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Determination of the Flavonoids from *Oscillatoria terebriformis* and *Chroococcus turgidus* Extract by High Performance Liquid Chromatography.

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Abstract

Flavonoids, one of the most abundant natural flavonoids, present in daily food. Flavonoid is of interest because of its pharmacological function. A simplified method for the detection of flavonoids was also developed using Reversed - Phase HPLC. Concentration of flavonoids in was *Oscillatoria terebriformis* and *Chroococcus turgidus* calculated based on calibration curve.

Keywords: *Oscillatoria terebriformis*, *Chroococcus turgidus* HPLC, Flavonoids.

Flavonoids (flavus- yellow) or bioflavonoids are a ubiquitous group of polyphenolic substances which are present in most plants, concentrated in the seeds, fruit skin, peel, bark, and flowers(D. Malesev et al 2007).Flavonoids are water soluble polyphenolic molecules containing 15 carbon atoms. Flavonoids belong to the polyphenol family. Flavanoids can be visualized as two benzene rings which are joined together with a short three carbon chain. One of the carbons of the short chain is always connected to a carbon of one of the benzene rings, either directly or through an oxygen bridge, thereby forming a third middle ring, which can be five or six-membered. The flavonoids consist of 6 major subgroups: chalcone, flavone, flavonol, flavanone, anthocyanins and isoflavonoids. Together with carotenes, flavanoids are also responsible for the coloring of fruits, vegetables and herbs. Flavonoids are polyphenolic compounds that are ubiquitous in nature and are categorized, according to chemical structure, into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones.

The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health-they have been reported to have antiviral, anti-allergic, antiplatelet, anti-inflammatory, and antitumor and antioxidant activities.

Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage.

The objective of this work was to analyzethe flavonoid content of *Chroococcus turgidus* and *Oscillatoria terebriformis* using HPLC.

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Materials and Methods

The micro algae

The effluent collected periodically from the factory premises was subjected to laboratory examination and the cyanobacteria were isolated from the effluent using serial dilution, standard plating, colony isolation and culture techniques. The *Oscillatoria terebriformis*, a thermophilic cyanobacterium and *Chroococcus turgidus* was identified following the monograph of Desikachary (1959) and Philipose (1967).

LABORATORY GROWTH CONDITIONS

The cultures were grown at 24 ± 1°C in a thermo-statically controlled environmental chamber illuminated with cool white fluorescent lamps (Philips 40w, cool daylight, 6500k) at an intensity of 2000 lux in a 12/12 h light/dark cycle. The Cyanobacteria were grown in various culture media. The Cyanobacterial nature of the culture was ascertained microscopically before cultivation and harvesting.

Biomass extraction: The biomass of *Oscillatoria terebriformis* and *Chroococcus turgidus* were washed briefly to remove the adhering medium components. To the biomass 2 ml of each solvent was added, kept for 15 min and a mild sonication for 5 min was given. The supernatant were separated from the debris by centrifuging for 15 min at 2000 rpm. Then the extracts itself were centrifuged for 15 min at 5000 rpm. The extracts was collected in a storage vial, allowed for complete evaporation of solvent and re-suspended with 1 ml of sterile water. The vial was stored in a refrigerator until further use.

Preparation of Cyanobacterial extract: 0.5g of dried *Oscillatoria terebriformis* and *Chroococcus turgidus* material were extracted in 20ml of acetone and methanol kept in an orbital shaker for overnight. The obtained extracts were filtered with Whatman no.1 filter paper and the filtrate was collected. The solvents were removed under reduced pressure at 50°C to yield a concentrated extract (12% and 11%) respectively.

HPLC analysis of Flavonoids

Standard preparation: Standard stock solution of gallic acid, Rutin & Quercetrin (Sigma) was prepared in methanol, at concentrations of 5mg/ml. Standard solution were filtered through 0.45 mm membrane filter and injected by autosampler.

Content of Flavonoids by HPLC Method

HPLC analysis was performed using a LDC Milon Roy CM 4000 gradient pump coupled to a Hewlett Packard 1100 diode-array detector. Flavonoid separation was carried out in a 5 mm Chrompack C18 column 250 mm_4.6 mm, protected by a Chrompack C18 pre-column 3.0 mm_10 mm. Cyanobacterial extracts were eluted at 1 ml/min20 μl injection volume) using as mobile phase a binary solvent system consisting in methanol, water, and phosphoric acid (100:100:1) equal volumes (about 20 µL) of each of the Standard solutions was injected and the Test solution into the chromatograph equal volumes (about 20 µL) of each of the Standard solutions was injected and the Test solution into

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the chromatograph . Record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of each flavonoid in samples.

Result and Discussion:

Reversed-phase HPLC has been used in a number of occasions for the analysis offlavonoids in cyanobacteria, it was used to distinguish species based on the quantitative variation of flavonoids among them. It has been applied especially for the identification of flavonoid derivatives. In the present investigation, flavonoids were quantified at 254nm using peak area by comparison to a calibration curve derived from the *Chroococcus turgidus* and *Oscillatoria terebriformis* the main difference was in peak eluted at 3.336 min.

Figure 1 :HPLC ANALYSIS OF STANDARD QUERCETIN

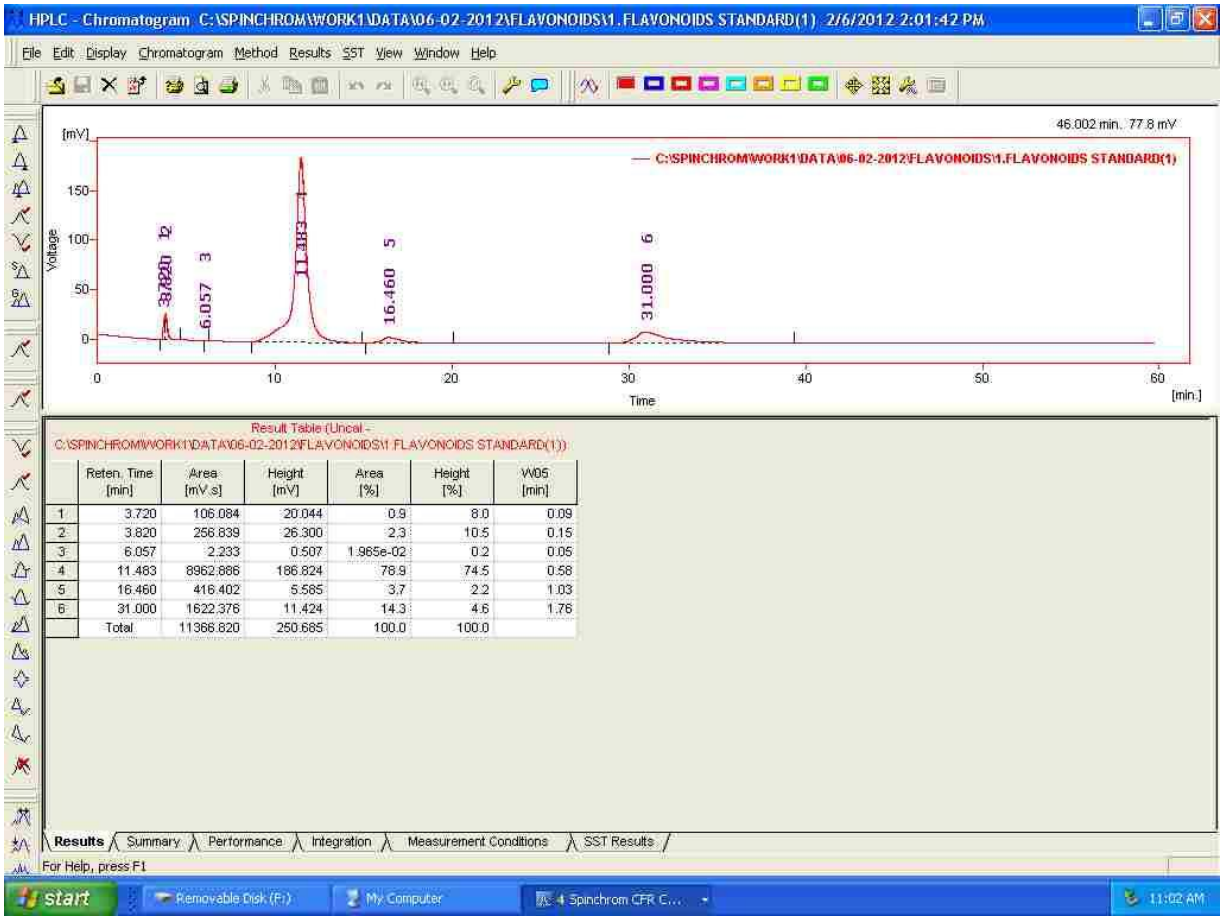

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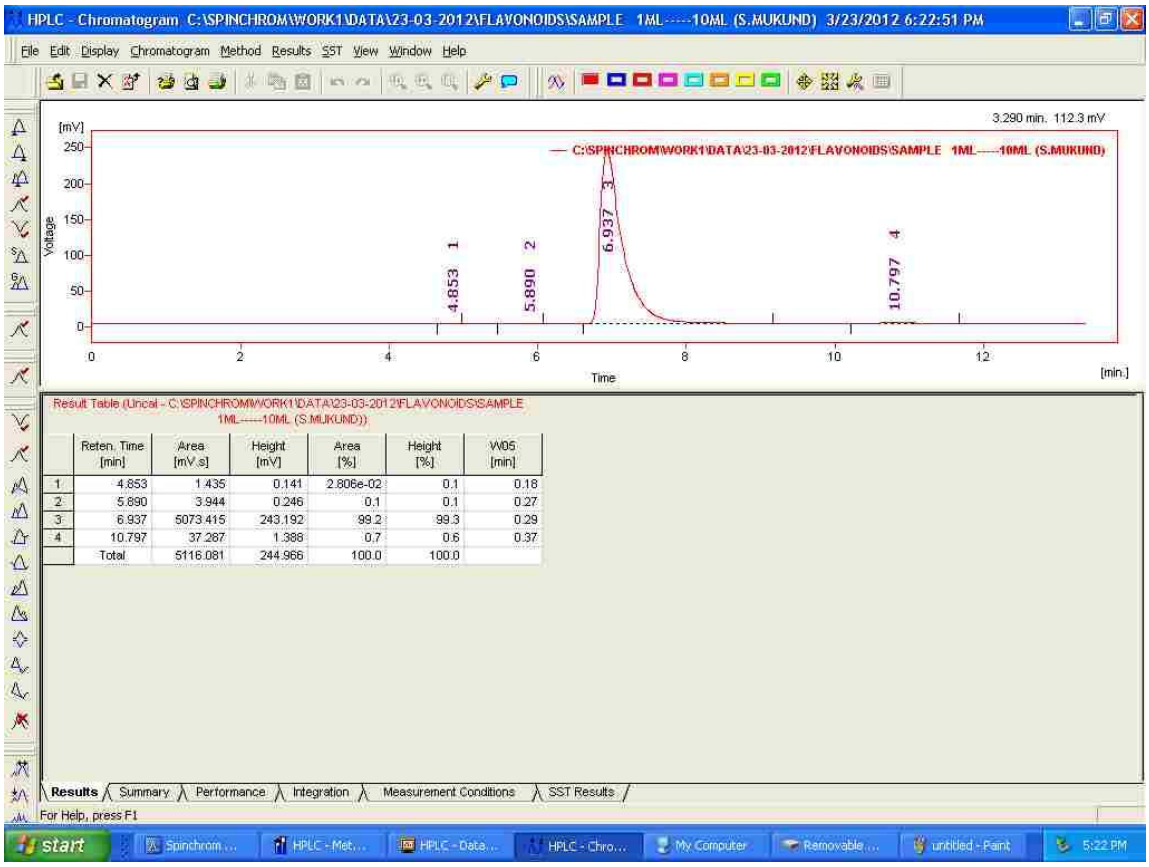
Figure 2-HPLC Analysis of *Chroococcus turgidus*



For 1ml of Sample – *Chroococcus turgidus*

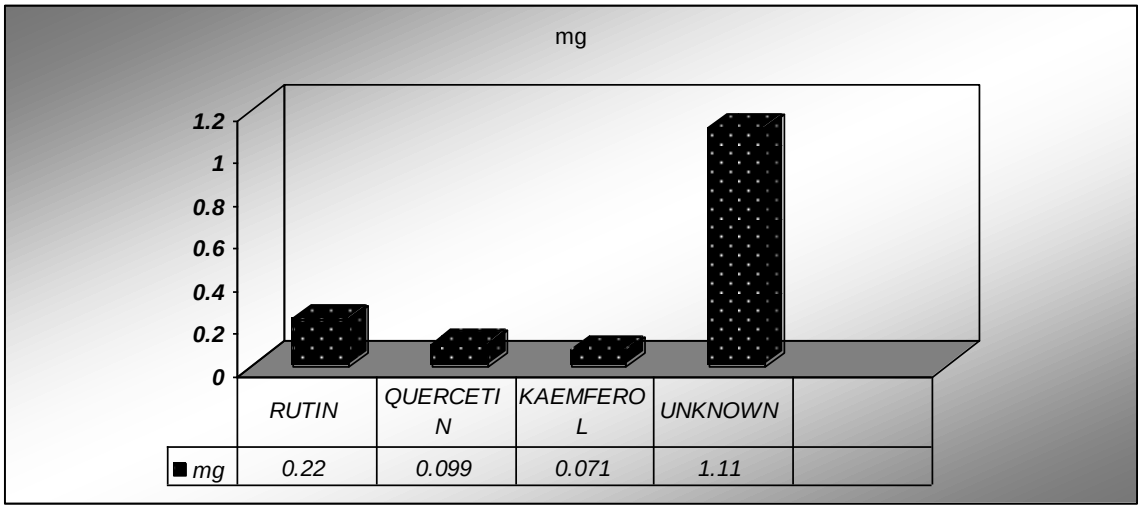


Figure 3: Total* Flavonoids Contents in *Oscillatoria terebriformis

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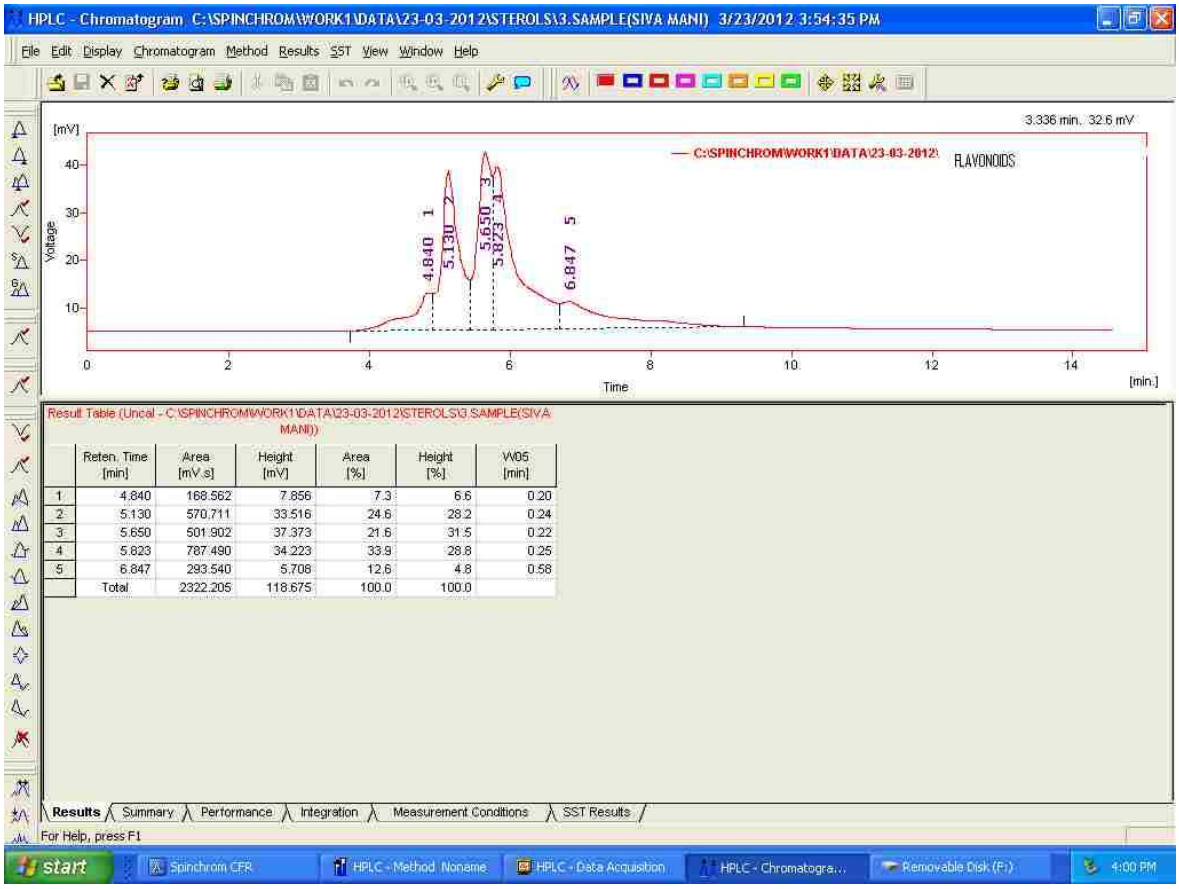
Figure 4- HPLC Analysis of *Oscillatoria terebriformis*.



For 1ml of Sample- OSCILLATORIA TEREBRIFORMIS

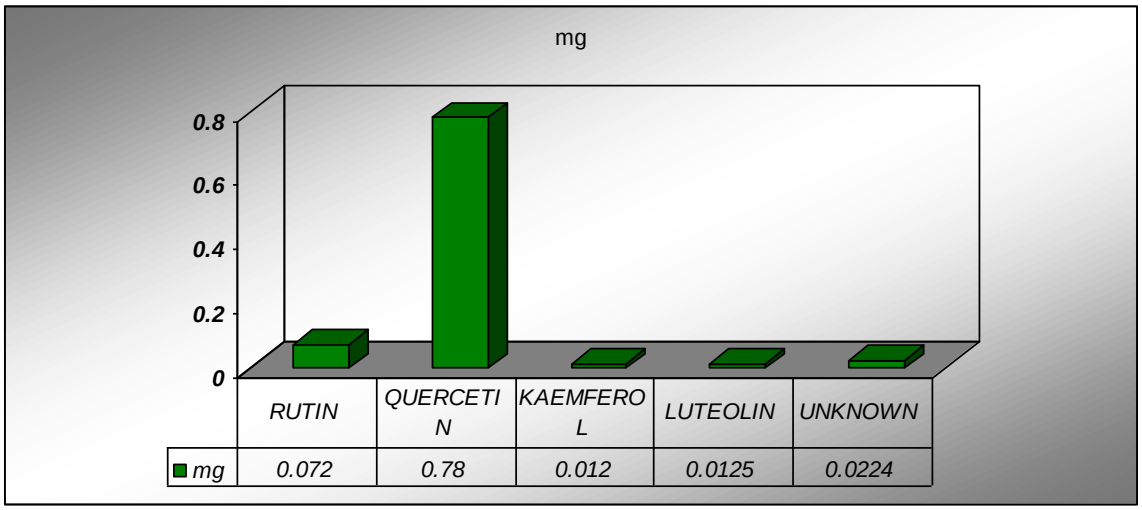


Figure 5:* *Total* Flavonoids Contents in *Oscillatoria terebriformis

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The peaks in this study showed marked increase in peak area in case of *Oscillatoria terebriformis* and *Chroococcus turgidus* and compared with standard quercetin (Fig-1).From the calibration curve results, the amount of Quercetin, in the sample injected was calculated. Distribution different types of flavonoids from *Oscillatoria terebriformis* and *Chroococcus turgidus* were found. The results are graphical represented for the two different cyanobacteria (Figure 3 and Figure5).The present method was applicable for analysis of flavonoids from cyanobacterial sample using HPLC technique.

Dietary flavonoids are natural antioxidants. (Kandaswami *etal* .,1994).They may be against cancer through limit of damaging oxidative reactions in cells, which may predispose to the development of cancer. Oxygen-derived free radicals appear to possess the propensity to initiate as well as to promote carcinogenesis. Lipid peroxidation products originating from dying cells could also exert a cancer promotional effect. (Halliwell *etal*., 1992).Oxidation of DNA is likely to be important causes of mutation that potentially can be reduced by antioxidants Flavonoids are chemically one-electron donors. They serve as derivatives of conjugated ring structures and hydroxyl groups that have the potential to function as antioxidants in invitro cell culture or cell free systems by scavenging super oxide anion, singlet oxygen, lipid peroxy-radicals, and/or stabilizing free radicals involved in oxidative processes through hydrogenation or complexing with oxidizing species. In vitro studies are able to demonstrate for flavonols, flavones, and most recently also for anthocyanins a considerable antioxidative activity, mainly based on scavenging of oxygen radicals. (Duthie *etal*., 1999). Theoretical underpinnings for the efficacy of flavonoids as antioxidants in vivo come from the inhibition of low-density lipoprotein (LDL) oxidation, likely due to their reductive capacity and protein-binding properties.(Wang *etal* .,1999)

Many researchers have conducted in vitro studies on the potential anticancer activity of flavonoids in diverse cell systems.Hirano and co-workers examined anticancer efficacy of 28 flavonoids on human acute myeloid leukemia cell line HL-60, and compared differences between antiproliferative activity and cytotoxicity of these compounds with those of four clinical anticancer agents. Eight of the 28 flavonoids showed considerable suppressive effects on HL-60 cell growth with IC50s ranging from10–940 ng/ml. The flavonoid genistein had the strongest effects almost equivalent to the effects of current anticancer agents with little cytotoxicity against HL-60 cells, whereas the regular anticancer agents had potent cytotoxicity.Kuntz *et al* (1999)screened more than 30 flavonoids for their effects on cell proliferation and potential cytotoxicity in human colon cancer cell lines Caco-2 and HT-29. Almost all compounds displayed antiproliferative activity without cytotoxicity. There was no obvious structure-activity relationship in the antiproliferative effects either on basis of the subclasses (i.e., isoflavones, flavones, flavonols, and flavonones) or with respect to kind or position of substituents within a class.

Conclusion

Flavonoids are generally nontoxic and manifest a diverse range of beneficial biological activities. The role of dietary flavonoids in cancer prevention is widely discussed. There is much evidence thatflavonoids have important effects on inhibiting carcinogenesis.Flavonoids could be useful anticancer agents, to date few clinical studies have demonstrated that these bioflavonoids retain anticancer properties in humans in vivo. In addition, clinical trials available have required intravenously administered flavonoids at concentrations around 1400 mg/m2 before effects are seen. These plasma concentrations are unlikely to be achieved using the dietary supplements currently available. Therefore, more

focused clinical studies are required to establish whether such dietary effects of these compounds can be exploited to achieve cancer preventive or therapeutic effects in human.

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