxicity and mutagenicity by tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and Ames test, respectively. Out of four extracts, ethanol extracts of *Aloe vera* and *Allium sativum* exhibited activity at MIC 5mg/mL to 7mg/mL and 3mg/mL to 5mg/mL, respectively and IC_{50} by MTT assay for combination of all fractions were 278.3mcg/100µL and 270.8mcg/100µL and in Ames assay M.I of TA98 were 0.14 and 0.07 and M.I of TA100 were 1.14 and 0.44, respectively. *Aloe vera* and *Allium sativum* extracts showed anti-mycobacterial activity against MDR-MTB isolates so, MIC of ethanol extracts of each plant and fractions of column chromatography had been checked. The MTT and Ames tests depicted that ethanol extracts of *Aloe vera* and *Allium sativum* were non-cytotoxic and non-mutagenic, and can be used in treatment of patients suffering from MDR-MTB.

Keywords: *Allium sativum, Aloe vera*, MDR-MTB, *Mycobacterium tuberculosis*, tuberculosis, TB in children

INTRODUCTION

Tuberculosis (TB) is one of the most critical infectious diseases, that the community has faced over the period of past few centuries (Daniel, 2006). It is an infection of different parts of the body and in most of the cases affect lungs and is mainly caused by the bacterium MTB in humans. It is a contagious disease and can spread from person to person through the air. If an infected person breathes, he or she exhales these infectious microbes capable of spreading the disease to the community (Buonanno *et al*., 2020). The basic symptoms of TB are low grade fever, cold, night sweats, weight loss and cough (Rahman *et al*., 2020). But the person who recently developed the active form of disease may not show these symptoms for months (Miller *et al*., 2000). This may lead to delay in the initialization of the treatment and the spread of the disease to the community. Basic treatment of TB includes an active six months course which includes 4 antimicrobials given with precaution and under supervision of health care professionals (Horsburgh Jr *et al*., 2010).

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Sometimes the antimicrobials, we give to treat the TB may get resistance by the TB causing microbes. This may lead to the development of MDR-TB, which cannot be treated by the Isoniazid and Rifampicin, the most powerful anti-TB drugs (Lange *et al*., 2018). There could be various reasons for the development of resistance. One of the main reasons is the usage of the inappropriate method of treatment and mismanagement of disease control. This may include the usage of poor standard drugs with substandard storage conditions. In most countries, it is being difficult to manage and treat properly the MDR-TB. The possible reason for that is the expensive treatment of the disease, unavailability of the effective medicines and the various adverse effects patient may experience during the treatment (Sotgiu *et al*., 2012).

Prevalence of TB in pediatrics and its proper method of management is largely being ignored by the WHO, health policymakers and clinical physicians (Swaminathan and Rekha, 2010). The possible reason for this is due to the impression that pediatrics rarely develops infectious diseases and they might not be involved in the epidemiology of the disease. However, pediatrics suffering from TB should be prioritized. Not only because

the children suffering from pandemic diseases should be properly treated but also because if the efforts of limiting the infectious disease at the pediatric level are ignored, the control of the disease in the overall community will be severely affected (Perez-Velez and Marais, 2012).

Traditionally, Medicinal plants like *Aloe vera* are being used in treatment of Tb (Nguta *et al*., 2015). *Aloe vera* has shown a potent anti TB activity due to its antimicrobial property. Apart from this, *Allium sativum* when checked against MDR-TB- has shown potent inhibitory effects against both MDR and non-MDR TB (Gupta *et al*., 2010). Various studies recommended that the *Allium sativum* has decreased chances of resistance in TB thus ceasing its development to the MDR-TB (Hannan *et al*., 2011a).

MATERIALS AND METHODS

Collection of plants

Wild *Aloe vera* was collected as a whole plant from Raja Jang, District Kasur Punjab, Pakistan. However, *Allium sativum* was purchased from the local market of Lahore. The voucher numbers were GC. Herb. Bot. L 3370 for *Aloe vera*. L and GC. Herb. Bot. 3371 for *Allium sativum* L as assigned by the herbarium of government college botany department, Lahore-Pakistan.

Method of Extraction

Both the plants were dried at 40°C in a hot air oven, followed by their grinding into fine powder. 50g powder of each plant was treated with n-hexane pharmaceutical grade as a solvent at ~60ºC, in Soxhlet apparatus, separately by making a thimble with Whatman's filter paper No.1. Extraction process was repeated several time to confirm the maximum yield of extract. The particulate material was separated by passing the extract through Whatman's filter paper No.1. Obtained extract was dried by using rotary evaporator, and the collected mass was stored at 2-8 ºC for further analysis. Moreover, the similar procedure was employed to obtain aqueous, ethanol and chloroform extract.

Study area, isolation and inoculation of culture

Samples for MDR *M. tuberculosis* were collected from early morning sputum and other body fluids of pediatric patients, suffering from MTB, who visited The Children's Hospital and Institute of Child Health (CH & ICH), Lahore, during the period of January 2016 to December 2017. The total number of patients in both years were 1718 (male=828 and female=890). Polymerase Chain Reaction (PCR) was used for confirmation of *Mycobacterium tuberculosis.* For detection of MDR-MTB isolates, activity against Rifampicin, Isoniazid, Streptomycin, Ethambutol and Pyrazinamide was checked by using Gene Xpert Dx system and BACTEC MGIT 960 system.

The sensitivity of plant extracts

In Middle brook 7H9 broth, sensitivity testing of plants extract was performed by using BACTEC MGIT 960 system (Becton Dickinson, Sparks, MD) (Njeru and Muema, 2020), and the whole activity was carried out at Microbiology Department of University of Health Sciences, Lahore.

Reconstitution of MGIT PANTA was done with 15ml of MGIT growth supplement and for the inoculation and incubation of the MGIT medium, MGIT tubes were labelled and 0.8ml of MGIT growth supplement was added in them along with 0.1ml of different concentrations of plant extracts. In all the tubes 0.5ml of the culture suspension (inoculum) was added except the growth control (GC) tube. For GC tube test, culture suspension was diluted by adding 0.1ml of test culture suspension in 10ml of sterile normal saline. After dilution 0.5ml was added in GC tube. All the tubes were incubated at 37ºC in BACTEC MGIT 960 instrument. MGIT tubes were incubated until the instrument depicted GC tube positive. All the tubes were scanned after the test was completed in 4 to 21 days as indicated by the instrument.

The results were recorded as susceptible (S), resistant (R) or intermediate (X). The growth unit (GU) values of the plant extract vials were evaluated by using manual reader, which had indicated S (when the growth unit of the drug tube was less than 100) and R (when the growth unit of the drug tube was 100 or more). The plant extract which showed sensitivity against MDR *M. tuberculosis* was further proceeded and the MIC was determined in the same way.

Column chromatography of effective plant extracts

Aloe vera

Column chromatography of ethanol extract of *Aloe vera* was performed by using silica gel (60 mesh) glass column, based on increasing solvent polarity. 10g of extract had been mixed with 25g of pre-activated silica gel, and its slurry was made with n-Hexane and loaded on the column and then ran firstly with 500 ml of n-Hexane (100%), followed by different solvents including, n-Hexane: Chloroform (1:1), n-Hexane: Chloroform (2:3), Chloroform (100%), Chloroform: Ethyl acetate (4:1), Chloroform: Ethyl acetate (1:1), Chloroform: Ethyl acetate (1:4), Ethyl Acetate (100%), Ethyl Acetate: Methanol (4:1), Ethyl Acetate: Methanol (1:1) and Methanol (100%). Each fraction was concentrated on rotary vacuum evaporator, buchi type at 40ºC. With the help of BACTEC MIGT 960 system anti-mycobacterial potential of all the column fractions was determined.

Allium sativum

Extraction of *A. sativum* was processed in similar way, as done for *Aloe vera*, but with the variation in the ratios of solvents, used. The ratios of solvents used were n-Hexane (100%), n-Hexane: Chloroform (4:1), n-Hexane:

Chloroform (1:1), n-Hexane: Chloroform (3:7), Chloroform (100%), Chloroform: Ethyl acetate (4:1), Chloroform: Ethyl acetate (3:1), Chloroform: Ethyl acetate (7:3), Chloroform: Ethyl acetate (65:35), Chloroform: Ethyl acetate (1:1), Chloroform: Ethyl acetate (1:4), Ethyl acetate (100%), Ethyl acetate: Methanol (4:1), Ethyl acetate: Methanol (1:1) and Methanol (100%). Each fraction was concentrated and anti-mycobacterial potential of all the column fractions was checked as above in *Aloe vera*.

The minimum inhibitory concentrations of the effective fractions were observed by BACTEC MIGT 960 system as described above.

In-vitro safety evaluation

MTT assay

MTT assay was performed for cytotoxicity (Dantu *et al*., 2012). The sample was prepared in 1% Dimethyl Sulphoxide (DMSO) solution having concentration ranges from 100mcg/100µl to 0.2mcg/100µl by two-fold serial dilutions. For cytotoxic evaluation of plant extracts plates having baby hamster kidney (BHK-21) cell lines were used. For respective dilutions, plates were used to mix 200µl of dilutions with cell culture medium after proper labelling. Each sample dilution was analyzed in duplicate. The lids of plates were closed and were incubated in CO_2 incubator for 72 hours. After discarding the cell culture media from cells, 20µl of MTT solution was added in each well along with 100µl of fresh media. Again, plates were incubated for further three hours. 100 µL of DMSO was added for dissolution of formazan crystals after removal of media. The intensity of the color of live cells (alteration of yellow MTT dye to purple color) was measured using an enzyme-linked immunosorbent assay (ELISA) reader and absorbance was taken at 570nm.

Control groups

Cell culture media served as a positive control, whereas, 100% DMSO was used as negative control. Cell survival percentage of BHK-21 cells was calculated by;

Cell survival (%) = (Mean OD of Positive dilution – Mean OD of negative Control + Mean OD of Positive Control) × 100

Ames assay

Muta-Chromoplate kit was used (Gonzalez-Avila *et al*., 2003). Two mutant strains (TA98 and TA100) of Salmonella typhimurium were purchased from Environmental Bio Detection Products Incorporation (EBPI) Canada. Sodium azide (5µg/ Petri plate) was used as positive control for TA-100 and potassium dichromate (5µg/ Petri plate) for TA-98. The assay was performed using background plate consisting of reagent mixture (2.5ml), De-ionised Water (17.5ml) and Salmonella test strain (0.005ml), along with standard mutagen plate, having mutagen standards (0.1ml), Reagent mixture (2.5ml), De-ionised Water (17.4ml) and Salmonella test strain (0.005ml). Whereas, the test sample plate contained the plant extract (0.005ml), Reagent mixture (2.5ml), De-ionised Water (17.5ml) and Salmonella test strain (0.005ml). The results were recorded by calculating mutagenic index (M.1) as;

$$M.I = \frac{\text{Number of Revertant colonies per plate with test chemical dose}}{\text{Number of natural Revertant colonies of negative control plate}}$$

Test concentration was considered mutagenic, if MI value is ≥2, test concentration was considered significantly mutagenic if MI value is ≥3, test concentration was considered very strongly mutagenic if MI value is ≥4 and test concentration was considered non-mutagenic if MI value is <2.

STATISTICAL ANALYSIS

Inhibitory concentration of each active fraction was calculated using GraphPad Prism version 8 on Windows 10. A log dose was plotted against calculated cell survival percentage (CSP) and IC_{50} was calculated using nonlinear regression fit.

RESULTS

Extraction of plants

Percentage yield of n-hexane, chloroform, 100% ethanol and water/aqueous extracts of *Aloe vera* was 1.9%, 2.1%, 15%, 19.8% *and* of *Allium sativum* was 0.8%, 0.6 %, 14.7% and 22.5% respectively.

Sensitivity of plant extracts

The chloroform and ethanol extract of *Aloe vera,* exhibited inhibition at 6mg/mL and 5mg/mL, respectively. In the case of *Allium sativum*, MICs were determined at concentrations of 1mg/mL- 5mg/mL, Hexane and Ethanol extract exhibited inhibitions at 3mg/mL (table 1 & 2) respectively.

Column chromatography of plant extracts

On the basis of MICs, ethanol extracts of *A. vera and A. sativum* were selected for further experiment. However, n hexane extract of *A. sativum* and chloroform extract of *A. vera* were not used for further experiment because of their low percentage yield.

MIC and anti-mycobacterial activity of different fractions

MICs and Anti-Mycobacterial activity of *Aloe vera* based 13 individual fractions and their mixtures were evaluated by manual MGIT reader at different concentrations (30mcg/ml to 50mcg/ml). The effective fractions were A_2, A_{11}, and A_{12} having MIC 40mcg/ml. Maximum activity was exhibited by the mixture of all fractions, having MIC 30mcg/ml (table 3).

Table 1: Anti mycobacterial activity and MICs of 4 different extracts of *Aloe vera*

S No.	Type of Extract	Minimum Inhibitory Concentration				
		3mg/mL	4mg/mL	5mg/mL	6mg/mL	7mg/mL
1.	Hexane Extract (AVHE)	*+	+	+	+	+
2.	Chloroform Extract (AVCE)	+	+	+	**-	-
3.	Ethanol Extract (AVEE)	+	+	-	-	-
4.	Aqueous Extract (AVAE)	+	+	+	+	+

Table 2: Anti-Mycobacterial activity and MICs of 4 different extracts of *Allium sativum*

S No.	Type of Extract	Minimum Inhibitory Concentration				
		1mg/mL	2mg/mL	3mg/mL	4mg/mL	5mg/mL
1.	n-Hexane Extract (ASHE)	*+	+	**-	-	-
2.	Chloroform Extract (ASCE)	+	+	+	+	+
3.	Ethanol Extract (ASEE)	+	+	-	-	-
4.	Aqueous Extract (ASAE)	+	+	+	+	+

Table 3: Anti mycobacterial activity and MICs of different fractions of ethanol extract of *Aloe vera*

S No.	Solvent Phase	Label	Minimum Inhibitory Concentration		
			30µg/mL	40µg/mL	50µg/mL
1	n-Hexane (100%) (1-5)	A_1	*+	+	+
2	n-Hexane: Chloroform (1-8) 1: 1	A_2	+	**-	-
3	n-Hexane: Chloroform (1-8) 2: 3	A_3	+	+	+
4	Chloroform (100%) (1,2,4 &5)	A_4	+	+	+
5	Chloroform (100%) (3)	A_5	+	+	+
6	Chloroform: Ethyl Acetate (3-5) 4: 1	A_6	+	+	+
7	Chloroform: Ethyl Acetate (1) 4: 1	A_7	+	+	+
8	Chloroform: Ethyl Acetate (2) 4: 1	A_8	+	+	+
9	Chloroform: Ethyl Acetate (1-4) 1: 1	A_9	+	+	-
10	Chloroform: Ethyl Acetate (1-3) 1: 4	A_{10}	+	+	+
11	Ethyl Acetate (100%)	A_{11}	+	-	
12	Ethyl Acetate: Methanol (1-3) 1: 1	A_{12}	+	-	-
13	Methanol (100%) (1-4)	A_{13}	+	+	+
14	A (Mixture of all fractions)	A	-	-	-

Table 4: Anti mycobacterial activity and MICs of different fractions of ethanol extract of *Allium sativum*

S No.	Solvent Phase	Label	Minimum Inhibitory Concentration		
			20µg/mL	30µg/mL	40µg/mL
1	n-Hexane: Chloroform 3: 7	G_1	*+	+	**-
2	Chloroform (1-2)	G_2	+	+	-
3	Chloroform (3)	G_3	+	+	-
4	Chloroform (4)	G_4	+	+	+
5	Chloroform: Ethyl Acetate (1-3) 4: 1	G_5	+	-	-
6	Chloroform: Ethyl Acetate (1-5) 65: 35	G_6	+	-	-
7	Chloroform: Ethyl Acetate (1-6) 1: 1	G_7	+	+	+
8	Chloroform: Ethyl Acetate (1-5) 1: 4	G_8	+	-	-
9	Ethyl Acetate (1-5)	G_9	+	+	-
10	Ethyl Acetate: Methanol (1-5) 4: 1	G_{10}	+	+	+
11	Ethyl Acetate: Methanol (6-8) 4: 1	G_{11}	+	+	+
12	Ethyl Acetate: Methanol (1-2) 1: 1	G_{12}	+	+	+
13	Ethyl Acetate: Methanol (3-9) 1: 1	G_{13}	+	+	-
14	Methanol (100%) (1-8)	G_{14}	+	+	+
15	G (Mixture of all fractions)	G	-	-	-

+ means growth is present, **- means growth is absent

Table 5: MTT assay

Fractions	MIC (µg/mL)	IC_{50} (w/v µg/100µL)	IC50 (w/v µg/mL)
A_2	40	8.58	85.8
A_{11}	40	3.61	36.1
A_{12}	40	665.5	6655
A (Mixture of all fractions)	30	278.3	2783
G_5	30	2.06	20.6
G_6	30	402.7	4027
G_8	30	173.5	1735
G (Mixture of all fractions)	20	270.8	2708

14 individual fractions based on *Allium sativum* and their mixture, of, were evaluated at different concentrations (20mcg/mL to 40mcg/mL). The effective fractions were G_5, G_6, and G_8 having MIC 30mcg/mL. Maximum activity was exhibited by the mixture of all fractions, having MIC 20mcg/mL (table 4).

Safety Profile for fractions of plant extract

MTT Assay

Cytotoxic potential of four most effective fractions of *A.vera* and *A.sativum* were evaluated by using BHK-21 cell line. IC_{50} calculated for A_2, A_{11}, A_{12} and A were 8.581mcg/100µl, 3.61mcg/100µl, 655.51mcg/100µl and 278.3mcg/100µl, respectively and for G_5, G_6, G_8 and G were 2.059mcg/100µl, 402.7mcg/100µl, 173.5mcg/100µl and 270.8mcg/100µl, respectively.

For *A. sativum*, G_5 was considered as not safe component by showing comparatively minimum value of IC_{50} i.e., 20.6µg/mL, having MIC 30µg/mL. On the other hand, for *A. vera*, A_{11} was considered cytotoxic component with IC_{50} of 36.1µg/mL with MIC 40µg/mL (table 5).

Ames assay

Out of 96 wells, the number of revertant colonies was 90, when $K_2Cr_2O_7$ was used as a standard for TA98, exhibiting mutagenic activity. When NaN_3 was used as standard for TA100, the number of revertant colonies was 86 out of 96 wells. The outcomes have indicated that NaN_3 is a mutagenic substance while background results remained non-mutagenic for both TA98 and TA100. By using TA98, the active fractions of both plants were subjected to mutagenicity, the mixture of all fractions of A and G have exhibited the non-mutagenicity, as M.I values were 0.14 and 1.14 respectively and the value of the mutagenic index is less than 2. However, rest of the fractions were found to be mutagenic having M.I more than 2. Similar was the case when TA100 was used.

Mutagenic index of

TA 98

For *Aloe vera* $M.I = \frac{2}{14} =$ 0.14

For *Allium sativum* $M.I = \frac{16}{14} =$ 1.14

Where, 2 and 16 were the number of revertant colonies per plate, and 14 were the natural revertant colonies of negative control plate

TA 100

For *Aloe vera* $M.I = \frac{2}{27} =$ 0.07

For *Allium sativum* $M.I = \frac{12}{27} =$ 0.44

Where, 2 and 12 were the number of revertant colonies per plate and 27 were the natural revertant colonies of negative control plate

DISCUSSION

MDR-MTB is considered to be a fatal disease in pediatric patients. The present studies were valuable, as it has conducted to evaluate the effectiveness of the easily available and common plants. In the most populated area (Lahore) of Punjab, Pakistan, the studies were quit significant to highlight the potential of *A. sativum and A. vera* against the MDR-*M. tuberculosis*.

Out of four different extracts of *A. sativum,* n-Hexane and ethanol based extracts (ASHE and ASEE), were found to be imparting pharmacological activities. However, other extracts, including chloroform and water based extracts were failed to inhibit the growth of microbes. The results presented in the studies confirmed that *A. sativum* exhibited satisfactory anti-tuberculosis activity against MTB, and these findings have strong evidence from the literature where, Sivakumar and Jayaraman have not only confirmed the anti-TB activity of ethanol extract of A. *sativum*, but also recommended it as the adjuvant therapy for tuberculosis (Sivakumar and Jayaraman, 2011; Nair *et al*., 2017).

The scientist has reported numerous studies, providing clues about the therapeutic effectiveness of medically effective plants, especially, against MDR-TB (Pandit *et al*., 2015). The extracts of A. *s. ativum* proved to be beneficial against MDR-TB. The results were in accordance with the recommendations, that the extract of *Allium sativum* can be used against MDR-TB, and one possible reason of this was that, it decreases the burden of drug resistance (Hannan *et al*., 2011b). According to a

study, the *in vitro* antimycobacterial activity of ethanol extract of garlic determined was 1-2mg/ml against MDR *M. tuberculosis* (Rajani *et al*., 2015). The results of present study were in accordance with the results of previous study which showed antimycobacterial activity at 100µg/mL of ethanol extract and 50µg/ml aqueous extracts against MTB (Sivakumar and Jayaraman, 2011). Similarly, fractions of ethanol garlic extract (G_5, G_6 and $G_{8)}$ have exhibited considerable pharmacological activities (MIC 30µg/mL). Furthermore, combination of all fractions, labelled as G has shown the maximum therapeutic potentials at comparatively low MIC, probably due to the possible synergism of all the constituents.

The present study described that, out of four, n-Hexane extract (AVHE) and aqueous extract (AVAE) of *A. vera* did not showed any inhibition against MDR. *M. tuberculosis*. However, chloroform extract (AVCE) and ethanol extract (AVEE), both have shown noticeable inhibitory activities. In other two studies, *Aloe vera* was also evaluated for antimycobacterial activity by using its gel at different concentration levels. The percentage of inhibition against MTB was exhibited at 25%, 35% and 56% when LJ medium was applied by using *Aloe vera* gel in pure form. Whereas, the 32%, 46% and 68% inhibition was exhibited against Multi drug resistant strains of TB when *Aloe vera* pure gel was used as 2,4 and 6 percent respectively by applying LJ medium (Vyas *et al*.; Bernaitis *et al*., 2013). As reported in literature, the MIC value against the tested strains of *Mycobacterium tuberculosis* was not same and the value vary against all tested strains (Nguta *et al*., 2016). The value of MIC against the Mycobacterium strain H37Ra was 2.5 mg/ml. This might be due to the fact that constituents of different varieties of *Aloe vera* have different concentration. The effective fractions were A_2, A_{11} and A_{12} having MIC 40µg/mL. Maximum activity was shown by combination of all fractions labeled as A having MIC 30µg/mL. The low value of MIC of A may be due to the synergistic effect of all components.

Toxicological study is mandatory before suggesting the *A. vera* extract and *A. sativum* garlic extract for anti-mycobacterial properties. Toxicological effects of medicinal plants cannot be ignored as they may be intrinsically toxic or become potentially toxic when taken in combination with other preparations. Because most of the time, extract preparations are usually not evaluated for purity and consistency of active compounds. Therefore, MTT assay and AMES test are of much importance. Cytotoxic evaluation of *Allium sativum* components by using MTT Assay revealed that fraction G_5 was not safe, while remaining effective fractions of *Allium sativum* were safe at their MICs. Similarly, cytotoxic potential of four most effective fractions of *Aloe vera* was evaluated by using BHK-21 cell line. Fraction (A_{11}) of *Aloe vera* was found not to be safe, as exhibited by MTT assay, however, rest of the active fractions of *Aloe vera* were not cytotoxic as their MICs were much below their cytotoxic IC_{50}. The results were found in accordance to the previous studies (Sanches *et al*., 2015). Furthermore, the AMES test indicated that constituents of both *Aloe vera* (A) and *Allium sativum* (G) were non-mutagenic. Therefore, one can use extracts of these two plants safely for the treatment of MDR- MTB.

CONCLUSION

In-vitro antimycobacterial activity of ethanol extracts of *Aloe vera* and *Allium sativum* was seen against MDR-MTB isolates collected from infected children and MIC of that extracts and of fractions obtained by Column chromatography had been checked by BACTEC MGIT960 system. Cytotoxic and mutagenic tests of effective fractions were performed by MTT and Ames assays, their results revealed that ethanol extracts of both plants were non-cytotoxic and non-mutagenic and can be used in treatment of MDR-MTB in pediatrics.

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REFERENCES

Bernaitis L, Shenoy VP, Ashok M, Nath M and Shenoy R (2013), Biological, Sciences C. Effects of selective medicinal plants against multi drug resistance *Mycobacterium tuberculosis* strains. *Res. J. Pharm. Biol. Chem. Sci.,* **4**(3): 812-817.

Buonanno G, Stabile L and Morawska L (2020). Estimation of airborne viral emission: Quanta emission rate of SARS-CoV-2 for infection risk assessment. *Environ. Int.*, 105794.

Daniel TM (2006). The history of tuberculosis. *Respir. Med,* **100**(11): 1862-1870.

Dantu AS, Shankarguru P, Ramya DD and Vedha HB (2012). Evaluation of *in vitro* anticancer activity of hydroalcoholic extract of *Tabernaemontana divaricata*. *Asian J Pharm Clin Res,* **5**(3): 59-61.

Gonzalez-Avila M, Arriaga-Alba M, De la Garza M, del Carmen HernándezPretelín M, Domınguez-Ortız MA, Fattel-Fazenda S and Villa-Trevino S (2003). Antigenotoxic, antimutagenic and ROS scavenging activities of a Rhoeo discolor ethanolic crude extract. *Toxicology in vitro,* **17**(1): 77-83.

Gupta R, Thakur B, Singh P, Singh HB, Sharma VD, Katoch VM and Chauhan SV (2010). Anti-tuberculosis activity of selected medicinal plants against multi-drug resistant *Mycobacterium tuberculosis* isolates. *Indian J. Med. Res.,* **131**(6): 809.

Hannan A, Ullah MI, Usman M, Hussain S, Absar and Javed MK (2011). Anti-mycobacterial activity of garlic (*Allium sativum*) against multi-drug resistant and non-multi-drug resistant *Mycobacterium tuberculosis*. *Pak. J. Pharm. Sci.,* **24**(1): 81-85.

Horsburgh Jr CR, Goldberg S, Bethel J, Chen S, Colson PW, Hirsch-Moverman Y, Hughes S, Shrestha-Kuwahara R, Sterling TR and Wall K (2010). Latent TB infection treatment acceptance and completion in the United States and Canada. *Chest.,* **137**(2): 401-409.

Lange C, Chesov D, Heyckendorf J, Leung CC, Udwadia Z and Dheda KJR (2018). Drug-resistant tuberculosis: An update on disease burden. *Diagnosis and Treatment,* **23**(7): 656-673.

Miller LG, Asch SM, Yu EI, Knowles L, Gelberg L and Davidson P (2000). A population-based survey of tuberculosis symptoms: how atypical are atypical presentations? *Clin. Infect. Dis,* **30**(2): 293-299.

Nair SS, Gaikwad SS, Kulkarni SP and Mukin AP (2017). *Allium sativum* constituents exhibit anti-tubercular activity *in vitro* and in RAW 264.7 mouse macrophage cells infected with *Mycobacterium tuberculosis* H37Rv. *Pharmacogn. Mag,* **13**(Suppl 2): S209.

Nguta JM, Appiah-Opong R, Nyarko AK, Yeboah-Manu D and Addo PG (2015). Medicinal plants used to treat TB in Ghana. *Int. J. Mycobacteriol.*, **4**(2): 116-23.

Nguta JM, Appiah-Opong R, Nyarko AK, Yeboah-Manu D, Addo PG, Otchere I and Kissi-Twum A (2016). Antimycobacterial and cytotoxic activity of selected medicinal plant extracts. *J. Ethnopharmacol.,* **182**: 10-15.

Njeru SN and Muema JM (2020). Antimicrobial activity, phytochemical characterization and gas chromatography-mass spectrometry analysis of *Aspilia pluriseta* Schweinf. *Extracts,* **6**(10): e05195.

Pandit R, Singh PK and Kumar V (2015). Natural remedies against multi-drug resistant *Mycobacterium tuberculosis*. *J. Tuberc. Res,* **3**(04): 171.

Perez-Velez CM and Marais BJ (2012). Tuberculosis in children. *N Engl J Med,* **367**(4): 348-361.

Rahman M, Mizanur I, Islam MM and Shahidul M (2020). A 65-year-old male with moderate anemia, low-grade fever and weight loss. *Bangabandhu Sheikh Mujib Med Univ J,* **13**(3): 97-110.

Rajani SD, Desai PB and Rajani D (2015). Anti-mycobacterial activity of garlic (Allium sativum) against multi-drug resistant and reference strain of Mycobacterium tuberculosis. *Int J Appl Res,* **1**: 767-70.

Sanches I, Carvalho A and Duarte R (2015). Who are the patients with extrapulmonary tuberculosis. *Revista Portuguesa de Pneumologia* (English Edition), **21**(2): 90-3.

Sivakumar A, and Jayaraman G (2011). Anti-tuberculosis activity of commonly used medicinal plants of south India. *J. Med. Plant Res,* **5**(31): 6881-6884.

Sotgiu G, Centis R, D'Ambrosio L, Alffenaar J-WC, Anger HA, Caminero JA, Castiglia P, De Lorenzo S, Ferrara G and Koh WJ (2010). Efficacy, safety and tolerability of linezolid containing regimens in treating MDR-TB and XDR-TB: Systematic review and meta-analysis. *Eur. Respir. J,* **40**(6): 1430-1442.

Swaminathan S and Rekha B (2010). Pediatric tuberculosis: Global overview and challenges. *Clin. Infect. Dis,* **50**(Supplement3): S184-S94.

Vyas RB, Shrivastava P and Parihar G (2018). Anti-mycobacterial activity of *Aloe vera* against *M. tuberculosis* and *M. avium* standard strain. *IJMDR,* 2(1): 6-13.