Anti Microbial activity of the various leaf extracts of Vitex negundo Linn.

Abstract: The antibacterial activity of the leaves of Vitex negundo was tested against three bacteria Viz., Staphylococcus aureus Escherichia coli and Klebsiella Pneumoniae. The fresh aqueous, heated aqueous extract, chloroform and methanolic extract of leaves were used for screening their antibacterial potential. The fresh and aqueous extracts of leaves in various dilutions were found to have antibacterial activity against the three bacteria.

Key words: Vitex negundo, antibacterial, S. aureus, E. Coli, K. Pneumoniae

INTRODUCTION

The use of plants and plant preparation has been in existence since pre-historic times. There are several reports on the use of plants in traditional healing (1,2,3,4,5,6,7,8).

The available synthetic antibiotics are found to have serious side effects like bone marrow depression, anemia and damage to vital organs like liver and kidney. So it is necessary to identify newer antibiotics from herbal sources which are devoid of such serious side effects.

The rapid increase in bacterial resistance to various organisms is due to the emergence of resistant genes. This occurs because of the chemicals used as antibiotics are inadvertently used.

The World Health Organization (WHO) reported that about 80% of the world’s population depend mainly on traditional medicine and the traditional treatment involve mainly the use of the plant extract (9).

This practice is commonly found in rural areas where synthetic drugs are not available or where available, are too expensive to purchase.

Traditional medicine in developing countries uses a wide variety of natural products in the treatment of common infection (10& 11). In India, a large number of medicinal plants occur in the wild state. Herbal medicines are an important part of the culture and traditions of African people. Today, most of the population in urban south Africa, as well as smaller rural communities, are reliant on herbal medicines for their health care needs (12).

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Medicinal plants have been used as sources of medicine in virtually all cultures. During the last decade, the use of traditional medicine has expanded globally and is gaining popularity. It has continued to be used not only for primary health care of the poor in developing countries, but also in countries where conventional medicine is predominant in the national health care system (13).

Existence of human beings on earth is possible because of the vital role played by plant kingdom. Many traditional societies all over the world value a large number of plant species for a wide variety of reasons viz., food, shelter, medicine etc.

Plant materials used as folk medicine have become the object of public attention.

The plant and most of its parts like leaves, barks, roots, seeds, flowers etc are useful to mankind in several ways. The most important utility is where they can be used as medicine. Higher plants are untapped reservoirs of various chemicals awaiting intensive exploitation for their biological properties (14).

Vitex negundo Linn belongs to Verbenaceae is a woody, aromatic and medicinal shrub or a small tree grows 2-5 Meters in height. It is one of the common plants used in Indian system of medicine. It is used in ayurveda medicine as anti-inflammatory, analgesic and anti-itching agent internally and externally (15).

In the present study an attempt has been made to test the invitro antibacterial activity of Vitex negundo against three bacteriae staphylococcus aureus, E.coli, Klebsiella pneumonia. Clinical isolates of these organisms isolated during the study period from pus for staphylococcus aureus and from urine for Escherichia coli and Klebsiella pneumonia were utilized for this study.

MATERIALS AND METHODS

Selection of the herb:
Fresh leaves of Vitex negundo were collected from local garden. They were authenticated by the Department of Botany, Kongunadu Arts and science college, Coimbatore, Tamilnadu. These leaves were cleaned with sterile distilled water so as to remove the external dust particles and were shade dried. The dried plant materials were powdered. Using he powder were prepared fresh extract, aqueous extract, heated aqueous extract (80oC for 30min), chloroform extract (20 ml for 100g) and ethanolic extract of the leaves named as A1,A2,A3,A4,A5.

Fresh Extract: (A1)
100gms of fresh leaves taken, washed well in running tap water, dried in shade, wiped clean with spirit, transferred into a sterile mortar and pestle after grinding near flame and extract was collected in a sterile bottle.

Aqueous Extract:( A2)
The above steps were repeated as for fresh extract preparation. 100ml of sterile distilled water was added and the aqueous extract was obtained in a sterile container.

Heated aqueous extract: (A3)
The above steps were repeated as for fresh and heated aqueous extract preparation. The
aqueous extract was heated in a sterile conical flask in water bath at 80°C for 30 minutes and the product obtained is stored in a sterile container.

**Chloroform Extract: (A4)**
Leaves were ground in mortar and pestles. The ground pulp was then placed in a percolator. 20ml of chloroform was added gently to pulp and allowed to stand for 30 minutes. The filtrate thus obtained was the chloroform extract.

**Methanol Extract: (A5)**
Equal volume of sterile distilled water is added to 100% methanol to obtain 50% methanol, which was added to ground leaves in the percolator to obtain 50% methanol leaf extract after allowing it to stand for 30 minutes.

All these extracts were inoculated on to blood agar and MacConkey medium to observe if there were any bacterial or fungal contaminants in them by incubating them at 37°C overnight. The result showed that the leaf extracts had no growth of contaminants.

**Organism used:**
The test organisms included the six clinical isolates of staphylococcus aureus, E.coli and Klebsiella pneumonia which were used in the present study.

**Preparation of Inoculum:**
The three test organisms chosen were grown in sterile peptone water over night. Then the turbidity of staphylococcus aureus is matched to Mc Farland standard one, the turbidity of Eschericia Coli and Klebsiella pneumonia are matched to 0.5 of Mc Farland standard.

**Preparation of agar medium:**
0.5 g of Muller Hinton agar was diluted with 100 ml of distilled water and it is then allowed to melt by heating method and 19.5 ml of this molten media was taken in each tube. The tubes were then autoclaved for 15. Minutes at 121°C and 15 lbs (pounds) pressure. Then the tubes were allowed to cool to a temperature of 40 to 50°C the tubes were labeled as to which extract they contain and 0.5 ml of the appropriate extract preparation was added to the 19.5 ml of sterile media in the tubes.

All these contents were poured into clean and dry Petri Plates and then the media was allowed to settle to the plats.

The sterile Muller Hinton Agar with the extracts is spot inoculated with the prepared Inoculum of the three bacteria in the undiluted form and since there was growth of the organisms dilutions of the inocula were carried out.

Initially the organisms were diluted to 1:10, 1:100, 1:1000 from the prepared inoculums using sterile distilled water. These diluted organisms were spotted on each of the extract containing sterile Muller Hinton Agar plates. There was growth in 1:10 & 1:100 but not in 1:1000. So, further dilutions of all three organisms to 1:200, 1:400, 1:600 & 1:800 with sterile water were done.

**RESULTS AND DISCUSSIONS**
Staphylococcus aureus grew only in 1:200 but not the other dilutions in fresh and aqueous extract, but grew up to 1:800 in all the other extracts.
Escherichia coli grew only in 1:200 & 1:400 in both fresh and aqueous extract but up to 1:800 in all the other extracts.

So we infer that fresh extract to be the most suitable for antibacterial activity against the three bacteria and the aqueous extract the next with regard to antibacterial action but not the other extracts. The exact phytochemical constituents responsible for such activity can be identified by various methods like structural elucidation techniques.

The needs of the hour are novel antibacterial chemicals and if they are from a natural source they have several advantages. This includes low cost of the drug, rapid improvement with least side effects, procurement in areas where there is non availability of modern antibacterials and the most important is prevention of multi drug resistance in pathogenic bacteria.

CONCLUSION

The observed in vitro biological activity of these extracts are to be confirmed by bioassay guided isolation and identification of the active principle in the extracts.

Using ethanobotanical approach to validate the various extracts of the herb Vitex negundo by in vitro methods and scientific validation of herbal medicine by the ethno pharmacological research is a primary concern in developing countries.

Toxicity testing, selectivity and stability of the compounds are the all critical attributes of drug development which should also be done for these herb in further. The value of this research lies in the only utilizing one of the common plants of our country but also to improve the traditional health care.

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