

An Investigation on the Antibacterial Activity of *Rauvolfia tetraphylla* Dry Fruit Extracts

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Issued 01 May 2009

Abstract

The antibacterial activity of the dry fruit extracts was assayed against eight human bacterial species including *Salmonella typhi*, *Salmonella paratyphi* A, *Salmonella paratyphi* B, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Klebsiella mobilis*, *Escherichia coli* and *Staphylococcus aureus* using the agar diffusion method. Dry fruits were extracted using petroleum ether, chloroform and ethyl acetate. Ethyl acetate extract showed maximum inhibition 26mm against showed *Salmonella paratyphi* A. It also showed inhibitory action against eight pathogen tested. *R. tetraphylla* dry fruit extracts is found to be more potent and out of the three solvents used. Ethyl acetate is found to be most effective. The study suggests that the dry fruit extracts is possesses potential broad spectrum antimicrobial activity.

Keywords: Antibacterial activity, *Rauvolfia tetraphylla*, Human pathogens, Disc diffusion, dry fruit extracts, growth inhibition.

Introduction

India is endowed with a rich wealth of medicinal plants. microbes are closely associated with the health and welfare of human beings some are beneficial and some are detrimental. The increasing failure chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infections agents have lead to the screening of several medicinal plants for their potential antimicrobial activity (Ritch – Kro *et al.*, 1996; Martins *et al.*, 2001). Antibacterial properties of various plants parts like leaves, seeds and fruits have been well documented for some of the medicinal plants for the past two decades (Leven *et al.*, 1979). Antibiotic principles are distributed widely among angiospermic plants. A variety of compounds are accumulated in plant parts accounting for their constitutive antimicrobial activities (Callow, 1983). *Rauvolfia tetraphylla* L. belongs to the family Apocyanaceae, herbaceous plant. It is used as cholera, fever, eye disease and diarrhoea. It is also used for remedy of antihypertensive, as well as in dysentery and intestinal disorders (Anonymous, 1969). *R. tetraphylla* – an introduced and naturalized species are economically important for their alkaloids. *Rauvolfia tetraphylla* native of West Indies (Anonymous, 1969). Five species are recorded in India including one, which is introduced and naturalized.

The Government of India restricted the export of crude drug to conserve the natural growth and thus reduce, its exploitations, resulting in the shortage of these alkaloids in the world markets (Anonymous, 1969). This led of an active search for these alkaloids in other related species like *Rauvolfia micrantha*, a rare species endemic to the Western Ghats (Gamble, 1921; Henry *et al.*, 1987) and *R. teraphylla* an introduced and naturalized species, which is very common. However, both are economically important (Roja *et al.*, 1987) as they contain alkaloids such as reserpine, serpentine, reserpiline, ajamalicine and sarpagine (Anonymous, 1969). Therefore an attempt has been made to study the antibacterial activity of the petroleum ether, chloroform and ethyl acetate extracts of *R. tetraphylla* dry fruit were investigated.

Materials and Methods

Collection of Plant Material

The plant material was collected from wild population of the Shevaroy Hills of Salem distinct, Tamilnadu. Dry fruit samples were thoroughly washed and then dried in shade at 30°C for about 10 days. The dried dry fruit samples were ground well into a fine powder in a mixer grinder and sieved to give particle size of 50 – 150mm. The plant powder was stored in air sealed polythen bags at room temperature before extraction.

Preparation of Plant extracts

The method of Alade and Irobi (1993) with little modification was adopted for preparation of plant extracts. A fixed weight (25gm) of powdered plant material was soaked separately in 50ml of distilled water, petroleum ether, chloroform and ethyl acetate for 72hours. Each mixture was stirred at 24 hours interval using a sterile glass rod. At the end of extraction each extract was passed through Whatman No.1 filter paper (Whatman, England). the filtrate obtained was concentrated in vacuum using rotator evaporator. Eight different Human pathogenic bacteria were obtained from Stanely Medical College Chennai, Tamilnadu, and they were identified by various biochemical tests such as staining, Indole methyl red, citrate, Triple sugar iron agar, H₂S production and catalase namely. *Salmonella typhi*, *Salmonella paratyphi A*, *Salmonella paratyphi B*, *Proteus vulgaris*, *Klebsilla mobilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*.

The media used for antimicrobial test was nutrient Agar / Broth of Hi media Pvt. Ltd, Mumbai, India. Muller-Hinton agar was prepared and sterilized. The medium was pored three times on to the plates after intermittent solidification thereby a 4mm thickness agar was prepared. Using well cutter, wells (6mm diameter) were prepared. In each step of well cutting the well cutter was thoroughly wiped with alcohol and sterilized and sterilized on a different flame.

Lawns of *Salmonella typhi*, *Salmonella paratyphia A*, *Salmonella paratyphi B*, *Proteus vulgaris*, *Klebsilla mobilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* were prepared using sterile swabs and labelled accordingly and kept for few minutes 100µl of the plant extracts and the corresponding antibiotics were loaded using a sterile micropipette in the respective well and the plates were labelled at the bottom. The plates were incubated at 37°C for 24 hours. The inhibition zone diameters was calculated by measuring to minimum dimension of the zone of no bacterial growth around the well.

Results and Discussion

<i>Salmonella typhi</i>	-	10	11	-	-	-	15	20	23
<i>Salmonella paratyphi A</i>	-	10	11	-	-	-	20	24	26
<i>Salmonella paratyphi B</i>	7	-	11	-	-	-	10	13	16
<i>Proteus vulgaris</i>	-	6	9	-	8	10	10	14	16
<i>Klebsiella mobilis</i>	1	11	12	-	-	-	7	10	13
<i>Pseudomonas aeruginosa</i>	-	-	-	-	5	8	13	18	20
<i>Escherichia coli</i>	-	-	6	-	-	-	6	11	15
<i>Staphylococcus aureus</i>	-	-	-	-	-	-	6	11	14

- Indicates no activity

Root bark of *R. obscura* acts as antidiarrhoeic agent by triple pronounced antibacterial, antiamoebic and antispasmodic action (Tona *et al.*, 1999). From the present study it is evident that *R. tetraphylla* have potential antimicrobial activity. The antibacterial activity of plant extracts was not likely to be due to any one main active constituent but to the combined action of additional other compounds (Essawi and Srour, 2000). Similar result was obtained from the antimicrobial activities of *Rauwolfia tetraphylla* and *Physalis minima* leaf and callus extracts (Shafriff *et al.*, 2006).

Conclusion

The results of antibacterial activity of three solvents (chloroform, petroleum ether, ethyl acetate) used in the present study. Ethyl acetate was exhibited more antibacterial agents. The dry fruit extracts used in the study *Rauwolfia tetraphylla*, was considered as the most antibacterial activity which is most significant against *Salmonella paratyphi A*, *Salmonella typhi*, *Salmonella paratyphi B*, and *Pseudomonas aeruginosa*. The drugs derived from plant may have the possibility of using in medicine because of its positive antimicrobial activity. The results confirm the use of these plants in traditional medicine for the treatment of infections. It can be concluded from these studies that dry fruit extracts should be evaluated further for their potential use as an effective antimicrobial agents. Because of their activity against bacteria, these extracts may be an economic and potential broad spectrum antimicrobial properties.

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