# Toxic effect of various plant part extracts on the causal organism of typhoid fever

Several microorganisms-derived antibiotics are currently in use to treat a variety of infectious human diseases. Many of them have, however, a limited antimicrobial spectrum due mainly to frequent evolution of drug-resistant mutant strains of the pathogen; some even lead to serious side effects. Efforts are thus directed to identify antibiotic sources other than the traditional microorganisms. Literature studies reveal that more than a hundred species of therapeutically important higher plants are listed and described in the ancient Indian treatise. The *Atharva veda* is still relevant in the Ayurvedic system of medicines, the application of several others in floklore is being increasingly reported. More recently, the antimicrobial activity has been tested in many of plant species[1–4].

The arid zone plants are rich in alkaloid and other phytochemical contents and many of them are effectively used to cure a wide range of ailments[5]. This study reports the antibacterial potential of different arid zone plants against *Salmonella typhi*, a causal organism of typhoid fever. In this study twenty five arid zone plants were studied for their antimicrobial efficacy but we report only the effective ones.

For the evaluation of the antibacterial effect of plants against *S. typhi*, aqueous and methanol extracts of root, stem, leaf and flowers of each plant were prepared. For extraction, fresh leaves were collected, washed 2–3 times with tap water and then rinsed with distilled water. Tissue sterilization was done by grinding 1.0 g of tissues either with 10 ml of distilled water or 90% methanol, for preparing aqueous and alcoholic extracts, respectively and left for 24 h at room temperature. After evaporating the methanol, 10 ml of distilled water was added and the macerates were squeezed through double-layered muslin cloth and filtered through filter paper. Filtrates were centrifuged at 10,000 rpm for 20 min. The supernatants were filtered through Whatman's no. 1 filter paper and sterilized by filtering through 0.2 µm filters. The filter-sterilized extracts were used for bioassays. *S. typhi* was obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India, and maintained on a nutrient agar. The disc diffusion method was used for testing antibacterial activity. The media (25 ml), inoculated with 'suspension of experimental organism, was poured into sterilized petri dishes and left to gel at room temperature. Whatman's no. 1 filter paper discs (5 mm diameter) were soaked in 0.2 ml aqueous and alcoholic extracts, as well as a 10 ppm solution of tetracyclin. Sterilized distilled water and methanol were used as controls. The filter paper discs were placed equidistantly on inoculated media and diffusion of solution was allowed to occur for 30 min at room temperature. Plates were incubated at 37°C for 24 h. Three plates were employed per treatment and the average zones of inhibition were recorded.

Table 1 shows the effect of different plant part extracts. It reveals that both the aqueous and alcoholic root extracts of *Fagonia cretica* and *Barleria acanthoids*, stem extracts of *Ocimum americanum*, *Tecomella undulata*, *Balanites aegyptiaca* and *Argemone mexicana*, leaf extracts of *Fagonia cretica* and *Tecomella undulata*, flower extracts of *Sarcostema acidum* showed the inhibition of the test organism. Only alcoholic stem extracts of *Tribulus terrestris*, *Cadaba fruticosa*, *Salvadora persica*, *Leptadaenia pyrotechnica* and *Tephrosia purpurea*, leaf extract of *S. persica*, leaf and flower extracts of *L. pyrotechnica* were also effective against the organism. It was observed that stem and leaf extracts were

**Table 1.** Effect of plant part extracts on *S. typhi*

| | Effective diameter of zone of inhibition after 24 h (mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Root extract | | Stem extract | | Leaf extract | | Flower extract | |
| Plant species | Aq. | Alc. | Aq. | Alc. | Aq. | Alc. | Aq. | Alc. |
| *Tribulus terrestris* | – | – | – | 1.6 | – | – | – | – |
| *Ocimum americanum* | – | – | 5.3 | 5.6 | – | – | – | – |
| *Cadaba fruticosa* | – | – | – | 2.06 | – | – | – | – |
| *Fagonia cretica* | 6.3 | 7.3 | – | – | 14.6 | 15.3 | – | – |
| *Salvadora persica* | – | – | – | 5.5 | – | 7.6 | – | – |
| *Tecomella undulata* | – | – | 5.3 | 6.0 | 10.0 | 5.6 | – | – |
| *Leptadaenia pyrotechnica* | – | – | – | 5.0 | – | 5.0 | – | 5.5 |
| *Balanites aegyptiaca* | – | – | 4.06 | 5.0 | – | – | – | – |
| *Argemone mexicana* | – | – | 5.6 | 5.3 | – | – | – | – |
| *Tephrosia purpurea* | – | – | – | 9.0 | – | – | – | – |
| *Sarcostema acidum* | – | – | – | – | – | – | 5.6 | 5.6 |
| *Barleria acanthoides* | 4.3 | 4.6 | – | – | – | – | – | – |
| SD ± | 1.41 | 1.90 | 0.68 | 2.17 | 3.25 | 4.74 | 0.00 | 0.05 |
| Methanol | 5.0 mm | | | | | | | |
| Sterilized DW | 0.00 mm | | | | | | | |
| Tetracyclin (antibiotic) | 13.06 mm | | | | | | | |

– = No inhibition.

**Table 2.** Flavonoidal content in the leaf extract of *Fagonia cretica*

| | Free quercetin | Bound kaempferol | Total content |
|---|---|---|---|
| | 1.89 | 0.88 | 2.77 |
| | 1.92 | 0.91 | 2.83 |
| | 1.88 | 0.94 | 2.84 |
| Average | 1.89 | 0.91 | 2.81 |
| S.D.± | 0.02 | 0.03 | 0.03 |

more effective against the organism. The highest inhibition of the organism was observed with *F. cretica* leaf extract, which was more effective than antibiotic tetracyclin (Table 1).

Considering the remarkable results with *F. cretica* leaf extract, phyto-chemical analysis was carried out in leaf extract (Table 2). For phyto-chemical studies, leaves were extracted with hot ethanol (80% 100 ml/g dry wt), filtered and the filtrates concentrated *in vacuo* separately. Each of the residues was re-extracted with petroleum ether, ethyl ether and ethyl acetate (fractions A, B and C) in succession[6]. Various ethyl acetate fractions (C) were hydrolysed (in 7% $H_2SO_4$, 2 h) and neutralized. Fraction A was rejected as being rich in fatty substances, whereas fraction B was analysed for free flavonoids and fration C for bound flavonoids. The ethyl ether and etheyl acetate (hydrolysed) fractions were separately pooled for further analysis. Each of the isolates was examined by thin layer chromatography (TLC, silica gel-g coated plates) along with the standard reference samples of apigenin, isorahm netin, isovitakin, kaempferol, lucteolin, myrecitin, quercetin and vitexin. The flavonoids were separated by TLC using *n*-butanol–acetic acid–water (4 : 1 : 5 upperlayer) as solvent system, observed under UV light, placed in a chamber saturated with ammonia and were sprayed with 5% ethanolic ferric chloride.

The compounds were removed from unsprayed TLC plates (observed under UV light) along with the silica gel. The mixtures were separately extracted with ethanol and the compound dried. The isolated compounds were crystallized separately in 50% ethanol and subjected to IR and UV spectra studies with the known flavonoid to make its presence sure. The presence of flavonoids in leaves of *F. cretica* confirmed its anti-bacterial potential, as flavonoids are said to be antimicrobial in nature[7].


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# Tissue culture of *Bulbothrix setschwanensis* (lichenized ascomycetes) *in vitro*

The tissue culture of several lichen taxa has been successfully achieved[1]. An attempt has been made here to derive cultures from the natural thalli of a lichen species *Bulbothrix setschwanensis* (Zahlbr.) Hale.

The lichen *B. setschwanensis* (Zahlbr.) Hale, producing atranorin and salazinic acid under natural conditions was used for this study. A part of the material has been preserved as herbarium specimen (India, Uttar Pradesh; Kumaon Hills, Nainital, Hanumangarh, alt. ca. 1900 m. about 3.22 km from Nainital, on trees, *U.V. Makhija*, 97.130-**AMH**).

Lichen culture was started a few days after collection of the lichen. The methods used for the culture are similar to those as reported by Yamamoto, Mitzuguchi and Yamada[2].

The culture media were selected to be nutrient-poor so as to ensure a proportionate growth of both the photobiont and the mycobiont to enable the formation of cell aggregates.

The following culture media were used: 1) malt–yeast extract medium (MY) containing malt extract 10 g, yeast extract 4 g, agar 15 g in 1 l of water; 2) water–agar medium, agar 15 g in 1 l of water; 3) Malt Extract Broth (MEB), 20 g per l of water; 4) Bold's Basal Medium (BBM)[3] and 5) Modified Bold's Basal medium. The composition of modified (by us) BBM is given in Table 1.

All chemicals used in this experiment were of analytical reagent grade (Hi-media Laboratories Pvt Ltd, India). The pH of all media used here was adjusted to 6.6 with 1 N NaOH or 1 N HCl .

About two months after incubation, the most actively growing tissues composed of mycobiont without contamination were selected and cut into smaller masses of tissue with the scalpel. Each of these masses was transplanted onto fresh media and was cultured under the same conditions as mentioned earlier. The trans-