Research Article

Evaluation of Antitubercular Activity of Methanolic Extract of *Citrus Sinensis*

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ABSTRACT

Tribal community are using their traditional knowledge system to cure various diseases. They use natural source of drugs through trial and error method and the process is experienced over hundreds of years, this says that the medicinal plant shave been in the focus as lifesaving drugs right from the beginning of the human civilization. *Citrus sinensis*is one such drug which has a strong research support of its use in biological activities such as antityphoid activity, antidiabetic activity, anticarcinogenic activity, antiproliferative activity, antioxidant activity, antibiotic activity, antifungal activity, antioxidant, anxiolytic effect, anti-lipidperoxidative effect, anti-inflammatory, lipoxygenase inhibitor, amylase inhibition, urease inhibition, Anti-hyperthyroidism, trypsin inhibition, inotropic effect, anti-arthritic, cardio-protective, anti-asthmatic, depression treatment, hepatotoxicity treatment, thrombosis treatment and rheumatic disease treatment. *Citrus sinensis*is a well-known herb which is found in the tropical and subtropical regions of India, China, Northern Australia and New Caledonia. Since *Citrus sinensis* has shown a wide variety of medicinal importance, we have here tested antitubercular activity by using Micro-plate Alamar Blue Assay (MABA) method.The dried peels of *Citrus sinensis*wereinitiallyde-fated and then were subjected to the methanolic extraction. The methanolic extract obtained was dissolved in various solvents such as water, methanol, ethanol, chloroform, diethyl ether separately and were subjected to evaluation of antitubercular activity against Mycobacterium tuberculosis by Microplate Alamar Blue Assay (MABA) method. The results concluded that the extract dissolved in water as solvent showed significant activity at 50µgm/ml.

Keywords: Antitubercular, *citrus sinensis,* MABA, methanolic extract, mycobacterium.

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INTRODUCTION

Citrus sinensis is a shrub that is subtropical rather than a tropical species. It is an important drug of Indian system of medicine and is used since time immemorial. During the last two decades, the drug has been subjected to extensive Phytochemical, pharmacological and clinical investigations and many interesting findings have been reported in various fields. It has a long history of uses by indigenous and tribal people and in Ayurveda or natural herbal medicines [1].

It is a small, shallow-rooted evergreen shrub or tree, about 6-13m height with an enclosed conical top and mostly with spiny branches. Twigs are angled with thick spines.Bark of thetrees is thin, smooth, and grey-brown to greenish in colour.The flowers are axillary, fragrant, single, few or cymose, and often perfect (having both functional stamens and pistils) or staminate. Petal colour range from whitish to pink. The fruits areglobose to ovoid, hesperidium, fleshy, indehiscent berry that ranges widely in size, colour, shape, and juice quality [2-4].

ThePeel extract of *citrus sinensis* has been reported for a wide range of biological activities such as anti-typhoid, anti-diabetic, anti-carcinogenic, sedative action, anti-hypercholestremic, antibiotic, antifungal, antioxidant, anxiolytic effect, anti-lipidperoxidative effect, anti-inflammatory, lipoxygenase inhibitor, amylase inhibition,

urease inhibition, Anti-hyperthyroidism, trypsin inhibition, inotropic effect, anti-arthritic, cardio-protective, anti-asthmatic, depression treatment, hepatotoxicity treatment, thrombosis treatment, rheumatic disease treatment [5-25].

Figure 1: *Citrus sinensis* tree

Figure 2: *Citrus sinensis* dried fruit peel

Since *Citrus sinensis* has shown a wide variety of medicinal importance, here we tested the antitubercular activity of the methanolic extract of the dried fruit peel of *Citrus sinensis* by using Micro plate Alamar Blue Assay (MABA) method.

METHODOLOGY

Collection of plant material:

The dried and authenticated peel of *Citrus sinensis* was obtained from Green Chem Pvt. Ltd, Bangalore

Extraction:

Methanolic extraction was done to the dried crude drug. The powdered peel of *Citrus sinensis* (1 kg) was extracted by refluxing with methanol (2.5 litre) in an extractor for 2 hours at 60-65˚C on water bath. After 2 hours methanolic extract was collected and the residue was again charged for the second extraction with methanol (2.5litre). Similarly third extraction was done and extract was collected. All three methanolic extract were collected and filtered using muslin cloth. The three extract were combined and subjected to vacuum distillation in Buchi Rota-vapour R-210.

Then the extract was dried in a hot air oven at a controlled temperature of 50˚C to get a course powder. The total percentage yield of the extract was found to be 10%. The mark obtained was discarded.

1g of the powdered extract was dissolved in various solvents such as water, methanol, ethanol, chloroform, and diethyl ether separately to get 100µgm/ml. These solutions were used as samples in the screening.

The Anti-mycobacterium activity of the methanolic was assessed against Mycobacterium tuberculosis using Alamar Blue Assay (MABA) method. This Methodology is non-toxic, uses a thermally stable reagent and shows correlation with proportional and BACTEC radiometric method.

Preparation of Medium:

Middle brook 7H9 Broth Base was prepared by mixing the ingredients mentioned in Table1 and the Middlebrooks OADC growth supplement was prepared by mixing the ingredients mentioned in (**Table 2**)**.**

Table 1: Middlebrook 7H9 Broth Base

Ingredients	Quantity (grams/litre)
Ammonium sulphate	0.50
Disodium phosphate	2.50
Monopotassium phosphate	1.00
Sodium citrate	0.10
Magnesium sulphate	0.05
Calcium chloride	0.0005
Zinc sulphate	0.001
Copper sulphate	0.001
Ferric ammonium citrate	0.04
L- Glutamic acid	0.50
Pyridoxine	0.001
Biotin	0.0005

Table 2: Middlebrook Oadc Growth Supplement

Ingredients	Quantity
Bovine albumin fraction V	2.50 grams
Dextrose	1.00 grams
Catalase	0.002 grams
Oleic acid	0.025 grams
Sodium chloride	0.425 grams
Distilled water	50.00ml

Prepared media was stored below 8˚C at pH 6.6 +/-0.2, protected from direct light.

2.35gm of Middle Brook 7H9TB broth base was suspended in 450ml distilled water which contains 5 ml glycerol sterilized by autoclaving at 15psi pressure at 121˚C for 15 minutes. Cooked to 400˚C and enriched with dextrose to a final concentration of 0.5% of bovine albumin fraction V.

Antitubercular screening:

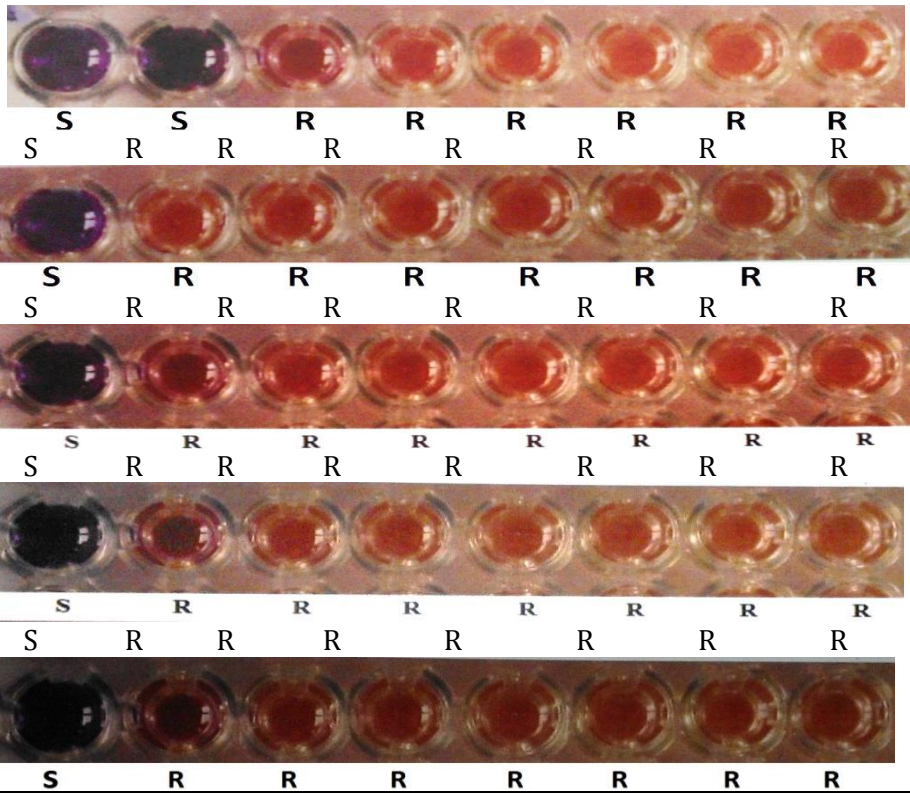
200µl of sterile deionised water was added to all outer perimeter wells of sterile 96 well plates to minimized evaporation of medium in the test wells during incubation. The 96 well plates received 100 µl of the Middlebrook 7H9 broth and serial dilution of compound were made directly on plate. The final drug concentration tested were 100µgm/ml to 0.8µgm/ml. Plates were covered and sealed with paraffin and incubated at 37˚C for five days. 25 µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hours [26].

RESULTS AND DISCUSSION

The antimycobacterium activity of the methanolic extract of *Citrus sinensis* peel was assessed against Mycobacterium tuberculosis by Alamar Blue Assay (MABA) method. The change in the colour in various concentrations 100 µgm/ml,50 µgm/ml,25 µgm/ml, 12.5 µgm/ml, 6.25 µgm/ml,3.125 µgm/ml,1.6 µgm/ml, 0.8 µgm/ml in different solvents like water, methanol, ethanol, chloroform and diethyl ether have been shown in the (**Table 3**).

Table 3: Antitubercular activity results of methanolic extract of *Citrus sinensis* peel using different solvents

Sl No.	Solvents	Final Drug Concentration (µg/ml) 100	50	25	12.5	6.25	3.125	1.6	0.8
1	Water	S	S	R	R	R	R	R	R
2	Methanol	S	R	R	R	R	R	R	R
3	Ethanol	S	R	R	R	R	R	R	R
4	Chloroform	S	R	R	R	R	R	R	R
5	Diethyl ether	S	R	R	R	R	R	R	R



Note: S-Sensitive, R-Resistant.

Here MIC is defined as the lowest drug concentration which prevents the colour change from blue to pink. Higher concentration of 100µgm/ml all the solvent showed sensitivity against Mycobacterium tuberculosis while lowering the concentration shows resistance by the bacteria. Water soluble portion showed significant activity also at 50µg/ml.

CONCLUSION

By the above experiment we conclude that the water soluble portion of the methanolic extract of the dried peels of *Citrus sinensis* shows better antitubercular activity at 50µg/ml and it may be used for further investigations.

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