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ANTIMICROBIAL ACTIVITY OF IMPORTANT MEDICINAL PLANT ADANSONIA DIGITATA

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ABSTRACT:-

Seed extracts of *Adansonia digitata* (Seed) was analyzed for their antimicrobial activities against human pathogens *Salmonella typhi, Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus* as human pathogens and plant pathogens *Aspergillus* spp., *Alternaria* spp., *Penicillium* spp. and *Fusarium* spp. These pathogens were subjected to Aqueous and ethanolic extracts of seeds. The phytochemical analysis was carried out for the detection of primary and secondary metabolic like alkaloids, steroids, terpeniods, flavonoids, flavones, gallic acid, tannins, catabolic tannins, reducing sugars and while carbohydrates, glycosides, saponins, phenols, proteins and amino acids. Experimental results have been discussed here.

Keyword: - Aantimicrobial, phytochemical screening and medicinal plant *Adensonia digitata*

Introduction:-

The world plant biodiversity is the largest source of herbal medicine and it is now clear that the medicinal value of these plants depends upon medicinal plant some organic compounds present in the plant which provide definite physiological action on the human body. During the present investigation medicinal plant *Adensonia digitata* was selected from the local forest in Marathwada region of Maharashtra. Phytochemical screening for presence

of secondary metabolites and qualitative phytochemical analysis of the selected part of plant with different solvent were selected viz. methanol, ethanol, chloroform, and acetone. Various plant parts act as source of large amount of crude drugs, comparing different groups such as antispasmodics, emetics, anti-cancer, antimicrobial etc. Number of the plants is claimed to possess the antibiotic properties in the traditional system and is also used extensively by the tribal people worldwide.

Materials and Methods:-

Materials:

Plant material

Given plant specimen was collected in local forest area in Marathwada region. This plant part such as leaves, seed, endosperm, were collected and these plant parts were dried under the shade and grounded into powder using the pulverized and collected to separate bottle.

Plant material extraction:

10gm of dry powdered plant part was taken and used for preparation of extract using water and solvent such as ethanol, methanol, acetone, chloroform, in 80% for up to 100ml concentration. These extracts were filtered with Whatman filter paper and stored it the cool condition in the refrigerator at 20^0c temperature. These extracts were used for qualitative and phytochemical screening.

Collection of pathogen:-

The cultures of *Salmonella typhi*, *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*, *Aspergillus* spp., *Alternaria* spp., *Penicillium* spp. and *Fusarium* spp. was obtained from Department of Botany, Govt. Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh (PB) India And were subcultured and maintained on Lowenstein Jensen media.

Determination of Minimum Inhibitory Concentration (MIC) of plant extract.

The MIC of the plant extract was performed using the broth micro dilution assay against the three *Mycobacterium strain* and three bacterial species also tests were performed in sterile 96-well micro plates by dispensing into each well a total volume of 200µl comprising 100µl of standardized suspension of test culture (110^6 cells/ml) 100µl of different concentration of chemical compounds and incubated up to 48 h at 37ºC. MIC was determined by absorbance measurement at 595 nm using thermo make Bio-Rad iMark absorbance Reader (Made in JAPAN). The MIC was defined as the lowest concentration of the sample that inhibited the growth of test microorganism.

Evaluation of the synthesized compounds as potential growth inhibitors of Mycobacterium species as well as bacterial species.

The three *Mycobacterium* strain was obtained from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh (PB), India and also studied two bacterial species. Bacterial species was subculture and maintained into Potato Dextrose agar and *Mycobacterium* strain was maintained into Lowenstein Jensen media. As per of

experimental standardization, initially 1mg/ml; concentration of plant extract was used for antimicrobial analysis and it was further take up to only 25ul/ml add into the well, however the clear zone of inhibition was observed under the experimental condition.

The sensitivity test of *Mycobacterium* strain and various bacterial species was demonstrable by agar diffusion Method. A 25µl volume of each of (1mg/ml) the plant extract was loaded into the well using sterile pipette. The plates were kept in refrigerator for pre diffusion of the sample and incubated at 37ºc for 48 hours. Growth of three *Mycobacterium species* and plant extract was observed after the diameter of inhibition zone was measured subtracting the well size Rifampcin, Streptomycin (10µg/ml), was used as reference standard.

All these plant extract was checked for antioxidant activity by DPPH (1, 1-diphenyl-2-picryl-hydrazil) method. The activity was Measured by the following method wherein 3 mL of each different concentration of all plant extract (20–100 lg/mL) was added to 1 mL methanolic solution of DPPH (20 ppm). Similar experiment was carried out simultaneously with ascorbic acid as standard antioxidant. The blank was also prepared by adding 1 mL DPPH to 3 mL of

methanol. The tubes were kept in dark for 30 min; then absorbance was measured at 517 nm using UV–Visible spectrophotometer.

Sr. No.	**Plant extract**	**Solvent extract**	**MIC of Plant Extract**	**Anti-Mycobacterial Activity of Plant Extract Inhibition zone diameter (mm ± SD)**

			M.tuberculosis	***M.pheli***	***M.avim***	***M.tuberculosis***	***M.pheli***	***M.avim***
1.	***Adansonia digitata* (Seed)**	Aqueous	2.10±0.25	1.20±3.5	2.10±2.5	11.2±5.1	12.2±3.1	31.2±3.1
		Ethanol 80%	2.38±0.08	4.28±4.8	3.18±2.8	13.3±61	23.3±6.1	13.3±4.1
		Chloroform 80%	1.5±0.14*	2.4±2.54*	1.4±3.54*	14.4±9.1	24.4±1.1	14.2±1.1
		Methanol 80%	4.10±2.03	2.11±1.23	1.11±1.23	13.3±3.1	13.3±2.1	23.3±2.1
		Acetone 80%	3.46±0.07	2.36±2.17	1.36±1.07	14.3±0.9	12.3±1.9	22.4±0.9
2.	***Adansonia digitata (Endosperm)***	Aqueous	1.12±1.34	3.42±1.34	2.32±1.14	13.0±1.2	15.0±2.2	23.1±2.2
		Ethanol 80%	1.33±1.32	2.13±1.32	0.13±0.32	22.3±1.3	12.3±3.3	11.4±1.3
		Chloroform 80%	3.12±0.3	3.2±0.3*	1.21±0.3	12.3±0.31	11.3±0.1	23.3±3.1
		Methanol 80%	4.32±4.3	3.42±1.0	3.33±1.0	23.7±2.45	13.7±3.0	12.±2.0
		Acetone 80%	3.22±0.7*	2.12±0.3*	1.32±0.1*	13.5±1.2	14.4±2.1	15.3±2.1
3.	**Rifampicin(10µg/ml)**	DMSO	1.41±0.12*	0.21±0.10	0.11±0.10	33.1±1.72	33.1±1.72	23.1±1.72
4.	**Icosonizid(10µg/ml)**	DMSO	2.11±0.12	1.11±0.10	2.41±0.10	23.4±2.32	33.4±2.32	13.4±2.32

Table 3: Results presented here are the mean values from three independent experiments ±S.D. Activity of plant extract against three *Mycobacterium* strains. The results summarized are the mean values of the three independent experiments**.** *P ≤ 0.05 as compared to standard drugs. The samples were tested against Mycobacterium tuberculosis cultured on Accumix made Lowestein Jensen media. The MICs were calculated using micro broth dilution assay. An Anti-Mycobacterial Acativity of Plant Extract Inhibition zone diameter (mm ± SD).

Result and discussion:-

Determination of Minimum Inhibitory Concentration (MIC) of plant extract.

The results of the antimycobacterial activity the synthesized compounds are summarized in (Table 3.), which clearly shows the differential sensitivity of *Mycobacterial* strains towards the test samples. The strain *Mycobacterium tuberculosis* was observed to be resistant towards the plant extract *M. elengi* (Leaf) ethanol 80% *M.avim* (MIC, 100 µg /mL), methanol 80% *M.tuberculosis* (MIC, 100 µg /mL), acetone 80% *M.avim* (MIC, 100 µg /mL). *A. digitata* (Seed) aqueous *M.avim* (MIC, 100 µg /mL), ethanol 80% *M.pheli* (MIC, 100 µg /mL), Chloroform 80% *M.pheli* (MIC, 100 µg /mL), methanol 80% *M.avim* (MIC, 100 µg /mL), acetone 80% *M.tuberculosis* (MIC, 100 µg /mL). *A. digitata* (Endosperm) aqueous *M.avim* (MIC, 100 µg /mL), ethanol 80% *M.tuberculosis* (MIC, 100 µg /mL), chloroform 80% *M.avim* (MIC, 100 µg /mL), methanol *M.tuberculosis* (MIC, 100 µg /mL), acetone 80% *M.avim* (MIC, 100 µg /mL). were found to be effective growth inhibitors of this strains. However, the compounds *W. fruticosa* (Leaf) aqueous *M.avim* (MIC, 200 µg /mL),ethanol 80% *M.pheli* (MIC, 200 µg /mL), chloroform 80% *M.tuberculosis* (MIC, 200 µg /mL), methanol 80% *M.tuberculosis* (MIC, 200 µg /mL), acetone 80% *M.avim* (22.6±0.8), *G. glauca* (Leaf) aqueous *M.pheli* (MIC, 200 µg /mL), ethanol 80% *M.tuberculosis* (MIC, 200 µg /mL), chloroform 80% *M.pheli* (MIC, 200 µg /mL), methanol 80% *M.pheli* (MIC, 200 µg /mL), acetone 80% *M.avim* (MIC, 200 µg /mL). *C. equisetifolia* (Leaf) aqueous *M.avim* (MIC, 200 µg /mL), ethanol 80% *M.avim* (MIC, 200 µg /mL), chloroform 80% *M.pheli* (MIC, 200 µg /mL), methanol 80% *M.pheli* (MIC, 200 µg /mL), acetone 80% *M.pheli* (MIC, 200 µg /mL).The results of the above studies signifies the importance as lead molecules in the design and development of novel and effective antimycobacterial agents.

Evaluation of the synthesized compounds as potential growth inhibitors of *M. tuberculosis*

The results of the antimycobacterial activity of the synthesized compounds are summarized in (Table-3), which clearly shows the differential sensitivity of *Mycobacterial* strains towards the test samples. The strain *Mycobacterium tuberculosis* was observed to be resistant towards the plant extract see in the (Table-3), all plants extract and there different solvent better activity against *Mycobacterium strains,* some of the solvent extract like *M. elengi* (Leaf) ethanol 80% *M.avim* (33.1±1.0), methanol 80% *M.tuberculosis* (37.5±1.1mm), acetone 80% *M.avim* (33.3±1.1mm). *A. digitata* (Seed) aqueous *M.avim* (31.2±3.1), ethanol 80% *M.pheli* (23.3±6.1), Chloroform 80% *M.pheli* (24.4±1.1), methanol 80% *M.avim* (23.3±2.1), acetone 80% *M.tuberculosis* (14.3±0.9). *A. digitata* (Endosperm) aqueous *M.avim* (23.1±2.2), ethanol 80% *M.tuberculosis* (22.3±1.3), chloroform 80% *M.avim* (23.3±3.1), methanol 80% 23.7±2.45 (*M.tuberculosis*), acetone 80% *M.avim* (15.3±2.1). *W. fruticosa* (Leaf) aqueous *M.avim* (17±2.7),ethanol 80% *M.pheli* (22.6±0.9), chloroform 80% *M.tuberculosis* (15.4±.5), methanol 80% *M.tuberculosis* (24.2±1.7), acetone 80% *M.avim* (22.6±0.8), *G. glauca* (Leaf) aqueous *M.pheli* (24.1±1.5), ethanol 80% *M.tuberculosis* (31.1±0.6), chloroform 80% *M.pheli* (22.3±2.3), methanol 80% *M.pheli* (32.3±0.6), acetone 80% *M.avim* (20.2±2.5). *C. equisetifolia* (Leaf) aqueous *M.avim* (22.3±1.6), ethanol 80% *M.avim* (23.6±0.66), chloroform 80% *M.pheli* (26.4±0.4), methanol 80% *M.pheli* (24.3±0.93), acetone 80% *M.pheli* (24.3±0.76). were found to be effective growth inhibitors of this strain. In these plants solvent extract might have relevance in imparting the growth inhibition activity against *Mycobacterium species*..The results of the above studies signify the importance of plant

extract as lead molecules in the design and development of novel and effective antimycobacterial agents.

Sr.No.	Plant extract	Solvent extract	MIC of Plant Extract		Anti-fungal activity of Plant Extract Inhibition zone diameter (mm ± SD)	
			C.albicans	*A.niger*	*C. albicans*	*A.niger*
1.	***Mimusops elengi*(Leaf)**	Aqueous	1.11±0.10	2.34±0.30	15.5±2.1	12.4±1.30
		Ethanol 80%	1.17±0.08	2.37±0.09	20.5±5.1	12.3±2.09
		Chloroform 80%	2.0±0.20*	3.12±0.40	17.5±4.1	13.2±2.40
		Methanol 80%	1.53±0.11	2.13±0.13	12.8±7.1	12.3±1.13
		Acetone 80%	5.50±0.15	1.23±0.31	17.2±8.1	11.3±1.1
2.	***Adansonia digitata* (Seed) *Bombacaeae***	Aqueous	1.65±0.08	3.23±0.45	18.3±71	17.1±4.5
		Ethanol 80%	2.5±0.14*	5.25±0.82	14.4±9.1	29.9±3.2
		Chloroform 80%	6.10±0.09	2.30±0.70	27.8±3.1	13.4±1.3
		Methanol 80%	5.56±0.07	5.56±0.07	28.3±0.9	29.4±2.0
		Acetone 80%	0.22±1.34	0.41±1.57	11.1±1.2	10.4±2.8
3.	***Adansonia digitata (Endosperm)Bombacaeae***	Aqueous	2.12±1.32	4.32±0.78	12.3±1.3	24.9±5.1
		Ethanol 80%	3.12±0.23	2.46±0.66	14.3±0.31	12.4±3.6
		Chloroform 80%	4.12±1.23	3.62±1.65	13.7.45	23.2±1.7
		Methanol 80%	4.11±0.5*	3.61±0.6*	25.5±1.2	24.1±1.4
		Acetone 80%	2.11±0.47	3.33±0.63	12.0±1.7	21.4±3.4
4.	***Woodfordia fruticosa*(Leaf) *Lythraceae***	Aqueous	2.21±1.23	5.61±1.66	12.6±0.9	29.9±2.0
		Ethanol 80%	5.34±0.98	3.67±0.55	29.4±1.5	18.7±1.5
		Chloroform 80%	2.31±1.23	1.61±1.69	14.2±1.7	14.1±2.4

		Methanol 80%	2.45±0.45	3.16±0.66	12.6±0.6	23.4±1.4
		Acetone 80%	3.45±0.34	2.45±0.64	14.2±1.5	12.3±1.9
5.	***Gindia glauca* (leaf)** ***Thymeleacea***	Aqueous	2.23±0.34	3.16±0.93	3.1±0.6	24.7±0.3
		Ethanol 80%	3.23±034*	1.23±0.7*	21.3±2.3	23.3±4.0
		Chloroform 80%	2.51±1.12	1.61±1.72	15.1±1.6	13.1±1.7
		Methanol 80%	4.23±0.78	2.88±0.78	23.7±3.5	12.5±0.7
		Acetone 80%	1.22±0.89	4.63±0.77	11.1±1.6	14.2±5.8
6.	***Casuarina equisetifolia*** **(leaf) *Casurinaceae***	Aqueous	2.33±1.4*	3.44±1.1*	13.6±0.66	13.3±1.7
		Ethanol 80%	3.78±0.57	4.66±0.55	12.5±0.4	24.6±1.5
		Chloroform 80%	2.34±0.4*	1.44±0.89	13.6±0.93	25.4±2.8
		Methanol 80%	2.45±0.53	3.26±0.88	11.3±0.76	23.6±3.8
		Acetone 80%	2.71±0.12	4.71±0.4*	12.1±1.72	34.1±4.3
7.	**Streptomycin.**	DMSO.	1.21±0.11	3.1±0.9	23.4±1.23	32.1±1.2

Table 1. 2: Results presented here are the mean values from three independent experiments ±S.D. Activity of plant extract against fungal species. The results summarized are the mean values of the three independent experiments**.** *P ≤ 0.05 as compared to standard drugs. The samples were tested against fungal species cultured on Accumix made Potato Dextrose Agar. The MICs were calculated using micro broth dilution assay. Anti-fungal activity of Plant Extract Inhibition zone diameter (mm ± SD)

Antioxidant activity

` The antioxidant activity of biosynthesized Ag NPs was evaluated by DPPH assay. In the present study the synthesized Ag NPs showed better activity then the previously reported study [15, 16]. The methanolic solution of DPPH was turned from blue to yellow on the addition of Ag NPs which is due to the scavenging of DPPH by donation of hydrogen to form the stable DPPH molecule [15, 17]. As the concentration of Ag NPs increased from 20 lg/mL to 100 lg/mL the absorbance at 517 nm was decreased indicating the increase in free radical scavenging activity (Fig. 2).

Fig. 2. Antioxidant activity of all these plant extracts p as compared to ascorbic acid as standard antioxidant

CONCLUSION

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