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RESEARCH ARTICLE

ANTIBACTERIAL ACTIVITY OF FLAVONOIDS ISOLATED FROM VITEX NEGUNDO.

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Abstract

There has been an increasing incidence of multiple resistances in human pathogenic microorganism in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of various diseases. The objective of present study is to isolate flavonoids from *Vitex negundo* a plant growing in wild in the forest of Baruasagar, of Jhansi region. Flavonoids consist of large group of polyphenolic compounds having a benzo- γ -pyrone structure and are ubiquitously present in plants. They are synthesized by phenylpropanoid pathways. Present work was carried out for screening of the antimicrobial potential of flavonoids extracted from *V. negundo* against some multidrug resistant pathogenic bacteria and the emphasis is to produce herbal formulations from *V. negundo*. Different plant parts (stem, root, and leaves) of *V. negundo* were collected and air dried and then Soxhlet extraction by using standard method for flavonoids extraction. After this, extracts were tested for their antimicrobial activity using well diffusion method by observing inhibition zones. Antibacterial activity of leaves, stem and root of *Vitex negundo* was evaluated against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Results showed maximum antibacterial activity against *E. coli* followed by *S. aureus* and the least antibacterial activity was found against *P. aeruginosa*.

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Introduction:-

Nature consists of vast number of plants which are very useful for health and even used as prevention and cure for various diseases. Plants constituted the basis of traditional medicine systems that have been in existence for thousands of years. Such extensive dependence of human being on "Mother Nature" has invoked tremendous interest in the scientific world, which ultimately led to the isolation of a vast number of chemical agents with potentials for multipurpose uses (Lalitha and Jayanthi, 2012). Plant-based antimicrobials represent a vast source for medicines. Exploration of plant antimicrobials is needed because antimicrobials of plant origin have enormous therapeutic potential. They may act as lead compounds for the pharmaceutical industry or as the base for the development of new antimicrobials ([Aiyegoro *et al.*; 2012, Aiyelaagbe, 2008).

Vitex negundo is one such plant which is widely used for various treatments such as arthritis, rheumatism, malaria, toothache, body ache and menstruation problems etc. This plant is widely found in Baruasagar region of Jhansi district in Uttar Pradesh. Since the plant is very important not only for local people but also for rest of India, so the

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phytochemical study of *V. negundo* was carried out. Of all the components present in it, flavonoids were extracted and tested for their antimicrobial activity. Synthetic drugs are not only expensive and inadequate for the treatment of diseases, but are also often with adulterations and side-effects, as a result, herbal formulations are developed on large scale to overcome such problems. (Padua *et al*; 1999, Jabeen *et al*; 2009)

The world is gradually turning to herbal formulations which are known to be effective against a large repertoire of diseases and ailments. More importantly, they are not known to cause any notable derogatory effects (Kirtikar and Basu, 1984) and are readily available at affordable prices (Sharma *et al*; 2008, Prajapati *et al*.2004).

Materials and Methods:-

Vitex negundo Linn:-

Vitex negundo Linn. (Verbenaceae) is a woody, aromatic shrub with tri or penta foliate leaves on quadrangular branches, which give rise to bluish-purple coloured flowers in branched cymes. It prefers to grow in humid places or along water courses in wastelands and mixed open forests and found in Afghanistan, India, Pakistan, Sri Lanka, Thailand, and Malaysia. (Vishwanathan and Basavaraju, 2010)

Preliminary Detection of Flavonoids:-

The aqueous extracts were prepared by soaking 50 g of dried powdered sample in 100 ml of distilled water for 12 h. The extracts were filtered using what man filter paper No. 1. Dilute ammonia solution (5 ml) was added to a portion of the aqueous filtrate of each part of the plant, followed by addition of concentrated H₂SO₄. A yellow colour observed in each extract indicated the presence of flavonoids. The yellow coloration disappeared on standing. Few drops of NaOH solution was added to each test extracts. A yellow colour observed in each extract. The colour disappeared after addition of dilute acid; indicate the presence of flavonoids (Subramanian and Nagarajan, 1969; Sofowara, 1993).

Extraction of Flavonoids:-

Flavonoids have been extracted from different parts of the plants viz. Root, stem and leaf. Hundred grams of finely powdered plant part was Soxhlet extracted with hot 80% methanol (500 ml) and filtered. Filtrate was re-extracted successively with petroleum ether (fraction I), ethyl ether (fraction II) and ethyl acetate (fraction III) using separating funnel. Petroleum ether fraction was discarded due to being rich in fatty substances, whereas ethyl ether and ethyl acetate fractions were analyzed for free and bound flavonoids, respectively. Ethyl acetate fraction was hydrolyzed by refluxing with 7% H₂SO₄ for 2 hours (for removal of bound sugars from the flavonoids). Resulting mixture was filtered and filtrate was extracted with ethyl acetate in separating funnel. Ethyl acetate extract thus obtained was washed with distilled water till neutrality. Ethyl ether (free flavonoids) and ethyl acetate fraction (bound flavonoids) were dried in vacuum and weighed (Harborne, 1973).

Selected Test Microorganisms:-

Three bacterial strains have been used to test the antibacterial activity of flavonoids extracted from different parts of the plants, namely, *Pseudomonas. aeruginosa*, *Staphylococcus. aureus* and *Escherechia coli*. The selected microorganisms were procured from Department of Botany and Industrial Microbiology, Bipin Bihari College Jhansi, India. the bacterial strains were grown and maintained on 'Muller-Hinton Agar Medium' (Beef extract 2.0 g; Peptone 17.5 g; Starch 1.5 g; Agar 17.0 g; in 1000 ml of distilled water; Final pH 7.4±0.2 at 37±2°C). After isolation strains were maintained at -20°C in deep freeze.

Determination of Antibacterial Assay:-

Antibacterial activity of the crude methanol extract was studied against gram positive and negative bacterial strains by the agar well diffusion method. Mueller Hinton Agar No. 2 (Hi Media, India) was used as the bacteriological medium. The extracts were diluted in 100% dimethylsulphoxide at the concentrations of 5 mg ml. The Mueller Hinton agar was melted and cooled to 48.50 °C and a standardized inoculums (1.5×10⁸ CFU mL⁻¹) (0.5 McFarland) was then added aseptically to the molten agar and poured into sterile petridishes to give a solid plant. Wells were prepared in the seeded agar plates. The test compound (100 µl) was introduced in the well (6 mm). The test compound (100 µl) was introduced in the well (6 mm). The plates were incubated overnight at 37°C. The antimicrobial spectrum of the extract was determined for the bacterial species in terms of zone sizes around each well [Djenane *et al*; 2012, Taleb-Contini *et al*; 2003]. The diameters of zone of inhibition produced by the agent were compared with those produced by the commercial control antibiotic streptomycin and ampicillin. For each bacterial and fungal strain, controls were maintained where pure solvents were used instead of the extract. The

control zones were subtracted from the test zones and the resulting zone diameter was measured with antibiotic zone reader to nearest mm. The experiment was performed in triplicate to minimize the error and the mean values are presented (Perez *et al*; 1990).

Results and Discussion;-

Quantitative Estimation of Flavonoids:-

All the parts (Stem, roots, and leaves) of *Vitex negundo* showed positive response in the preliminary detection test of flavonoids. Flavonoid contents estimated in each gram of dried plant material was recorded (Table-1). Content of free flavonoids were obtained maximum in leaves (7.50 mg/g.d.w) and minimum in roots (4.20 mg/g.d.w) whereas bound flavonoids was maximum in stem (0.47mg/g.d.w) and minimum in leaves (0.28mg/g.d.w). Total flavonoids yield was observed maximum in leaves (7.78 mg/g.d.w) whereas minimum in roots (4.55mg/g.d.w).

Table 1: Concentration of flavonoids in *Vitex negundo* extract in per gm dry weight of its stem leaf and root.

| Quantitative Estimation of Flavonoids of <i>Vitex negundo</i> | Flavonoids free (mg/g.d.w.) | Flavonoids bound (mg/g.d.w.) | Total Flavonoids (mg/g.d.w.) |
|---|-----------------------------|------------------------------|------------------------------|
| Stem | 4.65 | 0.47 | 5.12 |
| Root | 4.20 | 0.35 | 4.55 |
| Leaf | 7.50 | 0.28 | 7.78 |

Antibacterial Assay:-

Preliminary screening of antimicrobial activity was evaluated by using agar well method against three pathogenic bacteria are given in Table-2. The zones of inhibitions were maximum for *E. coli* followed by *Staphylococcus aureus* and *Pseudomonas aeruginosa*. But in more diluted condition *Pseudomonas aeruginosa* showed no zone of inhibition for stem and root extracts. Flavonoid extracts of *V. negundo* were screened for antibacterial activity (Table -2). Results presented in table indicates that all three extracts (leaf, stem, root) showed significant antibacterial activity and found active against all taken bacteria in less diluted conditions also but as soon as extract was more diluted, it shows no activity against *Pseudomonas aeruginosa* in the stem and root extract of *V. negundo*. Maximum antibacterial activity were recorded for leaf extract against *E.coli* IZ = 12 mm and minimum against *P. aeruginosa* IZ= 8 mm in low diluted condition IZ=10 mm and IZ=6.5 mm in high diluted condition and for stem extract against *E. coli*, *S. aureus* and *P. aeruginosa* have the inhibitory zone of 9 mm, 4 mm, 2 mm, in low dilution and 7 mm, 3 mm, and no zone in high dilution respectively. Many studies pertaining to the use of the plant as therapeutic agents were being carried out, especially those thought to have an effect against antibiotic resistant bacteria. In the present study crude flavonoids of *V. negundo* revealed the medical importance of this plant through the antimicrobial activity.

Table 2: Antibacterial Activity of Flavonoids of *V. negundo* against some Pathogenic bacteria

| Plant Parts | Extract Dilution | <i>Escherichia coli</i> | <i>Staphylococcus aureus</i> | <i>Pseudomonas aeruginosa</i> |
|-------------|------------------|-------------------------|------------------------------|-------------------------------|
| | | IZ(mm) | IZ (mm) | IZ (mm) |
| Leaf | A1 | 12 | 9 | 8 |
| | A2 | 10 | 8 | 6.5 |
| Stem | A1 | 9 | 4 | 2 |
| | A2 | 7 | 3 | - |
| Root | A1 | 10 | 5 | 3 |
| | A2 | 8 | 4 | - |

A1= 10⁻¹, A2=10⁻², IZ= Inhibition Zone

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