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QUANTIFICATION OF TOTAL PHENOLICS, TANNINS AND FLAVONOIDS FROM STEM BARKS OF VENTILAGO MADERASPATNA AND COCHLOSPERMUM GOSSYPIUM

1A.B. Kawde ,2 R.G Weginwar, [1]A.V.Bharti, [3]D. M.Akkewar, [2]G.S. Gond

[1]Ramdeobaba College of Engineering and Management, Nagpur, Maharashtra, India.
[2]Guru Nanak College Of Science, Ballarpur, Maharashtra, India.
[3]Institute of Chemical Technology, Hyderabad – 500607, Andhra Pradesh, India.

ABSTRACT

Ventilago maderaspatana and Cochlospermum gossypium are medicinal herbs used in traditional medicine for various diseases and disorders. In our previous work we have noticed that methanolic extracts of stem barks of these medicinal plants have exhibited strong antioxidant and significant antibiotic activity on gram negative bacteria and contain the major phytochemicals phytosterols, flavonoids and tannins were noticed in bioactive extractive. The aim of this study was to quantify some important antioxidant compounds of Stem bark extracts of Ventilago maderaspatana and Cochlospermum gossypium. Total phenolics (TP), total tannins (TT) and total flavonoids (TF) content were determined by colorimetric method. The results showed that the stem bark extracts of Ventilago maderaspatana and Cochlospermum gossypium contain total phenolics of 6.0821 and 5.0631 (mg GAE/ g dry matter) respectively, total tannins 2.248 and 1.782(mg GAE/ g dry matter) respectively, and total flavonoids 1.2032 and 0.8924 (mg QE/ g dry matter) respectively. Total phenolics contents of the Ventilago maderaspatana and Cochlospermum gossypium are likely to be a key for determining the antioxidant ability of these plants.

Keywords: Ventilago maderaspatana, Cochlospermum gossypium, Phytochemical, Antioxidant, Phenolics, Tannins,

INTRODUCTION

Nature has provided many things for humankind over the years, including the tools of the first attempts at therapeutic intervention. Ancient civilization depended on plant extracts for the treatment of various ailments. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannin, flavonoids andPhenolic compounds[1]. Nowadays, there is an upsurge of interest on phytochemical activities from antioxidants [2]. Various plant extracts have been utilized as natural antioxidant resources [3]. Natural antioxidants are compounds originated from plant or animals. Natural antioxidant comprises of phenolic acid (phenols), flavonoids/ bioflavonoid and tannic acid (tannins) named as polyphenols. Antioxidants are compounds responsible for preventing or delaying the oxidation of products by free radicals scavenging and reducing oxidative stress. There is a growing interest in the study of medicinal plants that possess antioxidant property. These natural antioxidants have been shown to be effective in the inhibition of oxidation of food, reduction of age related diseases[4, 5]

Ventilago maderaspatana is a medicinal herb belonging to family Rhamnaceae. The stem bark of *Ventilago maderaspatana* has been used for thousands of years for its medicinal properties such as appetite stimulant, treatment for gastro intestinal infection, to lower blood glucose in diabetes, for treatment of certain type of cancer and viral infections [6]. *Cochlospermum gossypium,* a medicinal herb belonging to family Cochlospermaceae, is a small deciduous tree. The tree yields a gum which is known as katira. Traditionally it is used in treating cough, diarrhea, dysentery, pharyngitis, fistula, gonorrhea, trachoma and syphilis.[7]

With this background, we have examined the extracts of stem bark for the presence of various phytochemicals and found that it is rich in phytosterols , ,flavonoids and phenolic compounds. We have also noticed significant broad spectrum antimicrobial activity of methanolic extract of stem barks of *Ventilago maderaspatana and Cochlospermum gossypium* against several gram-positive and gram-negative bacteria. In in-vitro antioxidant assay *Ventilago maderaspatana* showed 100% anti scavenging activity in our earlier studies.[8,9]

It is not understood yet which active ingredients are responsible for clinical usefulness. This research aims to determine

the total phenolics (TP), total tannin (TT) and total flavonoids (TF) content in methanolic extract of stem barks of *Ventilago maderaspatana and Cochlospermum gossypium* using colorimetric techniques.

MATERIALS AND METHODS

Collection of Plant Materials and Extraction Procedure:

The stem bark of *Ventilago maderaspatana* and *Cochlospermum gossypium,* were collected from forest of Chandrapur district, Maharashtra state, India. They were washed, dried, powdered and stored for further studies. The air dried and fine powdered stem bark material was extracted with solvent methanol using Soxhlet extraction apparatus by percolation for 8 hours under reflux. 100grams bark material of *V. maderaspatna and C. gossypium yielded* 12 gm of extract and 10 grams of extract respectively.

Determination of Total Phenolics (TP) Content

The phenolic content was determined by colorimetric assay [10, 11]. An aliquot extracts of stem barks of 200 µL, 800 µL deionized water, and 100 µL of Folin-Ciocalteu reagent were mixed and incubated for 3 min at room temperature. Sodium carbonate (Na_2CO_3) (20 % w/v) 300 µL was added and incubated for 2 hours at room temperature under dark condition. The absorbance was determined using Jasco V550, UV-VIS spectrophotometer at 765 nm. A blank was prepared with distilled water instead of aliquot extract. Gallic acid standard curve was first prepared from 0 – 200 mg/L and total phenolic content was expressed in mg gallic acid equivalent / g dry matter. The total phenolics were expressed in mg gallic acid equivalent (GAE)/ g dry matter, calculated from the prepared standard curve with 0 to 100 mg/ gallic acid (GA).

Determination of Total Tannins (TT) Content

The tannin content was determined using Folin Ciocalteu assay. Aliquot extract of 100 µL was added to 750 µL of distilled water, 500 µL Folin- Ciocalteau reagent and 1000 µL of 35 % sodium carbonate (Na2CO3). The mixture was shaken vigorously after diluting to 10 mL of distilled water. The mixture was incubated for 30 min at room temperature and read at 725 nm using Jasco V550, UV-VIS spectrophotometer Distilled water was used as blank. Gallic acid standard solutions were prepared as described above. The total tannins content were expressed as GAE/ g dry matter, as calculated from the prepared standard curve with 0 - 100 mg/ GA [12].

Table: 1 Total phenolics and tannin contents of methanolic extracts of *V. maderaspatna and C. gossypium* stem barks.

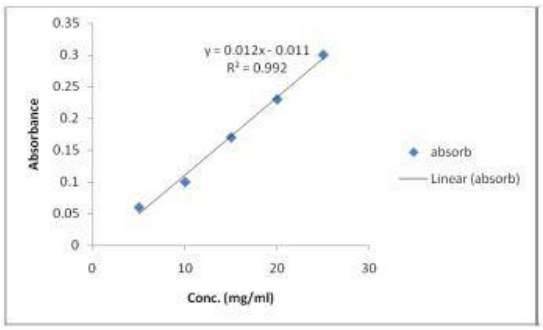
Bioactive compounds	Expressed as mg Gallic Acid Equivalents/g dry matter	
	V. maderaspatna	C. gossypium
Total phenolics	6.0821	5.0631
Total tannins	2.248	1.782

Determination of Total Flavonoids (TF) Content

Flavonoid content was determined by colorimetric analysis [13]. A mixture of 200 µL extract and 150 µL of sodium nitrite ($NaNO_2$) (5 % w/v), was first incubated for 6 min at room temperature. Next, 150 µL of aluminium chloride hexahydrate $AlCl_3.6H_2O$ (10 % w/v) was added and incubated for 6 min at room temperature. NaOH (10 % w/v) solution 800 µL was added and incubated at room temperature for 15 min. For blank, extract was replaced with distilled water. Absorbance was read by Jasco V550, UV-VIS spectrophotometer at 510 nm. A standard curve of quercetin dissolved in 80 % ethanol was initially prepared from 0 - 500 µg/mL. Total flavonoids was expressed in mg Quercetin equivalent (QE)/ g dry matter.

Table: 2 Total flavonoids contents of methanolic extracts of *V. maderaspatna* and *C. gossypium* stem barks

Sr. No.	Bioactive compound	Expressed as mg Quercetin equivalent /g dry matter	
		V. maderaspatna	C. gossypium
1	Total flavonoids	1.2032	o.8924



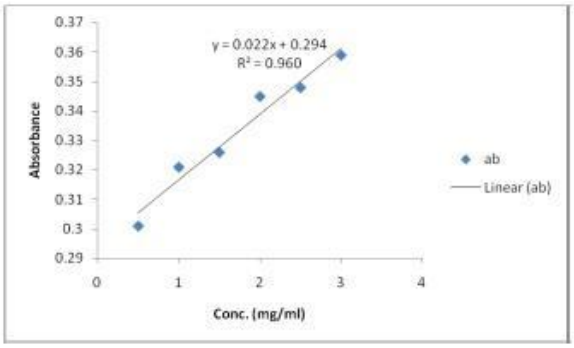


Fig. 1: Standard curve for Quercetin Fig. 2: Standard curve for Gallic acid

RESULTS AND DISCUSSION

Gallic acid was used as standard for total phenolic acid and tannin content [11]. The results showed that the stem bark extracts of *Ventilago maderaspatana* and *Cochlospermum gossypium* contain total phenolics of 6.0821 and 5.0631 (mg Gallic acid equivalents / g dry matter) respectively, total tannins 2.248 and 1.782(mg Gallic acid equivalents / g dry matter) respectively, and total flavonoids 1.2032 and 0.8924 (mg Quercetin equivalents / g dry matter) respectively. The Phenolics and tannins are very important plant phytochemicals that has several hydroxyl groups. These hydroxyl groups have been shown to be responsible for these chemicals radical scavenging ability. This ability makes them to act as antioxidants.. In the Folin-Ciocalteau assay, the molybdotungstate present in the reagent oxidizes the phenolics to yield a coloured product which absorbs around 725 nm.

Stem bark extract of *V.maderaspatna* show higher concentration of Total phenolics, tannins and flavonoids as compared to the stem bark extract of *C. Gossypium*. In our previous studies we have already reported that methanolic extracts of stem barks of these plants showed strong antibacterial and antioxidant activity.

CONCLUSION

From our study and with previous literature survey we come to conclusion that the stem barks of *V.maderaspatna* and *C. gossypium* are rich in phytochemicals which have free radical scavenging activity.

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