

guidance during this work, of Professor A. G. Ramachandran Nair of our department.

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ESTIMATION OF QUERCETIN IN THE LEAVES OF *MAESA INDICA* WALL BY A NEW COLORIMETRIC METHOD

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THE leaves of *Maesa indica* are used in curries in North India and also as fish poison. Extracts of the branchlets, leaves, bark and stem have insecticidal activity¹. Locally the plant is called 'vavding'. It is used as a blood purifier and as an anthelmintic by the local people. This communication reports isolation and estimation of quercetin from the leaves *M. indica* from Goa.

The methods reported in the literature for quercetin estimation are Folin–Ciocalteu spectrophotometry^{2–5}, fluorimetry⁶, colorimetry by using Folin–Ciocalteu reagent⁷, and by treating with NaOAc, HOAc (3:1) and AlCl₃ (ref. 8). In the present work a simple and sensitive method that gives very stable colour for more than one hour is described. In the Folin–Ciocalteu method the authors described the method for general phenolic substances from apple juice, while the present method is specific for quercetin only. In the NaOAc, HOAc and AlCl₃ method the colour obtained is stable for 20 min whereas in the present method the colour is stable for more than one hour. The present method is more sensitive than the earlier methods, and is good up to 2 µg quercetin.

Mature leaves of *M. indica* were collected at the

beginning of winter from the Morlem forests of Sattari taluka in Goa. A fruiting voucher specimen of the plant is preserved in the phytochemistry research lab., Goa College of Pharmacy. The leaves were washed in running tap-water, air-dried and powdered. The leaf powder (100 g) was defatted with petroleum ether. The marc was then extracted with methanol, and the extract reduced to 50 ml and mixed with 6% HCl (50 ml). The solution was heated on a water bath for 45 min. The hydrolysate was extracted thoroughly with ether in a separating funnel. On separation the ether layer was concentrated to residue⁹. The residue was digested with H₂O, filtered and then dissolved in *n*-propanol. It was further purified by preparative TLC (silica gel G, using EtOAc:HCOOH:AcOH:H₂O, 100:11:11:27)^{10,11}. The compound obtained was recrystallized from methanol and was confirmed from UV and IR spectra as quercetin.

A new colorimetric method was developed for quantitative analysis of quercetin by reacting it with acidic ammonium molybdate in *n*-propanol medium using Speckol Spectrocolorimeter (Carl Zeiss Jena).

Quercetin (10 mg) was dissolved in 100 ml of *n*-propanol to obtain a reference solution. 10 g of leaf powder was defatted with petroleum ether and thoroughly extracted with methanol in a continuous hot extractor. The methanolic extract was filtered and concentrated to 5 ml. This concentrate was subjected to acid hydrolysis as in the isolation procedure and the volume was made up to 50 ml. Two ml of this was made up to 100 ml with *n*-propanol to obtain a test solution.

Three activated silica gel G plates were streaked with 0.25, 0.5 and 0.75 ml of the reference and test solutions. The solvent system used was using EtOAc:HCOOH:AcOH:H₂O (100:11:11:27). Scrapings of the band corresponding to quercetin were transferred to a stoppered test tube and extracted vigorously with 5 ml of *n*-propanol. After centrifuging the supernatant liquid was filtered through Whatman No. 42 filter paper into a 10 ml volumetric flask. To the above filtrate, 0.5 ml of 0.1 N H₂SO₄ and 1 ml of 10% (w/v) aqueous ammonium molybdate were added. The resulting yellow chromogen was measured at 420 nm. A graph of absorbance versus concentration was plotted and was found to be linear over the concentration range 2 to 14 µg/ml, indicating conformity with Beer's law.

The quercetin content of the leaves of *M. indica* was found to be 3.03%. Quercetin has also been reported in *Maesa macrophylla*¹².

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CATION EXCHANGE CAPACITY OF SOIL AND ADSORPTION OF *AZOSPIRILLUM* TO SOIL PARTICLES

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AZOSPIRILLUM occurs as a predominant diazotroph in the rhizosphere of grasses and cereals¹. The population of *Azospirillum* in soil and the rhizosphere zone of plants is influenced by many factors, of which soil is an important one. Microorganisms in

soil mostly occur adsorbed to soil particles and rarely as unadsorbed cells². The influence of the cation exchange capacity (CEC) of soil on the adsorption of *Azospirillum* and other heterotrophic bacteria on soil particles is reported.

Three different soil types were used in these studies. Soil samples were collected from the top 0–10 cm, air-dried, and pounded and sieved through a 50 mesh sieve. The total heterotrophic bacteria in the soils were enumerated by the dilution plate technique on yeast extract glucose agar³. CEC of the soils was determined as described earlier⁴. One hundred grams of each soil was taken in a beaker and leached thrice with 1 N ammonium acetate, pH 7.0. A standardized cell suspension of *Azospirillum brasilense* strain S.3, which is resistant to both streptomycin and chloramphenicol at 100 µg ml⁻¹ of each and contains a pink chromogen (obtained from D. Hubbell, University of Florida, USA), was added to the soil samples and mixed well. Three replications were maintained in each treatment.

After overnight incubation, the populations of the inoculated *Azospirillum* and the total heterotrophic bacteria were estimated using yeast extract glucose agar. The soil samples were then leached thrice with sterile distilled water with minimum disturbance to the soil, after which the populations of the inoculated *Azospirillum* and total heterotrophs in the soil samples were again determined. As strain S.3 was pink the colonies of *Azospirillum* on the agar medium were easily counted. The percentages of inoculated *Azospirillum* and heterotrophs retained in the soil samples were calculated.

Some physical properties of the three soil types are presented in table 1. Adsorption of *Azospirillum* and heterotrophic bacteria on soil particles is given in table 2. The study revealed that the CEC of soil is closely associated with the adsorption of not only cells of *Azospirillum* but also of the heterotrophic bacteria of the soil. The higher the soil CEC, higher was the percentage of adsorbed cells. Alluvial (clay) soil with a CEC of 339 m.e. retained 82.8% of *Azospirillum* in adsorbed state. The cations present in the soils might have contributed to the increase in the adsorption of *Azospirillum* cells to soil particles. Calcium ion-mediated adsorption has been suggested for root nodule bacteria⁵. It is believed that when microorganisms are adsorbed to solid surfaces they enjoy nutritional advantages. Cells adsorbed to surfaces in water take up substances in solution more easily than unadsorbed cells which are likely to be carried away. Other advantages to the bacteria