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Anti-Tubercular Activity of Neem Flower Extract

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Abstract

Neem – *Azadirachta indica*, has been used in ayurvedic medicine for more than 4000 yearsdue to its medicinal properties. Neem has many biological activities like anti-inflammatory,anti-fungal, anti-bacterial, anti-oxidant and anti-viral etc. In our research, we collected neemflowers and shade dried them. The flowers were extracted using ethanol solvent. Initiallyweighed quantities of shade dried flowers (100g) were soaked in ethanol (500mL) for 48hrs.Then the soaked mixture was filtered and flowers are kept aside. The ethanol is separatedfrom the extract using rotavapour at a temperature of 120^0C. The thick extract was collected atthe end and stored in separately. Flowers were soaked again with ethanol for 24 hrs and theprocess was continued. All the collected extracts were combined and chemical analysis wasdone to find out the presence of chemical constituents like triterpenoids, phenols, flavonoidsetc. Anti-tubercular activity was performed using MABA method for the ethanolic extract ofneem flowers with a standard rifampicin. The MIC of the standard was found to be 12.5 µg/mL, whereas the MIC of neem flower ethanolic extract was found to be 25 µg/ml.

Keywords Neem flower, Ethanolic extract, Anti-tubercular activity, MABA, MIC, Rifampicin

Introduction

Neem or Margosa is a botanical cousin of mahogany. It belongs to the family Meliaceae. Thelatinized name of Neem – *Azadirachta indica* - is derived from the Persian. It has great potential in the fields of pest management, environment protection and medicine.Neem tree is about 12-18 metres in height with a circumference up to 1.8-2.4 metres(1). Neem is a flowering plant which will produce flower on 3-5 years of agein which the flowers are 4-7mm in length and 6-10mm in width.The neem tree can be found growing in countries located in the equatorial belt. Two species of *Azadirachta* have been reported, *Azadirachta indica A. Juss* – native to Indian subcontinent and *Azadirachta excels Kack*. – confined to Philippines and Indonesia(2). The most important active constituent is Azadirachtinand the others are Nimbolinin, Nimbin, Nimbidin(3), Quercetin and ß-sitosterol(4).

Materials and Methods

Neemfloweres were taken and shade dried. The solvet used was ethanol AR.

Extraction Procedure of Neem Flower

500 ml round bottomed flask was taken, to this added 50gm of shade dried neem flowers and soaked in 250ml ethanol for 48hrs.The above mixture was refluxed for 10 mins.Cooled and filtered the mixture and collected the filtrate.Poured this filtrate into pear shaped flask and fixed this to rota evaporator in Fig. 1. Rotavapour apparatus (Roteva) was set with temperature below the boiling point of ethanol (78.37°C).Set the specific rotation to 70 rpm, after stabilization

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Fig 1. Shade dried Neem flowers soaked in ethanolic extract in round bottomed flask

Fig 2. Rotavapour (Roteva)

and switch on the vacuum (Equitron vacuum pump- 40 mbar). Separated ethanol solvent was collected and poured into flowers for re-extraction in Fig. 2.

Measure the extract and ethanol volume, extract was kept aside.The obtained ethanol was poured into the pre soakedneem flowers. It was soaked again for 24 hrs, again the extract and solvent are separated. The above process was repeated with 5 batches of neem flowers with each batch weighing 50 gm. All the collected extracts were combined and kept at room temperature.

Results and Discussion

Test For Chemical Constituents

Test for Flavonoids(5)

1ml of ethanolic extract of plant material was taken in a test tube and added few drops of dilute NaOH.An intense yellow colour was observed in the test tube and it becomes colourless on addition of few drops of dilute acid.This shows the presence of Flavonoids.

Test for Triterpenoids(6)

5 ml ethanolic extract was dissolved in 2 ml chloroform and 1ml acetic anhydride was added. Concentrated sulphuric acid was added to the above solution. Formation of reddish-violet colour appeared.This shows the presence of Triterpenoids.

Test for Phenols(7,8)

Take 3 ml of ethanolic extract of neem flowers in a test tube, add freshly prepared ferric chloride solution in it drop wise, Blue colour appeared.This Indicates the presence of Phenols.

Anti Tubercular Activity

Maba Method

A blue colour in the well was interpreted as no bacterial growth and pink colour was scored as growth(9). The MIC was defined as lowest drug concentration which prevented the colour change from blue to pink(10).From the extract of neem flower we got comparable results with the standard drug rifampicin. Rifampicin has showed the anti tubercular activity i.e 25 µg/ml in Fig. 3. The anti- mycobacterial activity of the ethanolic extract of neem flowers could be due to its

Anti-Tubercular Activity

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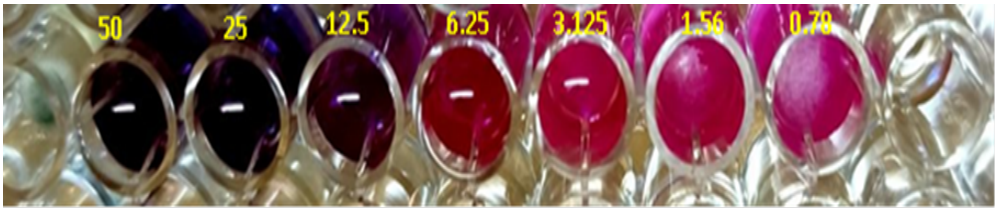

Fig 3. MABA METHOD- Standard Drug : Rifampicin 12.5 µg/ml

chemical constituents like flavonoids, triterpinoids or phenols. The preliminary evaluation gave a positive result with these chemical constituents.

Conclusion

The neem extract MIC was 25 µg/ml compared to that of standard first line drug i.e Rifampicin 12.5 µg/ml. The chemical constituents identified were triterpenoids, flavanoids, phenols.

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