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BLOOD ANTICOAGULANT AND ANTIBACTERIAL ACTIVITIES OF SOME PLANT EXTRACTS

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ABSTRACT

Eighteen plant extracts based on different organic solvents were prepared from four plants, viz., Eucalyptus, Parthenium, Papaya and Banana. These extracts were evaluated for antibacterial activity against some human pathogenic bacteria as well as blood anticoagulant activity. Out of total extracts tested for antibacterial and blood anticoagulant activity, the maximum antibacterial activity was exhibited by chloroform extract of Congress grass for *Shigilla dysenteriae* followed by ethanol extract of papaya for *Staptococcus epidermidis* and benzene extract of Congress grass for *Aeromonas hydrophila*. The maximum blood anticoagulant activity was shown by hexane extract of papaya followed by Congress grass, banana and water extract of eucalyptus.

Key words: Anticoagulant, Antibacterial Activity, Plant Extracts, Pathogenic Bacteria

INTRODUCTION

Ayurveda says there is no disease, which cannot be treated through the plants. In India medicinal plants are widely used by all sections of people, either directly as folk remedies or in different indigenous system of medicine or indirectly in the pharmaceuticals preparations of modern medicine **[1]**. Researchers are increasingly turning their attention to folk medicine looking for new leads to develop better medical preparations and drugs against cancer as well as viral and bacterial infections **[2-3]**. Although several plant species have been tested for anti-microbial properties **[4-6]** and few of them for

blood anticoagulant activity **[7-8]**, the vast majority has not yet been evaluated. Even the plants, familiar to us and are being used in our daily life for several other purposes such as food, timber and ornaments have not been evaluated for these activities.

The substances showing antithrombotic and thrombolytic effects were isolated from root of Paeonia suffructicosa **[9]**, Paeonia anomala **[10]** and Paeonia lutea **[11, 12]**. Blood clotting inhibitors were also isolated from medicinal plants like Porana volubilis, Listea cerbeba, Parameria laevigate and Piper betle, collected in Asian countries: Korea, China, Indonesia and Malaysia **[13]**.

Here, an attempt has been made to evaluate some plant extracts for their blood anti coagulant activity and antimicrobial activity against various human pathogenic bacterial cultures.

MATERIALS AND METHODS

Preparation of Plant Extracts

Plant leaves were collected and washed with distilled water and then were sterilized with 0.0% $HgCl_2$ and subsequently washed with distilled water. There after, 50g of leaf samples were weighed separately and crushed using mortar and pestle. The crushed leaves were kept in 500ml conical flask containing the chemical whose extract is to be obtained. The crushed leaves were fully immersed in the chemical. This was left undisturbed for 48hrs. After this, the extract obtained was filtered and left in open air for evaporation of solvents. Meanwhile, the leaves remains were transferred to another 500 ml conical flask containing the second chemical whose extract is to be obtained. Similarly, other extracts were also obtained in the same manner.

Bacterial Cultures

The test organisms used in the present study included *Escherichia coli, Vibrio cholerae, Staphylococcus epidermidis, Bacillus subtilis, Salmonella paratyphy, Shigella dysenteriae, Klebsiella pneumoniae, Streptococcus pneumoniae, Salmonella typhi, Aeromonas hydrophila* and *Proteus murabilis.*

Antibiotic Assay

For screening of antibacterial activity of different plant extract Agar well diffusion method **[14-15]** was employed. The test bacterial cultures were prepared as follows: a loop full of cells from master culture were inoculated in to 100 ml sterilized nutrient broth contained in 250 ml conical flack and inculcated on an orbital shaker at 37°C for 12hr. The cultures obtained were centrifuged in a cooling centrifuge at 10,000 rpm for 15 min. The resulting pellet was resuspended in sterilized cool distilled water for washing and repeated the centrifugation twice. After the final washing the cells were diluted with

saline solution (1:1000) to give suspensions of about $1X10^8$ microorganisms per ml **[16]**. About 0.1 ml of the suspension was then spread on sterilized nutrient agar test plates and thereafter, the wells were cut out using sterile cork borer (Perfect, India). Then, about 100ml of each extracts were poured into these wells. The plates were then incubated at 37^0C for 24 hrs. Similarly, plates were for the control using antibiotic discs. The antimicrobial activity was evaluated by measuring the diameter of inhibition zones were calculated.

Anticoagulant Assay

This was carried out according to the method of Afonne *et al.* **[17]** with a slight modification. Briefly, in the moisture chamber, which was prepared using cotton soaked in distilled water and being kept in side of petriplates. This created the moisture atmosphere inside plates. Then in each plate a glass slide was kept which was divided in to two parts. Fresh venous blood was taken out from a healthy donor and about 100µl of this blood sample was dropped down on each part of the slide. Equal amount of different plant extracts were added to each blood sample and mixed well using sterile toothpicks. The slides were then left undisturbed inside the moisture chambers for 8 hrs at room temperature. Similarly, slides were prepared for the control as well. EDTA (0.4g/10ml) standard solution was used as positive control, whereas, normal saline was used as negative control. The anticoagulant assay was carried out in duplicate for each plant extract obtained.

RESULTS AND DISCUSSION

Among hexane extracts, only parthenium showed intermediate antibacterial activity against *Escherichia coli, Bacillus subtilis* and *Shigella dysenteriae*. Among benzene extracts, parthenium showed potent antimicrobial activity against *Shigella dysntriae* and *Aeromonas hydrophilla* and intermediate activity against *Streptococus epidermis.* Benzene eucalyptus extract also showed moderate activity against *Vibrio cholerae* and *Aeromonas hydrophila*. Among chloroform extracts, parthenium showed potent antimicrobial effect against all the 11 bacterial strains tested. Chloroform eucalyptus extract showed intermediate activity against Escherichia coli, *Vibrio cholerae*, *Bacillus subtillis Aeromonas hydrophilla* and *Proteus microbillis*. Ethanol parthenium extract showed potent antimicrobial activity against *Escherchia coli, Streptococcus epidermis, Salmonenella paratyphi, Shigella dysenteriae, Salmonella typhi* and *Proteus microbilis* and intermediate effect against *Vibria cholerae* and *Streptococcus pneumoniae*. Ethanol

eucalyptus extract showed potent antimicrobial activity against all 11 bacterial strains tested **(Table-1)**. Ethanol papaya extract showed good antimicrobial effect against *Vibrio cholerae, Bacillus subtilis* and *Proteus microbillis* and intermediate activity against *Eacherichia coli, Klebsiella pneumonae* and *Streptococcus pneumoniae*. Ethanol banana extract showed intermediate activity against *Escherichia coli. Vibrio cholerae, Bacillus subtilis* and *Salmonella typhi*. The water extracts of any of the plant did not show any antimicrobial effect. There are many reports available indicating antimicrobial activity of various plant extracts **[1, 16, 18-21]**.

Only few reports are available describing blood anticoagulant property of plant extracts. Like anticoagulant activity is previously reported in *Tanacetum cilicium*, *Tanacetum macrophylum* **[22]** and *Sydisia scrabida* **[23]**. Albuquerque **[24]** reported anticoagulant activity of a sulphated polysaccharids of *Diclyota menstrualis*. In this study, good blood anticoagulant activity was exhibited by hexane extract of all four plants extract **(Table 2)**. Benzene extracts of Eucalyptus and Papaya showed good anticoagulant activity. Chloroform and ethanol extracts did not show any anticoagulant activity, which was comparable to EDTA.

Table 1: Inhibition of Plant Extracts on Bacterial Growth

SOLVENTS USED	PLANTS	BACTERIAL STRAINS										
		01	02	03	04	05	06	07	08	09	10	11
HEXANE	PARTHENIUM	±	-	-	±	-	±	-	-	-	-	-
	EUKALYPTUS	-	-	-	-	-	-	-	-	-	-	-
	PAPAYA	-	-	-	-	-	-	-	-	-	-	-
	BANANA	-	-	-	-	-	-	-	-	-	-	-
BENGENE	PARTHENIUM	-	-	±	±	-	±	±	-	-	+	±
	EUKALYPTUS	-	±	-	-	-	-	-	-	-	±	-
	PAPAYA	-	-	-	-	-	-	-	-	-	-	-
CHLOROFORM	PARTHENIUM	+	+	+	+	+	+	+	+	+	+	+
	EUKALYPTUS	±	±	-	±	±	-	-	±	±	±	±
	PAPAYA	-	±	-	-	±	-	-	-	-	-	-
ETHANOL	PARTHENIUM	+	±	+	+	+	+	+	±	+	+	+
	EUKALYPTUS	+	+	+	+	+	+	+	+	+	+	+
	PAPAYA	±	+	+	+	+	±	±	±	±	±	+
	BANANA	±	±	±	±	±	±	-	-	±	-	-
WATER	PARTHENIUM	-	-	-	-	-	-	-	-	-	-	-
	EUKALYPTUS	-	-	-	-	-	-	-	-	-	-	-
	PAPAYA	-	-	-	-	-	-	-	-	-	-	-
	BANANA	-	-	-	-	-	-	-	-	-	-	-

Note: Bacterial Strains: 01=*Escherichia coli*, 02=*Vibrio cholerae*, 03=*Streptococcus epidermis*, 04=*Bacillus subtilis*, 05=*Salmonella paratyphy*, 06=*Shigella dysenteriae*, 07=*Klebsiella pneumoniae*, 08=*Streptococcus pneumonae*, 09=*Salmonella typhi*, 10=*Aeromonas hydrophila*, 11=*Proteus mirabilis*; Inhibition Zones: (-) = <12mm. (±) =12-15mm. (+) =>15mm

Table 2: Anticoagulant Activity of Plant Extracts

SOLVENTS	PLANTS	ANTICOAGULANT ACTIVITY
HEXANE	PARTHENIUM	+
	EUKALYPTUS	+
	PAPAYA	+
	BANANA	+
BENGENE	PARTHENIUM	-
	EUKALYPTUS	+
	PAPAYA	+
CHLOROFORM	PARTHENIUM	-
	EUKALYPTUS	-
	PAPAYA	-
ETHANOL	PARTHENIUM	-
	EUKALYPTUS	-
	PAPAYA	-
	BANANA	-
WATER	PARTHENIUM	-
	EUKALYPTUS	+
	PAPAYA	-
	BANANA	+

Note: (+) = Anticoagulant Activity; (-) = Negative Anticoagulant Activity

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