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ANTI-MYCOBACTERIAL POTENTIALITY OF ARGEMONE MEXICANA.

LINN (LEAVES AND STEM EXTRACT)

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Received on 30-04-2016

Accepted on 28-05-2016

Abstract:

Emergence of multi-drug resistant (MDR) and extensive drug resistant strains of mycobacterium increases the complications in the treatment of TB. Due to this serious problem there is an urgent need of finding newer anti-TB agent to overcome this problem. Medicinal plants offer a hope for developing alternative medicine for the treatment of TB. The plants from ayurveda have been successfully used for the treatment of TB and having less toxicity and side effects as compared to allopathic medicines. The present investigation was carried out to determine the anti-mycobacterium activity of *Argemone Mexicana Linn*. The result suggests that the methanol extract could be a rich source of anti-tubercular agent.

Keywords: Anti-tuberculosis, Methanol extract, Medicinal plant, Natural.

Introduction:

Tuberculosis is a infectious disease and has been considered as a major health problem with 95% cases & 98% death from very ancient time. This is occurring in developing countries including India. Despite the availability of many effective chemotherapeutic agents, the emergence of multidrug resistant has increases the complication in the treatment of TB. These chemotherapeutic agents classified into main two types: first line agent & second line agent. Both classes of drug having ADR and these strains of M.tuberculosis developed resistant against these drugs. In the world plant is the largest source of herbal medicine. It is now clear that the medicinal value of this plant depend on the organic and inorganic constituent present in the plant which shows a valuable therapeutic effect on human body. In this work the selected plant *Argemone Mexicana Linn*, evaluated for qualitative and quantitative Phytochemical screening by taking

different solvent extract. The extracts were obtained by using different solvent system like n-butanol, methanol and water. Plant shows that it contains a large number of alkaloids, terpenoids, flavonoids, steroids, gallic acid, tannin, tannic acid, reducing sugar, all carbohydrates, glycosides, steroids, saponins, phenols, proteins and amino acids.(1,2,3,4,5) the argemone Mexicana linn. Plant contains a high amount of medicinal value which shows activity against M.tuberculosis. The strain of mycobacterium tuberculosis (MTCC-300), M.pheli (MTCC-1723), M.avim (MTCC-1724) was observed to be sensitive toward the plant extract.

Argemone Mexicana Linn is an exotic weed indigenous in South America but has widespread distribution in many tropical and sub-tropical countries including West Africa. (1) It is an herb with brances, yellow flowers and yellow juice and commonly known as prickly poppy or Mexican poppy and found widely throughout India. (2) It grows commonly in abandoned and cultivated fields of south-west Nigeria.(4,5) A Mexicana linn. Is known by many names in Nigeria, it is called “kaju” in Yoruba, “Ahon ekun” in Ijebu land.

A. mexicana is considered as an important medicinal plant in India; the yellow juice, which exudes when the plant is injured, has long been used in India as traditional medicine for dropsy, jaundice, ophthalmia, scabies and cutaneous affections. Different parts of this plant are used in chronic skin diseases, and also as emetic, expectorant, demulcent and diuretic; the seeds oil are employed as a remedy for dysentery, ulcers, asthma and other intestinal affections. (6,7) Leaves and seeds are also reported to find application in maintaining normal blood circulation and cholesterol level in human body; these plant parts possess anti-venom property as Flowers are found to be expectorant and have been used in the treatment of coughs. Seeds of the plant are used as purgative, laxative and digestive while its latex is used against conjunctivitis. (8) Besides, its infusion finds application against hypertension in Brazil. The fresh juice of the leaves and the latex both are reported to be used externally as a disinfectant for open wounds and cuts. In Mexico the seed is used as an antidote to snake poisoning and the fresh yellow milky seed extract contains protein-dissolving substances, effective in the treatment of warts, coldsores, cutaneous infections, skin diseases, itches and also dropsy and jaundice. The tincture of the entire plant is reported to be used orally for bronchitis and whooping cough.(13)

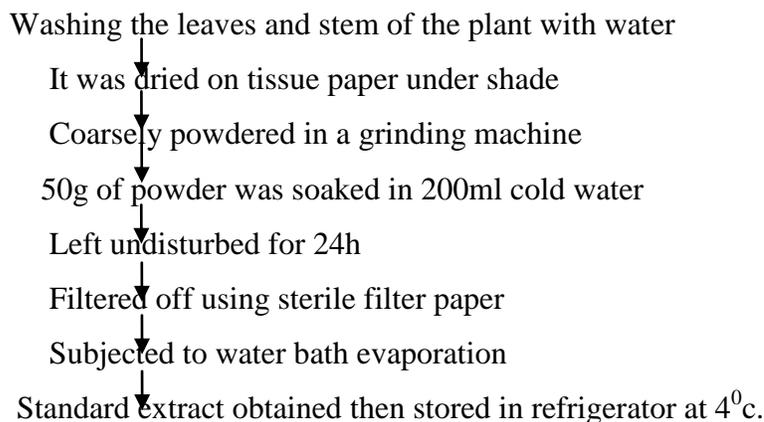
Materials and Methods

Plant material: The leaves and stem of plant Argemone Mexicana plant was collected from young matured plant from the District of Latur, during the month of Nov-Dec and identified by the botanist, School of life science,

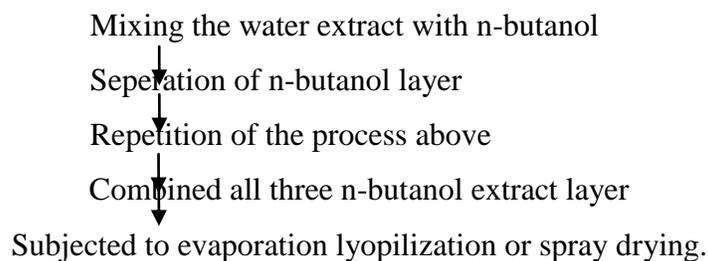
S.R.T.M.U. Nanded by comparing with the voucher specimen present in the herbarium. After authentication fresh plant materials were collected in bulk, washed under running tap water in order to remove adhering dust, dried under shade and pulverized in a mechanical grinder. The coarse powder was used for the extraction.

Extraction:

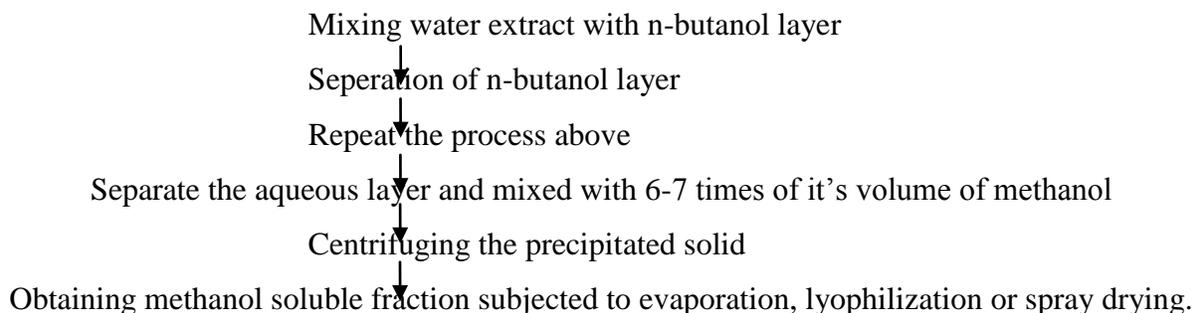
Aqueous extract:



N-butanol fraction:



Methanol soluble fraction:



The strain *Mycobacterium tuberculosis* (MTCC-300) *M.pheli* (MTCC-1723), *M.avim* (MTCC-1724) was observed to be resistant towards the plant extract.

Phytochemical screening:

Test for reducing sugars

To an aliquot of 2 mL of any extract, an aliquot of 5 mL of a mixture (1:1) of Fehling's solution I and II was added and the mixture was boiled for 5min; a brick-red precipitate indicated the presence of free reducing sugars(9).

Test for the presence of anthraquinones

An aliquot of 0.5 ML of the extract was shaken with 10 ML of benzene, filtered and an aliquot 5 ML of 10% ammonia solution was added to the filtrate and the mixture was shaken, the presence of a pink, red or violet colour in the ammoniac (lower) phase indicated on nutrient agar slants at 4°C(10).

Test for saponins

An aliquot of 0.5 ML of an extract was dissolved in an aliquot of 10 ML of distilled water in a test-tube was shaken vigorously for 30 s and then allowed to stand for 45 min. The appearance of a frothing, which persists on warming indicated the presence of saponins. (9).

Test for flavonoids

To a portion of the dissolved extract, a few drops of 10% ferric chloride solution were added. A green or blue colour indicated the presence of flavonoids. (9).

Test for steroids/terpens

A lot of 500 mg of the extract from the rotary evaporator was dissolved in an aliquot of 2 ML of acetic anhydride and cooled at 0 to 4 0⁰c to which a few drops of 12 N sulphuric acid were carefully added. A colour change from violet to blue-green indicated the presence of steroidal nucleus. (9).

Test for tannins

A fraction of 0.5 g of the extract was dissolved in 5 ML of water followed by a few drops of 10% ferric chloride. A blue black, green, or blue-green precipitate would indicate the presence of tannins. (9).

Test for alkaloids

A lot of 0.5 g of ethanol extract (from rotary evaporator) was stirred with an aliquot of 5 ML of 1% Hcl on a steam bath and filtrated; to an aliquot of 1 ML of the filtrate, a few drops of Mayer's reagent was added, and to another aliquot of 1 ML of the filtrate, a few drops of Dragendorff's reagent were added. Turbidity or precipitation in tubes due to either of these reagents indicated the presence of alkaloids in the extract. (9).

Test for resins

To an aliquot of 10 ML of the extract an aliquot of 10 ML of copper acetate solution 1% was added and shaken vigorously and, a separate green colour indicated the presence of resin. (10).

Test for glycosides

An aliquot of 5 ML of each extract was mixed with an aliquot of 2 ML of glacial acetic acid (1.048-1.049 g/ML), one drop of ferric chloride solution (1%), and mixed thoroughly. To this mixture, an aliquot of 1 ML of 12 N H₂SO₄ was added. A brown ring at the interface indicated the presence of glycosides. (9).

Evaluation of Anti-mycobacterial activity

The MIC of the plant extract was performed using the broth micro dilution assay against the three *Mycobacterium strain* and two 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 (110⁶ cells/ ml) 100µl of different concentration of chemical compounds and incubated up to 48 h at 37°C [17]. 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*.

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 [10]. 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 37°C 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 Rifampicin, Isoniazid (10µg/ml), was used as reference standard.

Result and Discussion: The above finding shows that Argemone Mexicana Linn. of different solvent extract have the ability to inhibit the growth of mycobacterium species as well. Out of different solvent plant extract, the methanolic extract of Argemone Mexicana shows better anti-tubercular activity as compared to other solvent extract. Hence the

presence anti-tubercular drug in Argemone Mexicana justifies to some extent ethnomedicinal use of this species as a

Anti-TB remedy.

Sr. no	Plant extract	Solvent extract	MIC of Plant Extract			Anti-Mycobacterial Activity of Plant Extract Inhibition zone diameter (mm ± SD)		
			M.tuber-culosis	M.pheli	M.avim	M.tuber-culosis	M.pheli	M.avim
1.	Argemone Mexicana extract.	Aqueous	1.11±0.1	2.23±0.10	1.1±0.0	25.5+1	20.2+3.0	34.3+1.0
		n-butanol	2.25±0.13	2.45±0.8	1.32±0.0	17.5+2.3	14.2+4.0	33.3+1.1
		Methanol soluble	4.4±0.22	5.0±0.12	2.2±0.12	38.5+1.2	13.5+1.2	23.4+3.1
2	Rifampicin(10µg/ml)	DMSO	1.43±0.13	0.22±0.10	0.11±0.12	33.3±1.73	33.2±1.2	23.1±1.4
3	Isonizide(10µg/ml)	DMSO	2.23±0.14	1.13±0.12	2.43±0.11	24.4+2.32	34.4+2.3	14.4+2.6

Conclusion:

The argemone Mexicana linn plant contain a valuable medicinal activity which clearly show sensitivity of M. strains towards the plant extracts. Our findings suggest that the methanol extract could be a rich source of anti-TB agent as compared to Aqueous and n-butanol extract. This investigation provides a important baseline information for further research interventions. To our knowledge it is for the first time, that it has been possible to demonstrate experimentally in the laboratory that Argemone Mexicana Linn. Plant extract possesses remarkable anti-TB activity. The result assumes significance and throws some light on the basis of ancient use of the tree in our traditional system of medicine.

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