IN-VITRO QUANTIFICATION OF ANTIOXIDANTS OF *Cyanotis Fasciculata* Var., Fasciculata

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Summary

Cyanotis fasciculata is one among the under exploited plants of medicinal value. The phytochemical analysis coupled with TLC studies of various extracts revealed the presence of many antioxidant phytoconstituents including flavonoids & phenolics. Hence all the extracts were subjected to estimation of Total Phenolic Content (TPC), Total Flavonoidal Content (TFC), and Total Antioxidant Capacity (TAC) in terms of catechol, Rutin and Ascorbic acid equivalent values, respectively. The maximum 35.33 $\pm$ 1.8 mg/g TPC was found in methanolic extract, where as the 70% ethanolic extract showed highest 131.8 $\pm$ 2.2 mg/g TFC and TAC was highest with 70% ethanolic extract followed by methanolic, ethyl acetate and chloroform extracts.

Key words: *Cyanotis fasciculata,* Total Phenolic Content, Total Flavonoidal Content, Total Antioxidant Capacity.

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Introduction

Free radicals, especially Reactive Oxygen Species (ROS) are undesirable but inevitable emitants of aerobic metabolic pathways. These being highly reactive can initiate & propagate many disastrous chain of interactions such as lipid peroxidation, denaturation of polypetides, fragmentation of nucleic acid strands etc., leading to extensive cellular damage that culminates in to quite a lot of denegenerative diseases ranging from inflammation to ischemic heart disease and cancer.[1] It is well documented that plant phenolics, tannins and flavonoids can efficiently quench these larcenous free vadicals and retards the process of oxidative damage to tissues and organs[2]. Hence, this has become impetus for search of herbal phytoconstituents with antioxidative and organ protective actions.[3]

Cyanotis fasciculata (Commelinaceae) is a small, terrestrial, annual herb of 4-10 inches long at branches, commonly found on dry grass lands and rocks. Flowers blue, purple or pink in colour and are present in auxiliary or terminal position with 3 petals united into a tube below. Stems are slender, slight pinkish, with cottony cob webby appearance. Leaves are broadly ovate to narrowly linear usually obtuse, juicy, woolly Cob- Webby. [4, 5, 6]

The juice from succulent leaves used to treat skin fungus disease and mouth sores. [7,8] The hydroalcoholic extract of entire plant is reported to be useful in lymphatic leukemia, possess diuretic and antiviral properties.[9,10]

In the present study an attempt is made to standardize various extracts of the plant material wit h respect to TFC, TPC & TAC. So that the extract exhibiting superior results can be taken for further biological screening & phytochemcial investigation.

Materials & Methods

The plants of *Cyanotis fasciculata* var., fasciculata were collected from Fort-hill top of Bellary, Karnataka in the month of September and were authenticated by Dr. Kotresh, Department of Botony, Karnataka University, Dharwad, Karnataka. The voucher specimens of these plants were preserved in the herbarium of the pharmacognosy department of this institution.

The plants were air-dried in shade and were pulverized in a mechanical grinder to cottony lumps. The powder was exhaustively extracted with 70% ethanol (AE), chloroform (CF), methanol (ME) and ethyl acetate (EA) individually by soxhlation; extracts were dried in rotary vacuum evaporation and relevant yields were calculated and stored in airtight containers at 4^0C. The different concentrations of extracts were prepared using corresponding solvents for quantification studies.

Preliminary phytochemical studies

The individual extracts were subjected to qualitative chemical investigation for the identification of various phytochemical constituents[11].

Total Phenolic Content (TPC)

To determine total phenolic content working stock solutions of the extracts (AE, ME, EA & CF) were prepared with distilled water to a suitable concentration for analysis. TPC was assessed approximately by using Folin-Ciocalteau Phenol reagent[12]. 50µl of extracts were made upto 3ml with distilled water and were added 0.5ml of Folin – Ciocaltean Phenol reagent, incubated for 3 mins at room temperature. Later 2ml of sodium carbonate solution (20% w/v) was added, incubated for 1 minute in boiling water bath. Absorption at 650nm was measured against a reagent blank in a UV–Viscible Spectrophotometer. The experiment was conducted in triplicate and TPC was expressed as mean $\pm$ standard deviation of catechol equivalent in milligrams per gram of sample using a standard curve generated with catechol.

Total Flavonoidal Content (TFC)

To determine the total flavonoid content, stock solutions of the all four extracts were prepared with corresponding solvents to a suitable concentration for analysis. Total flavonoid content was measured according to the method previously reported by Helmija et al[13] with slight modification using standard curve generated with Rutin.

Aliquots of each extract (AE, ME, CF & EA) were pipetted out in series of test tubes and volume was made upto 0.5ml with distilled water; sodium nitrate (5%; 0.3ml) was added to each tube & inclubated for 5 min at room temperature. Aluminium chloride solution (10%; 0.06ml) solution was added and incubated for 5 min, at room temperature; sodium hydroxide (1M; 0.25ml) was added and total volume was made to 1ml with distilled water. Absorbance was measured at 510nm against a reagent blank using Schimadza model 150–02 Double Beams Spectrophotometer and concentration of flavonoids in the test sample was determined in triplicates and expressed as mg of Rutin equivalent per gram of sample $\pm$ standard deviation.

Total Antioxidant Capacity (TAC)

Total antioxidant capacity was measured according to the method previously reported by Pricto[14], with slightly modification. In brief 100 µg of extracts, BHA (Butylated hydroxyl Anisole – as standard) were taken in 0.1ml of alcohol, combined separately in a Eppendroff tube with 1.9ml of reagent solution (0.6m sulphuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The tubes were caped and incubated in a thermal block at 95^0C for 90 minutes. After the samples were cooled to room temperature the absorbance of the aqueous solution of each was measured at 695nm against a blank. A typical blank solution contained 1.9mL of reagent solution and appropriate volume of the sample solvent used for the sample and it was incubated under the same conditions as the rest of the samples. For the samples of unknown composition antioxidant capabilities are expressed as equivalent of ascerbic acid. Ascorbic acid equivalents were calculated using standard graph of ascorbic acid. The experiment was conducted in triplicate and values are expressed as ascorbic acid equivalents in µg per mg of extract (mean $\pm$ SD).

Results

Preliminary phytochemical screening of both extracts viz. ME and AE showed the presence of sterols, triterpenoids, flavonoids, phenols, tannins, coumarins and alkaloids. Whereas, flavonoids were major constituents in EA and CF extracts in addition to phenolics and alkaloids, respectively.

TPC estimation revealed presence of high quantities of phenolic constituents in ME and AE viz., 35.33 ± 1.8 mg/g and 32.77 ± 1.9 mg/g respectively; EA fraction showed moderate TPC of 16.68 ± 2.4 mg/g; CF extract turned out to be the least in TPC with 2.73 ± 1.1 mg/gm (Table 1).

Rutin equivalent flavonoidal content was found to be highest in AE with 131.8 ± 2.2 mg/gm. Whereas ME, EA and CF extracts exhibited 105.88 ± 1.7 mg/gm, 100.49 ± 2.8mg/gm and 95.75 ± 0.9 mg/gm respectively (Table 1).

TAC estimation claimed AE with highest antioxidant capacity followed by ME, EA & CF as depicted in Table No. 1.

Table No.1: Total Phenolic Content (TPC), Total Flavonoidal Content (TFC) and Total Antioxidant Capacity (TAC) of various extracts

Extracts	TPC (Catechol equivalent)	TFC (Rutin Equivalent)	TAC (Ascorbic acid Equivalent)
AE	32.77 ± 1.9 mg/gm	131.8 ± 2.2 mg/gm	78 ± 0.8 %
ME	35.33 ± 1.8 mg/gm	105.88 ± 1.7 mg/gm	70 ± 2.1 %
EA	16.68 ± 2.4 mg/gm	100.49 ± 2.8 mg/gm	67 ± 3.1 %
CF	2.73 ± 1.1 mg/gm	95.75 ± 0.9 mg/gm	63 ± 1.4 %
BHA	-	-	71 ± 1.9 %

Values are mean ± SD (n=3)

Discussion

After comparing the TPC & TFC values of various extracts especially of EA & CF with their TAC values it was evident that flavonoids of this plant appears to contribute predominantly to TAC rather than its phenolics. Further, the data documented in the present communication justifies that AE has drawn maximum of antioxidant phytoconstituents and proved to be superior to BHA with respect to TAC. Altogether, it can be concluded that the same could be effective against free radical mediated pathological events; hence there is good scope for evaluation of its organ protective activity in various *in vitro & in vivo* models.

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