

Short Communication

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Partial Purification and Antibacterial Studies of Extracts from *Eugenia jambolana* Linn and *Vinca rosea* Linn

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During the last few years it has repeatedly been reported by many investigators that some plant juices and extracts show antibacterial activity. [1-23].

Present communication deals with the antibacterial activity of ethanolic extracts and partially purified alkaloidal and non-alkaloidal fractions of *Eugenia jambolana* and *Vinca rosea* which are commonly used in medicines.

The extraction of *E. jambolana* and *V. rosea*, air dried flowers, leaves, fruits, stem, roots and seeds were performed by maceration in EtOH for two weeks, after crushing by an Ultraturax apparatus. The crushed material was extracted with

EtOH five times, such that the last extract obtained, was almost colourless. All EtOH extracts were concentrated under reduced pressure to a brownish gummy mass. This gummy extract was acidified with 10% HCl (Merck) and extracted with CHCl_3 (Merck) to remove non-alkaloidal compounds. The acidic aqueous fraction was then basified with NH_3 and extracted thoroughly with CHCl_3 .

Antibacterial assay. The crude extract as well as the partially purified fractions were used to determine the antibacterial activity. 5 mg/ml aliquots, dissolved in distilled water, were used in the test. Antibacterial activity was tested against ten different Gram positive and Gram negative bacteria. Freeze dried cultures were procured from ATCC and were maintained on Difco Nutrient agar, stored at 4° and subcultures were made after 4-week intervals.

The tests were run in triplicate. Petri plates (10 cm diam.) were prepared with trypticase soya agar Bhakuni [4], 0.1 ml of the diluted overnight culture was poured and spread on each plate and the plates were stored for 30 min. at 37°. Wells of 6mm diam, were cut with a sterile cork borer in the inoculated agar. The wells were filled with the plant extract. 50% Ethanol

TABLE 1. ANTIBACTERIAL ACTIVITY OF CRUDE ETHANOLIC EXTRACTS.

S. No.	Name of plant		Part of the plant used	Name of microorganism					
	Botanical name	Vernacular name		<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus pyogenes</i>	<i>Streptococcus agalactiae</i>	<i>Corynebacterium diphtheriae</i>	Control
GRAM POSITIVE MICROORGANISMS									
1.	<i>Eugenia jambolana</i> L.	Jaman	Leaf	12mm	—	8mm	2mm	8mm	—
			Stem	8mm	—	8mm	2mm	4mm	—
			Root	8mm	6mm	8mm	4mm	6mm	—
			Seed	12mm	10mm	10mm	10mm	8mm	—
2.	<i>Vinca rosea</i> L.	Sadabhar	Leaf	8mm	—	10mm	10mm	—	—
			Stem	—	—	8mm	8mm	8mm	—
			Flower	8mm	—	12mm	14mm	16mm	—
			Root	8mm	—	10mm	14mm	8mm	—
GRAM NEGATIVE MICROORGANISMS									
1.	<i>Eugenia jambolana</i> L.	Jaman	Leaf	10mm	4mm	—	—	4mm	—
			Stem	8mm	2mm	—	4mm	—	—
			Root	10mm	8mm	—	6mm	10mm	—
			Seed	4mm	12mm	8mm	8mm	12mm	—
2.	<i>Vinca rosea</i> L.	Sadabhar	Leaf	10mm	—	8mm	16mm	10mm	—
			Stem	6mm	—	10mm	—	10mm	—
			Flower	8mm	—	8mm	12mm	10mm	—
			Root	10mm	6mm	24mm	22mm	8mm	—

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TABLE 2. ANTIBACTERIAL ACTIVITY OF THE ALKALOIDAL AND NON-ALKALOIDAL FRACTIONS OF LEAF, STEM, ROOT, FRUIT AND FLOWER OF *VINCA ROSEA* AND *EUGENIA JAMBOLANA*

S. No.	Name of microorganism	Alkaloidal								Non-alkaloidal							
		<i>Vinca rosea</i>				<i>Eugenia jambolana</i>				<i>Vinca rosea</i>				<i>Eugenia jambolana</i>			
		Leaf	Stem	Flower	Root	Leaf	Stem	Fruit	Seed	Leaf	Stem	Flower	Root	Leaf	Stem	Fruit	Root
GRAM POSITIVE																	
1.	<i>Bacillus subtilis</i>	-	2mm	-	6mm	15mm	10mm	15mm	15mm	4mm	-	-	-	-	-	-	-
2.	<i>Staphylococcus aureus</i>	-	-	10mm	14mm	10mm	8mm	15mm	10mm	-	-	-	-	-	-	-	-
3.	<i>Streptococcus pyogenes</i>	-	-	-	10mm	8mm	8mm	10mm	8mm	2mm	-	-	-	-	-	-	-
4.	<i>Streptococcus agalactiae</i>	-	10mm	12mm	6mm	10mm	8mm	12mm	8mm	8mm	-	-	-	-	-	-	-
5.	<i>Corynebacterium diphtheriae</i>	-	-	8mm	18mm	10mm	10mm	12mm	8mm	2mm	-	-	-	-	-	-	-
GRAM NEGATIVE																	
1.	<i>Escherichia coli 97</i>	-	6mm	-	8mm	10mm	10mm	15mm	10mm	6mm	-	-	-	-	-	-	-
2.	<i>Pseudomonas aerogenosa</i>	4mm	8mm	6mm	16mm	8mm	8mm	8mm	10mm	-	-	-	-	-	-	-	-
3.	<i>Salmonella paratyphi</i>	-	8mm	6mm	12mm	15mm	12mm	15mm	15mm	4mm	-	-	-	-	-	-	-
4.	<i>Shigella boydi</i>	-	6mm	18mm	10mm	10mm	10mm	14mm	12mm	2mm	-	-	-	-	-	-	-
5.	<i>Aerobacter hydrophilia</i>	-	12mm	-	10mm	10mm	10mm	12mm	10mm	6mm	-	-	-	-	-	-	-

-ve = No zone of inhibition; Control = 50% Ethanol water.

in water v/v was used as control.

The plates were incubated for 24 hrs. at 37°. At the end of the incubation period, the inhibition zones were measured to the nearest mm. ((Tables 1 - 2).

The data obtained for the antibacterial activity of the crude ethanolic extracts of different parts of *E. jambolana* and *V. rosea* are summarized in Table 1. The results show that all eight plant materials tested exhibited antibacterial activity against most of the Gram Positive and Gram Negative bacteria used in the study. The results are in conformity with the results obtained by Dhar *et al.* [8] and Baqir *et al.* [13]. The most significant activity, with a maximum zone of inhibition of about 24 mm, was observed for the root of *V. rosea* against *Salmonella typhi*.

The crude extract of both plants were fractionated into partially purified alkaloidal and non-alkaloidal fractions by chemical methods. The results are summarized in Table 2.

The different parts of *V. rosea* also showed remarkable antibacterial activity. The present study confirms the results of Farnsworth [3].

The present preliminary study in *E. jambolana* and *V. rosea* establishes the occurrence of antibacterial components. Structure elucidation of the active compounds and further work will be reported in due course.

Key words: *Eugenia jambolana*, *Vinca rosea*, Antibacterial activity.

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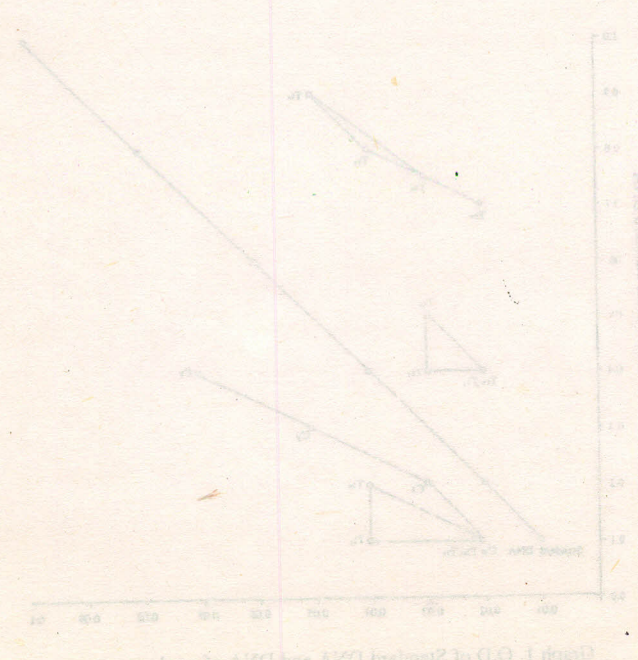
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The dishes were kept covered in dark place for 4 days at room temperature after interval of 24 hrs, one seed was taken from each petri dish and crushed in mortar with pestle in cold methanol. This process was carried out for all seeds individually and the materials were filtered separately.

Insoluble pellets were washed with cold methanol, cold 0.2M perchloric acid and cold ethanol separately. The insoluble pellets were detached with ethanol. Ethanol (1:1) at 20 for 30 min. After that the nucleic acid was extracted with 15 ml of 3% perchloric acid in 70 for 40 min. Supernatant was taken and dried at 300 mm and 200 mm.

A series of standards was prepared by taking 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 ml of DNA standard solution and the volume made upto 1 ml with 0.5% perchloric acid. Added 2 ml of freshly prepared diphenylamine reagent (13), heated in boiling water bath for 20 min. Kept at room temperature, readings were taken in 600 nm against blank (1 ml of 0.5M perchloric acid and 3 ml diphenylamine reagent). Whereas in sample, 1 ml of seed extract + 3 ml of diphenylamine was taken.

The experiment was repeated three times and the results have been calculated (Tables I-3 and Graph I). Table I shows the total nucleic acid present in seeds. The results indicate that control seeds grow normally whereas T₁, T₂ exhibit a growth decline after 72 hrs. Table 2 shows the DNA in



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During the germination of *Triticum aestivum* (wheat) seeds, the effect of epichlorohydrin in the growing wheat shoot at different intervals was found. The study has been carried out on the change of DNA and RNA replicating. The work is being studied spectrophotometrically in PCSR Laboratories Complex, Karachi.

Living organisms are exposed to various mutagens and carcinogens which are present in the surrounding. The DNA and RNA components of the nucleic acid are made of nucleotides [1-2]. Chagaff et al. [3] developed the method of synthesis of nucleosides and nucleotides which was first developed by Todd [4]. The study of functions of nucleic acid which has been continued [5-6] and has direct correlation to present experiment. Ojawa [7] suggested that the RNA of corydons of bean is used during growth of seedling and their results indicate that degradation of the storage depends on the pace of the embryo, the nucleic acid changes in the wheat were investigated. Epichlorohydrin, a well known mutagen and carcinogen is widely used as a raw material for production of important industrial chemicals [8]. It is reported to be responsible for production of mutations [9] and their reactions with nucleic acid as bifunctional alkylating agents [10-11]. Some work using epichlorohydrin has been done on nucleotide recently [12].

To carry out the determination of effect of epichlorohydrin in growing seeds, the spectrophotometer Photo-100 (Ema Co., Tokyo) photometer, cotton wheat seeds (Indo-Japan Agriculture University of Pakistan) were taken epichlorohydrin (HCl, diphenylamine, perchloric acid, 10% (GOD) and call thymine DNA (ATCC) have been used.

When seeds (*T. aestivum*) family *Fabaceae* (Gramineae) variety Blue Silver, were surface sterilized with 0.1% mercuric chloride solution and washed thoroughly with distilled water to remove mercuric chloride completely.

Small quantity of cotton was spread in four sets of petri dishes and dishes were labelled as T₁, T₂ and C (control). In T₁ = 0.1%, T₂ = 0.01%, T₃ = 0.001% solution of epichlorohydrin (aqueous) and in C small quantity of distilled water