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Antibacterial, Antifungal and Antitubercular Activity of Methanolic Extracts of *Adansonia digitata* L

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Abstract: *This study was aimed with an objective to investigate the antimicrobial, antifungal and antitubercular activity of different parts of Adansonia digitata L. The leaves, Root bark, and Fruit pulp were extracted with methanol. The antibacterial and antifungal activity of the extracts were determined using Broth dilution and Agar disc diffusion method and antitubercular activity was determined using L. J. agar (MIC) method. The antibacterial and antifungal activities of the extracts were tested against four Gram-positive strains Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumonia and Bacillus subtilis. Four Gram-negative strains- Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Vibrio cholera and three fungal strains - Aspergillus niger, Aspergillus clavatus, Candida albicans. The results were compared with different standards like Ampicillin, Ciprofloxacin, Norfloxacin and Chloramphenicol for antibacterial activity and Nystatin and Griseofulvin for antifungal activity. The highest minimum inhibitory concentration (MIC) was showed by fruit pulp extract against E. coli and C. albicans at dose of 62µg/ml and 250µg/ml. Zone of inhibition studies revealed that the maximum inhibition was showed by fruit pulp extract. Antitubercular activity of the extracts was tested against bacteria Mycobacterium tuberculosis (H37Rv) and found that minimum inhibitory concentration was showed by root bark extract at dose 62.5µg/ml. The overall results of the minimal inhibitory concentration and zone of inhibition indicates that methanolic extracts of A. digitata L. contains some potent antibacterial, antifungal and antitubercular phytoconstituents.*

I. Introduction

India has a remarkable wealth of herbal medicines and have a vast knowledge of traditional use of medicinal plants for prevention and cure of several diseases (Gupta and Tandon, 2004; Sharma, 1998). Many of the Indian medicinal plants have long been used as antimicrobial agents. *Adansonia digitata* L. has the vast potential of as a source for antibacterial and antifungal agent. A systematic investigation was undertaken to screen the local plant A. digitata L. for antibacterial and antifungal and antitubercular activity.

A. digitata L., family Bombaceae, is known by various names such as Baobab and Lemonade tree. It is endemic to Africa. In India it grows naturally in Mandu region of Madhya Pradesh. It is very common in Andhra Pradesh and also cultivated in Uttar Pradesh, Bihar, Tamil Nadu and Maharashtra (Nadkarni and Nadkarni, 2000). It is one of the largest and reportedly longest living species (6000 years) of the world (Ramadan et al., 1994; Shukla et al., 2001).

The plant is called as Baobab tree (English), Gorakamali (Hindi), and Gangerukie (Sanskrit) in Indian traditional medicine (Chopra et al., 1992).

The different plant parts are widely used as food, medicine, clothing and shelter. In folk medicine it is used as antipyretic, febrifuge, astringent in diarrhoea and dysentery, also as substitute for cinchona in various systems of medicine (Ramesh et al., 1992). The pith of the fruit contains high levels of ascorbic acid (vitamin C), tartaric acid, and citric acid and is used in producing a refreshing drink. Seeds are eaten fresh or dried. They can also be ground into a powder and used as a substitute for coffee. The leaves are said to be rich in vitamin C, sugars, potassium tartrate, and calcium. The leaves are freshly cooked as a vegetable or dried and crushed for later use by local people (Van wyk et al., 2005; Esterhuyse et al., 2001). The leaves are used medicinally against fever by reducing sweating and as an astringent by tightening mucous membranes thus reducing mucous secretions. In West Africa, the leaves and bark are used for treating urinary disorders and diarrhoea. Young roots are cooked and eaten (Wickens, 1982; Van wyk et al., 2005; Esterhuyse et al., 2001). Root bark and leaf extract of *A. digitata* has shown significant antiviral activity against Herpes simplex, Sindibis and Polio virus (Anani et al., 2000). Antibacterial and anti-Trypanosome activities have also been indicated by the plant extracts (Hudson et al., 2000; Atawodi et al., 2003). Toxicological, pharmacological, anti-inflammatory, analgesic, antipyretic antibacterial and antiviral effects of the various plant parts of A. digitata have been studied (Kamatou et al., 2011). A variety of phytochemicals constituents such as terpenoids, flavonoids, sterols, vitamins, amino acids, carbohydrates, and lipids (Shukla et al., 2001) have been reported from A. digitata. The fruit pulp of the

plant shows the presence for sterols, triterpenes, saponins, tannins, carbohydrates, and glycosides (Ramadan et al., 1994; Kamatou et al., 2011).

II. Material And Method

Collection and authentication of plant material

Fresh leaves, fruits and root bark of *A. digitata* L. were collected from Mandu, Madhya Pradesh, India, in the month of September 2010. The Plant was authenticated by Dr. Pramod Patil, Professor, Department of Botany, M.L.B Girls Post Graduate College, Bhopal, India. Voucher specimen 00919 has been deposited in their laboratory for future reference.

Preparation of plant extract

The plant material was washed well with water to separate the adhering soil material and dried in the shade. Dried leaves, fruit pulp and bark were comminuted to form coarse powder. Dried leaves, fruit pulp and bark (500 g each) were extracted with petroleum ether (60-80º) for 24 h to remove fatty substances. Defatted dried marc was further extracted with methanol. All the extracts obtained were evaporated to dryness under vaccum in rotary evaporator at 40°C.

Antibacterial and antifungal activity

Test microorganisms and growth media

Staphylococcus aureus (MTCC 96), *Streptococcus pyogenes* (MTCC 442), *Streptococcus pneumoniae* (MTCC 1936), *Bacillus subtilis* (MTCC 441) (Gram-positive bacteria), *Escherichia choli* (MTCC 443), Pseudomonas aeruginosa (MTCC 424), *Salmonella typhi* (MTCC 98), *Vibrio cholerae* (MTCC 3906) (Gram-negative bacteria), *Candida albicans* (MTCC 227), *Aspergillus niger* (MTCC 282), *Aspergillus clavatus* (MTCC 1323) (Fungi) were chosen based on their clinical and pharmacological importance. All MTCC cultures were obtained from the Institute of Microbial Technology, Chandigarh, India.

The bacterial and fungal cultures were incubated for 24 hours at 37°C on nutrient agar and potato dextrose agar (PDA) medium respectively, following refrigeration storage at 4°C. Mueller-Hinton broth was used as nutrient medium to grow and dilute the drug suspension for the test.

Sample preparation

DMSO was used as diluent to get desired concentration of plant extracts against standard bacterial strains. Serial dilutions were prepared by obtaining 2000 microgram/ml concentration as a stock solution for each drug. In primary screening 1000 µg /ml, 500 µg/ml and 250 µg/ml concentrations of diluted drugs were taken. Data were not taken due to high DMSO concentration (10%). The drugs found active in this primary screening were further tested in a second set of dilutions against all organisms. In secondary screening, the active drugs from primary screening were further diluted to obtain concentrations ranging from 200µg/ml - 5µg/ml.

Preparation of inocula

From a pure overnight culture the material is picked from at least 3-4 colonies, suspend totally in 4ml saline in tubes and mix. The inoculum were adjusted to 0.5 McFarland standard (approximately 108 colony forming unit (CFU) per milliliter by comparing turbidity) using a nephelometer (McFarland, 1987).

Determination of minimum inhibitory concentration (MIC)

Microbroth dilution method was used to determine the minimum inhibitory concentration (MIC) (Rattan A; 2000). Tubes containing 10ml Muller-Hinton broth with two fold dilution of plant extracts are inoculated with 50µl inoculums suspension of the bacteria using a multichannel pipette and incubated at 37°c overnight for bacteria and 22°c overnight for fungi. For purity control the tube containing no antibiotic is inoculated with 10µl inoculum suspension of bacteria and put for incubation at 37°c overnight. The MIC of the control organism is read to check the accuracy of the drug concentrations. The lowest concentration inhibiting 99% growth of the organism is recorded as the MIC. The amount of growth from the control tube before incubation (which represents the original inoculums) is compared. Control experiments were carried out under similar condition by using ampicillin, chloramphenicol, ciprofloxacin, and norfloxacin for antibacterial activity and nystatin and griseofulvin for antifungal activity as standard drugs.

Determination of zone of inhibition (ZOI)

Agar disk diffusion method was used to determine zone of inhibition. Mueller-Hinton agar plates (with 10cm diameter and uniform agar depth of approximately 4mm) are inoculated with 50µl inoculums suspension of the bacteria and antimicrobial disks (5 disks) are placed on the inoculated agar plate containing a known

amount of a standardized antimicrobial agent. The plate is incubated for 18 to 24 hours at 37ºc for bacteria and 48 to 96 hours for fungi at 28°c. During incubation the antimicrobial agent diffuses into the agar and inhibits growth of the microorganism, producing a zone of inhibition around the disk. The diameter of this zone is measured and the results are interpreted as values <8 mm were considered as not active against microorganisms. To verify the purity agar plates are inoculated with 10µl inoculum suspension of bacteria and put for incubation at 37 0C for 16 to 18 hours in ambient air. Control experiments were carried out under similar condition by using ampicillin, chloramphenicol, ciprofloxacin, and norfloxacin for antibacterial activity and nystatin and griseofulvin for antifungal activity as standard drugs.

Antitubercular activity

Test microorganisms and growth media

MIC of the test compounds against *M. tuberculosis* H37Rv was determined by L.J. agar (MIC) method (Desai et al.,1984; Shah et al., 1985; Anargyros et al., 1990). The Lowenstein Jensen medium, more commonly known as L.J. medium, is a growth medium (Elbir H; 2008), specially used for culture of *Mycobacterium*, notably *Mycobacterium tuberculosis*. When grown on L.J. medium, *M. tuberculosis* appears as brown, granular colonies (sometimes called "buff, rough and tough").

Sample preparation

For all the test extracts dilutions were prepared in DMSO. Serial dilutions were prepared by obtaining 2000 microgram/ml concentration as a stock solution for each drug. In primary screening 1000 µg /ml, 500 µg/ml and 250 µg/ml concentrations of diluted drugs were taken. The drugs found active in this primary screening were further tested in a second set of dilutions against all organisms. In secondary screening, the active drugs from primary screening were further diluted to obtain concentrations ranging from 200µg/ml - 5µg/ml.

Preparation of inocula

A culture of M. tuberculosis H37Rv growing on L.J. medium was harvested in 0.85% (4ml) saline in bijou bottles. The ten fold dilution of standard 1mg/ml *M. tuberculosis* suspension19 were streaked on L.J medium for determining CFU in the presence and absence of plant extracts. An *M. tuberculosis* suspension of 1mg/ml is equivalent to McFarland standard-120. One loopful 6µl of this suspension was streaked on the L.J. slants using 3mm external diameter loop.

Determination of minimum inhibitory concentration (MIC)

Dilutions of plant extracts were added to liquid L.J. medium and then media were sterilized by inspissation method. The tubes containing diluted plant extracts in L. J. medium were then inoculated with standard M. tuberculosis suspension and incubated at 37°C for 42 days. Readings was taken weekly. For comparison extract free control slants were used. The concentration at which no development of colonies occurred or less than 20 colonies was taken as MIC of test compound. The standard strain *M. tuberculosis* H37Rv was also tested with known drug Rifampicin and Isoniazid in the same batch of media for comparison of CFU on drug free control slants.

III. Results And Discussion

Extractive values and preliminary phytochemical screening

The leaves (ASLE), root bark (ASBE), fruit pulp (FPE) of *A. digitata* afforded methanolic extract in the percent yield of 3%, 2.3% and 1.4% respectively. Preliminary phytochemical screening of all the extracts revealed the presence of flavanoids, steroids, tannins, glycosides and amino acids.

High performance thin layer chromatography

High performance thin layer chromatographic study of the methanolic extract of leaf (ASLE) in the solvent system, toluene: ethyl acetate : diethylamine (7:3:0.5) revealed five spots with Rf values 0.10 (yellow), 0.64 (yellowish green), 0.82 (black), 0.84 (green) and 0.90 (green) when observed under visible light while fruit pulp extract (FPE) in the same solvent system showed four spots with Rf value 0.34 (light blue), 0.64 (faint), 0.71 (light blue), 0.84 (blue) when observed at 366nm. Methanolic extract of the bark (ASBE) in the solvent system, toluene: ethyl acetate (9:1) revealed one major and seven minor spots with Rf values 0.12 (white), 0.16 (red), 0.26 (sky blue), 0.28 (red), 0.37 (blue), 0.50 (red), 0.60 (blue), 0.66 (red) when observed at 366nm.

Antibacterial and antifungal activity

Minimum inhibitory concentration

The Minimum inhibitory concentration (MIC) values of the extracts and standard drugs tested against both gram positive and gram negative bacteria were shown in Table.1-2. The MIC values were found in the range of 62.5-250 µg/ml against the tested bacterial microorganisms. The MIC values against the gram-positive bacteria ranged from 100-250 µg/ml and against gram-negative bacteria from 62.5-250 µg/ml. The finest minimum inhibitory concentration was showed by fruit pulp extract against E. coli (Gram-negative bacteria) at dose of 62.5 µg/ml. Antibacterial potency of plant extracts against these bacteria expressed as MIC indicated that the plant extract is more effective against gram-negative at lower concentration than that against gram-positive bacteria.

Table-1: Minimum inhibitory concentration of methanolic extracts of *A. digitata* L. and standard drugs against Gram positive organisms.

Antibacterial activity					
Minimum inhibitory concentration (µg/ml)					
S.N.	Extract Code	*S. aureus* (MTCC 96)	*S. pyogenes* (MTCC 442)	*S. pneumoniae* (MTCC 1936)	*B. subtilis* (MTCC 441)
Methanolic extracts of *A. digitata* L.					
1	ASLE	250	200	250	100
2	ASBE	100	125	100	125
3	FPE	100	200	200	250
Standard Antibiotics					
4	AMP	250	100	100	250
5	CMP	50	50	50	50
6	CIP	50	50	50	50
7	NOR	10	10	10	100

Methanolic extracts of *A. digitata* - ASLE: Leaves extract; ASBE: Root bark extract; FPE: Fruit pulp extract.
Standard antibiotics-AMP: Ampicillin; CMP: Chloramphenicol; CIP: Ciprofloxacin; NOR: Norfloxacin.

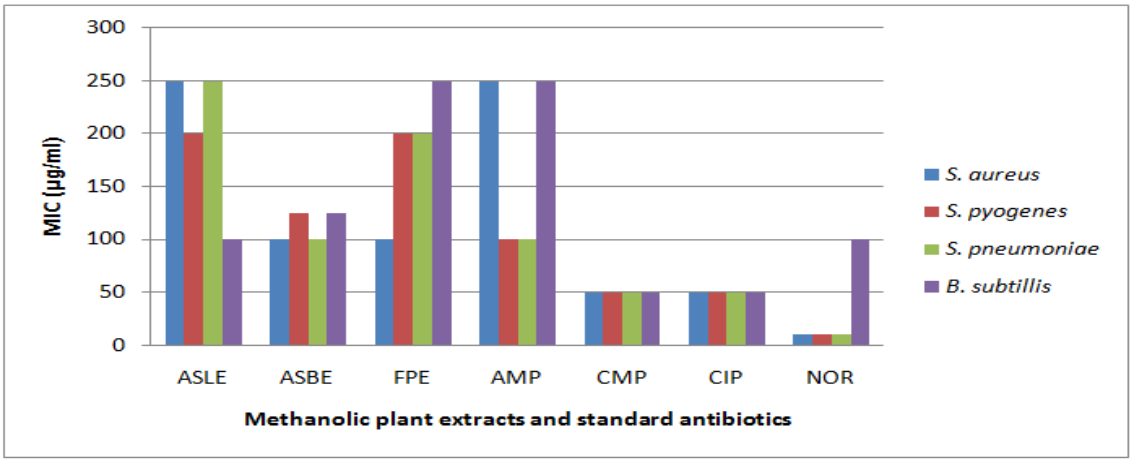

Fig. 1: Minimum inhibitory concentration of *A. digitata* and standard drugs against Gram positive organisms.

Table-2: Minimum inhibitory concentration of methanolic extracts of *A. digitata* L. and standard drugs against Gram negative organisms.

Antibacterial activity					
Minimum inhibitory concentration (µg/ml)					
S.N.	Extract Code	*E. coli* (MTCC 443)	P. aeruginosa (MTCC 424)	*S. typhi* (MTCC 98)	V. cholerae (MTCC 3906)
Methanolic extracts of *A. digitata* L.					
1	ASLE	250	125	100	125
2	ASBE	125	100	250	200
3	FPE	62.5	125	200	250
Standard Antibiotics					
4	AMP	100	-	100	100
5	CMP	50	50	50	50
6	CIP	25	25	25	25
7	NOR	10	10	10	10

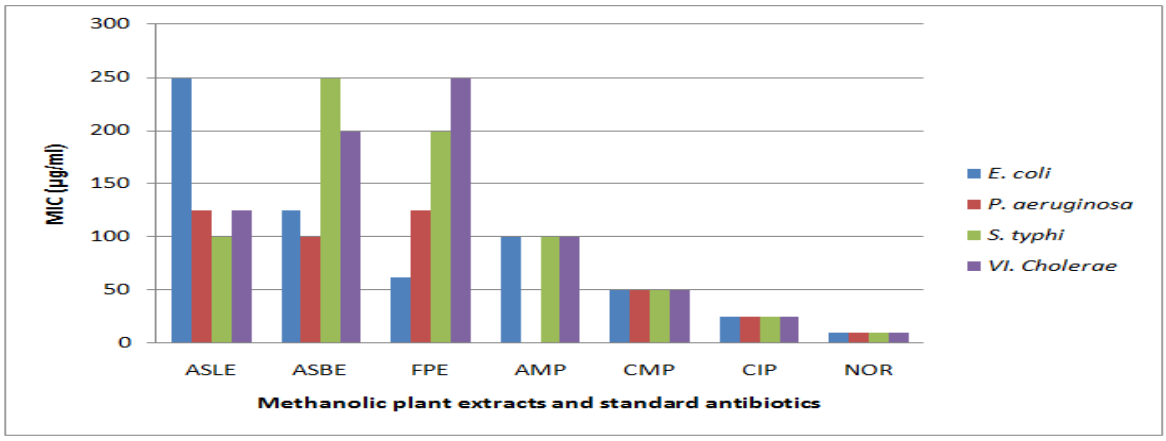

Fig. 2: Minimum inhibitory concentration of *A. digitata* and standard drugs against Gram negative organisms.

Minimum fungicidal concentration

The Minimum fungicidal concentration of extracts and standard drugs tested against fungal microorganisms were shown in Table.3. The fungicidal values of tested plant extracts were found in the range of 250-1000 µg/ml but in case of root bark extract against *A. niger* and *A. clavatus* it is >1000 µg/ml. The finest minimum fungicidal concentration was showed by fruit pulp extract against *C. albicans* at a dose of 250 µg/ml. Leaves extract showed minimum fungicidal concentration at a dose of 500 µg/ml against *C. albicans*. Root bark extract also showed minimum fungicidal concentration against *C. albicans* at a dose of 1000 µg/ml. The overall results of antifungal activity indicated that the tested plant extracts are more effective against fungi *C. albicans* while against *A. niger* and *A. clavatus* they showed significant antifungal activity.

Table-3: Minimum inhibitory concentration of methanolic extracts of *A. digitata* L. and standard drugs against fungal organisms.

Antifungal activity				
Minimum inhibitory concentration (µg/ml)				
S.N.	Extract Code	*C. albicans* (MTCC 227)	*A. niger* (MTCC 282)	*A. clavatus* (MTCC 1323)
Methanolic extracts of *A. digitata* L .				
1	ASLE	500	1000	1000
2	ASBE	1000	>1000	>1000
3	FPE	250	500	500
Standard Antibiotics				
4	NYS	100	100	100
5	GRI	500	100	100

Methanolic extracts of *A. digitata* - ASLE: Leaves extract; ASBE: Root bark extract; FPE: Fruit pulp extract.
Standard antibiotics-NYS: Nystatin; GRI: Griseofulvin.

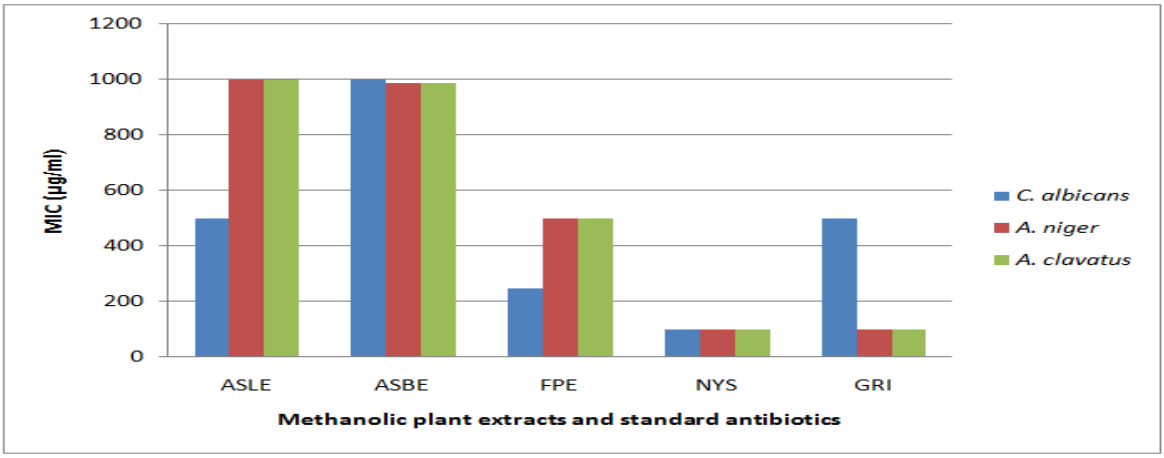

Fig-3: Minimum inhibitory concentration of *A. digitata* L. and standard drugs against fungal organisms.
Zone of inhibition against bacterial strains

Zone of inhibition studies of methanolic extracts of *A. digitata* L. against bacterial strains and standard drugs were represented in table.4-5. It reveals that the maximum zone of inhibition against bacterial strains; *E. coli, P. aeruginosa, S. aureus, S. pyogenes* and *B. subtilis* were showed by fruit pulp extract and highest with 28 mm diameter of zone of inhibition against E. coli at the concentration range of 250 µg/ml but in case of *V. cholera, S. pneumoniae* the maximum zone of inhibition with 21 mm and 20 mm diameter were showed at higher concentration 250 µg/ml by leaves extract. In case of *S. typhe* the maximum zone of inhibition with 23 mm diameter was showed by root bark extract at higher concentration 250 µg/ml. The zone of inhibition measured ranged from 10 to 28 mm for all the sensitive bacteria.

Table-4: Zone of inhibition of methanolic extracts of *A. digitata* L. and standard drugs against Gram positive bacterial strains.

Antibacterial activity table																					
S.N.	Code	Zone of inhibition (mm)																			
		S. aureus (MTCC 96)					S. pyogenes (MTCC 442)					S.pneumoniae (MTCC -1936)					B. subtilis (MTCC 441)				
		5	25	50	100	250	5	25	50	100	250	5	25	50	100	250	5	25	50	100	250
Methanolic extracts of A. digitata L.																					
1	ASLE	10	13	14	16	18	11	14	16	18	19	12	13	15	19	20	10	13	15	17	19
2	ASBE	12	14	19	20	21	10	13	19	20	20	11	13	16	17	18	10	12	14	16	17
3	FPE	17	19	21	22	22	16	19	21	21	22	10	13	15	16	18	11	13	15	18	21
Standard Antibiotics																					
4	AMP	10	13	14	16	18	11	14	16	18	19	11	12	14	17	17	10	12	13	16	16
5	CMP	12	14	19	20	21	10	13	19	20	20	13	14	18	19	21	12	14	15	17	18
6	CIP	17	19	21	22	22	16	19	21	21	22	13	14	15	19	21	12	13	15	17	19
7	NOR	19	22	25	26	28	18	19	20	21	21	17	19	21	22	26	12	16	18	19	21

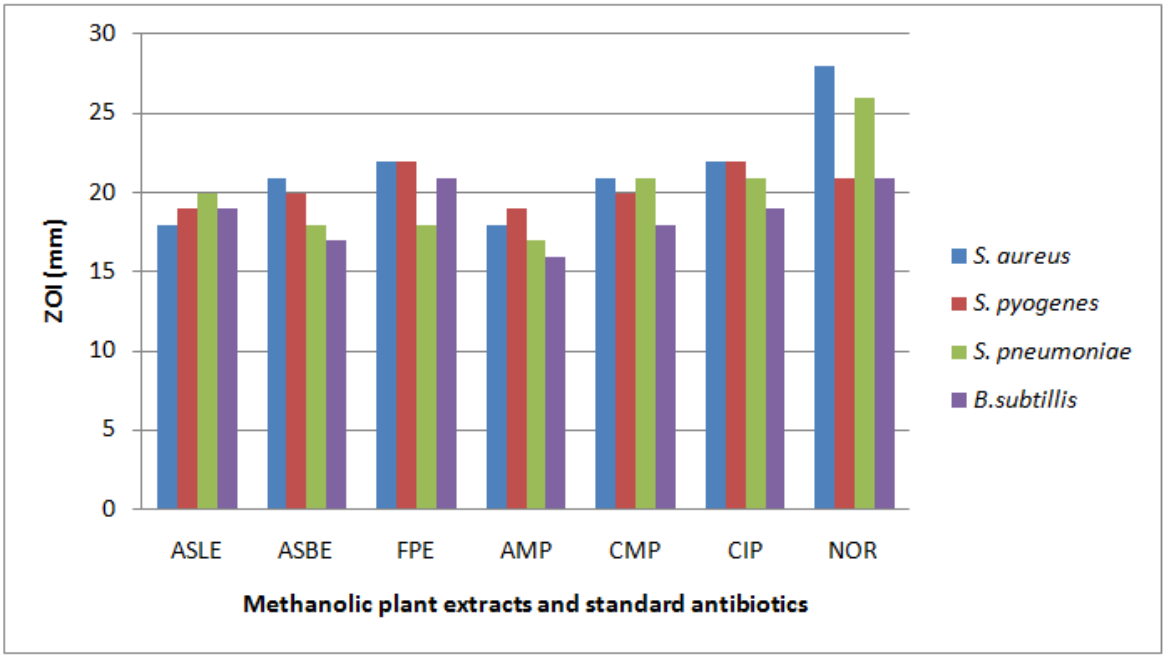

Fig-4: Zone of inhibition of *A. digitata* L. and standard drugs against Gram positive organisms.

Table-5: Zone of inhibition activities of methanolic extracts of *A. digitata* L. and standard drugs against Gram negative bacterial strains.

Code	Zone of inhibition (mm)																			
	E. coli (MTCC 443)					P. aeruginosa (MTCC 424)					S. typhi (MTCC 98)					VI. cholerae (MTCC 3906)				
	5	25	50	100	250	5	25	50	100	250	5	25	50	100	250	5	25	50	100	250
ASLE	14	15	16	19	20	14	15	15	18	20	10	13	15	19	20	10	12	14	20	21
ASBE	14	17	23	23	23	14	17	18	19	21	11	12	15	21	23	10	13	15	17	19
FPE	20	23	28	28	28	20	23	24	26	27	12	15	19	21	22	12	14	16	19	20
AMP	14	15	16	19	20	14	15	15	18	20	12	14	16	19	23	10	11	14	15	17
CMP	14	17	23	23	23	14	17	18	19	21	14	15	18	20	21	11	14	16	17	17
CIP	20	23	28	28	28	20	23	24	26	27	14	15	17	19	23	15	16	17	18	20
NOR	22	25	26	27	29	18	19	21	23	23	16	19	20	24	26	16	18	20	22	24

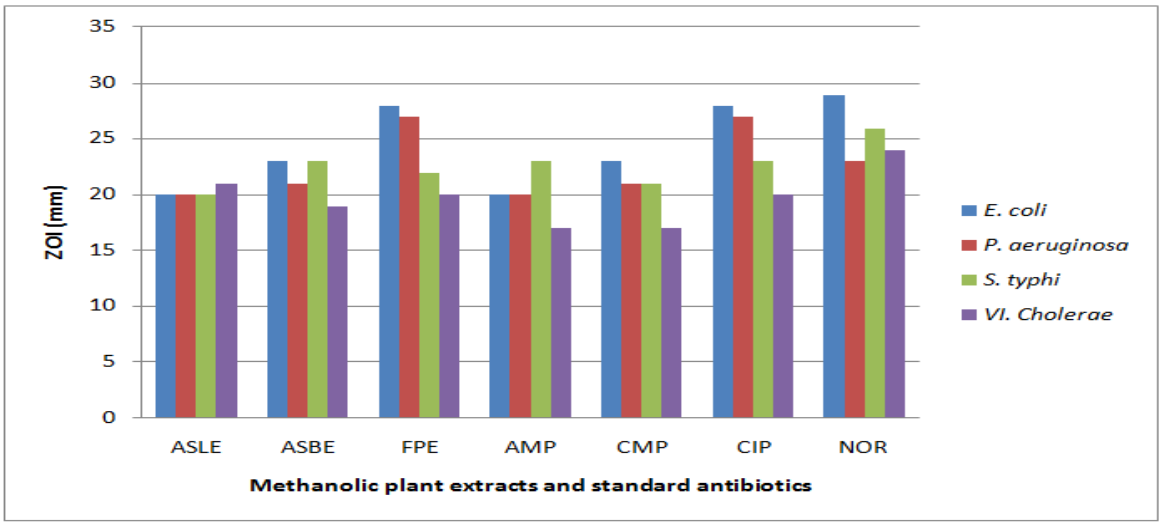

Fig-5: Zone of inhibition of *A. digitata* L. and standard drugs against Gram negative organisms.

Zone of inhibition against fungal strains

Zone of inhibition studies of methanolic extracts of *A. digitata* L. against fungal strains and standard drugs were represented in Table.6 and Fig. 6. In case of fungal strains the maximum zone of inhibition against *A. niger, A. clavatus, C. albicans* were showed by fruit pulp extract, which is highest at a concentration 250 µg/ml with 29 mm diameter of zone of inhibition against *C. albicans*. Leaves extract also showed maximum zone of inhibition with 23 mm diameter against *C. albicans* which is higher at 250 µg/ml while bark extract was very less susceptible to *C. albicans* and moderately susceptible against *A. niger* and *A. clavatus* with 21 mm of inhibition at higher concentration 250 µg/ml respectively. The zone of inhibition ranged from 12 to 29 mm for all fungal strains.

Table-6: Zone of inhibition activities of methanolic extracts of *A. digitata* L. and standard drugs against fungal strains.

Antifungal activity																
Zone of inhibition (mm)																
		C. albicans (MTCC 227)					*A. niger* (MTCC 282)					*A. clavatus* (MTCC 1323)				
S.N.	Code	5	25	50	100	250	5	25	50	100	250	5	25	50	100	250
Methanolic extracts of *A. digitata*																
1	ASLE	17	20	20	21	23	14	15	17	18	21	14	16	18	20	20
2	ASBE	12	15	17	19	20	14	17	18	20	21	14	18	20	20	21
3	FPE	21	23	28	28	29	20	21	24	26	26	19	21	24	25	26
Standard Antibiotics																
4	NYS	11	13	17	19	22	12	15	17	18	21	13	17	18	20	22
5	GRI	10	11	13	16	19	14	17	17	19	21	14	16	19	20	22

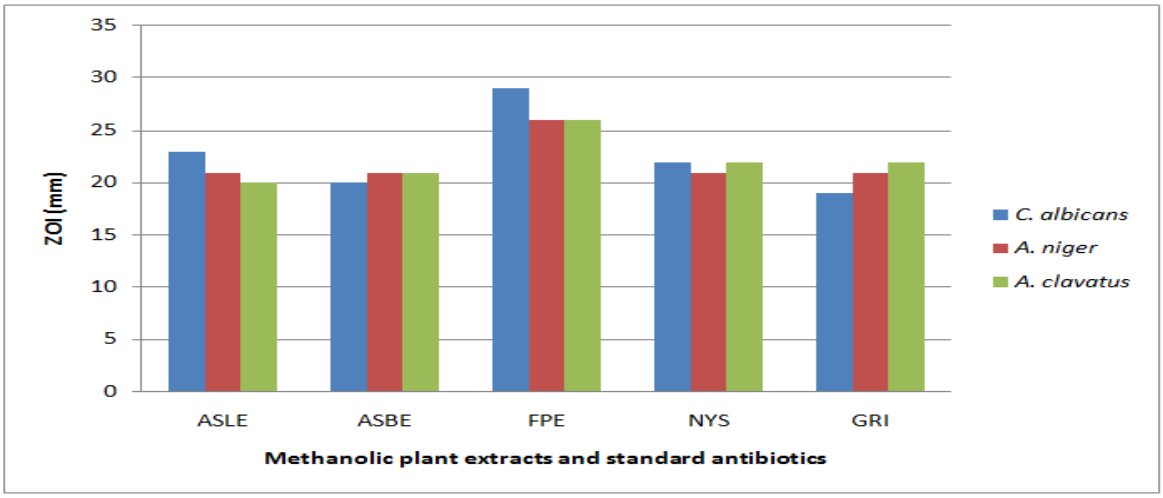

Fig-6: Zone of inhibition of *A. digitata* L. and standard drugs against fungal organisms.

Antitubercular activity

Minimum inhibitory concentration

The antitubercular activity of methanolic extracts and standard drugs tested against the microorganism *M. tuberculosis* was represented in Table 7. It was found to be more susceptible to the root bark extract with MIC value of 62.5µg/ml and moderately susceptible to fruit pulp extract with MIC value of 100 µg/ml and showed very less susceptibility to leaves extract with higher MIC value of 250 µg/ml. All three extracts were found to be less effective than the standard antibiotics used in the present study. The observation of MIC assay suggested that the extracts have bacteriostatic property.

Table-7: Antitubercular activity of methanolic extracts of *A. digitata* L. and standard drugs against the microorganism *M. tuberculosis*.

Antituberculosis activity		
Minimum inhibitory concentration (µg/ml)		
S.N.	Code	*M. tuberculosis* (H37Rv)
Methanolic extracts of *A. digitata* L.		
1	ALE	250
2	ABE	62.5
3	FPE	100
Standard Antibiotics		
4	ISZ	0.20
5	RIF	0.25

Methanolic extracts of *A. digitata* - ASLE: Leaves extract; ASBE: Root bark extract; FPE: Fruit pulp extract. Standard antibiotics-ISZ: Isoniazid; RIF: Rifampicin.

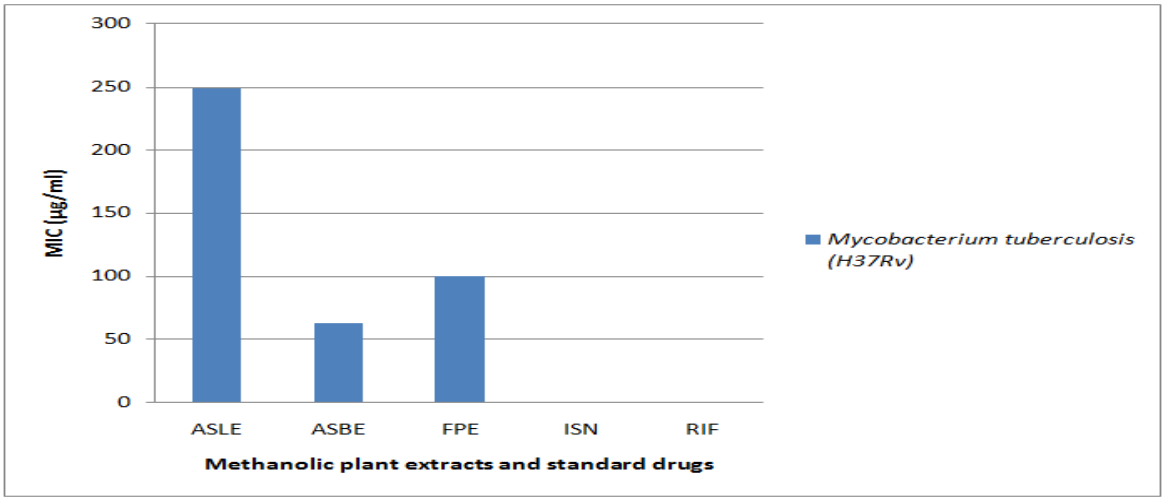

Fig-7: Minimum inhibitory concentration of *A. digitata* L. and standard drugs against microorganism *M. tuberculosis*.

In conclusion, the methanolic extracts of leaves, root bark and fruit pulp of *A. digitata* contain compounds which can be potentially used as an antimicrobial, antifungal as well as antitubercular agent. We can conclude that the traditional use of this plant extracts has also been justified in infectious diseases through this study. However to evaluate the potential effectiveness in human being further studies are needed.

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References

[1]. Alzoreky, N.S., Nakahara, K (2003): Antibacterial activity of extracts from some edible plants commonly consumed in Asia. International Journal of Food Microbiology. 80: 223-230.
[2]. Anargyros, P., Astill, D.J.S., Lim, I.S.L (1990): Comparison of improved BACTEC and Lowenstein-Jensen media for culture of mycobacteria from clinical specimens. Journal of Clinical Microbiology. 28: 1288–1291.
[3]. Anani, K., Hudson, J.B., de Souzal, C., Akpaganal, K., Tower, G.H.N., Amason, J.T., Gbeassor, M (2000): Investigation of medicinal plants of Togo for antiviral and antimicrobial activities. Pharmaceutical Biology. 38: 40-45.
[4]. Atawodi, S.E., Bulus, T., Ibrahim, S., Amed, D.A., Nok., A.J., Mamman, M., Galadima, M (2003): In vitro trypanocidal effect of methanolic extract of some Nigerian savannah plants. African Journal of Biotechnology. 2: 317-321.
[5]. Balick, M.J (1990): Ethnobotany and the identification of therapeutic agents from the rainforest. Ciba Found. Symp. 154: 22-31.
[6]. Bauer, A.W., Kirby, W.M.M., Sherris, J.C., Turck, M (1966): Antibiotic susceptibility testing by standardized single disc method. American Journal of Clinical Pathology. 45: 493–496.

[7]. Chopra, R.N., Nayar, S.L., Chopra, I.C., Asolkar, L.V., Kakkar, K.K., Chakre, O.J., Varma, B.S (1992): Glossary of Indian Medicinal Plants (with Supplement). Council of Scientific and Industrial Research, New Delhi.
[8]. Desai, N.C., Shukla, H.K., Tahker, K.A., 1984. Some new 2-aryl-3-isonicotamido-4-thiazolidinones and their 5-carboxymethyl homologues as potential antitubercular and antibacterial agents. Indian journal of Chemistry. 61, 239–240.
[9]. Elbir, H., Abdel-Muhsin, A.M., Babiker, A (2008): A one-step DNA PCR-based method for the detection of Mycobacterium tuberculosis complex grown on Lowenstein–Jensen media. American Journal of Tropical Medicine and Hygiene. 78(2): 316–367.
[10]. Esterhuyse, N., Von Breitenbach, J., Suhnge, H., 2001. Remarkable Trees of South Africa. Briza Publication, Pretoria.
[11]. Gupta, A.K., Tandon, N (2004): Reviews of Indian medicinal plants, Indian Council of Medical Research, Vol-11. New Delhi, India.
[12]. Hudson, J.B., Anani, K., Lee, M.X., De Souzâ, C., Arnason, J.T., Gbeassor, M (2000): Further investigationson the antiviral activities of medicinal plants of Togo. Pharmaceutical Biology. 38: 46-50.
[13]. Kamatou, G.P.P., Vermaak, I., Viljoen, A.M (2011): An updated review of Adansonia digitata: A commercially important African tree. South African Journal of Botany. 77: 908-919.
[14]. McFarland, J (1987): Standardization of bacterial culture for the disc diffusion assay. Journal of American Medical Association. 49: 1176-1178.
[15]. Nadkarni, K.M., Nadkarni, A.K (2000): Indian Materia Medica-2, Third edn. Popular Prakashan, Bombay, pp. 37-39.
[16]. Ramadan, A., Harraz, F.M., El-Mougy, S.A (1994) Antiinflammatory, analgesic and antipyretic effects of the fruit pulp of Adansonia digitata. Fitoterapia. 65: 418-422.
[17]. Ramesh, D., Dennis, T.J., Shingare, M.S (1992): Constituents of Adansonia digitata root bark. Fitoterapia. 63: 278–279.
[18]. Rattan, A (2000): Antimicrobials in laboratory medicine. Churchill B. I, Livingstone, New Delhi, pp 85-108.
[19]. Rios, J.L., Recio, M.C., Villar, A (1988): Screening methods for natural products with antimicrobial activity-a review. Journal of Ethnopharmacology. 23: 127-149.
[20]. Shah, R.R., Mehta, R.D., Parikh, A.R (1985): Studies on isoniazide derivatives: preparation and antimicrobial activity of 2-aryl-3-(pyridylcarbomyl)-5-carboxymethyl-4-thiazolidinones. Journal of Indian Chemical Soc. 62: 255–257.
[21]. Sharma, S.K (1998): Medicinal plants used in Ayurveda. New Delhi, India: National Academy of Ayurveda, Ministry of Health and Family Welfare, Government of India.
[22]. Shukla, Y.N., Dubey, S., Jain, S.P., Kumar, S (2001): Chemistry, biology and uses of Adansonia digitata - a review. Journal of Medicinal and Aromatic Plants Sciences. 23: 429-434.
[23]. Van wyk, B., Van Oudtshoorn, B., Gericke, N (2005): Medicinal Plants of South Africa. Briza Publication, Pretoria.
[24]. Wickens, G.E (1982): The baobab-Africa's upside-down tree. Kew Bulletin. 37: 173-209.