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Spectroscopic Determination Of Total Phenolic And Flavonoid Contents Of Tribulus terrestris Fruits

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Abstract: Herbal drugs have been in use since ancient times for the treatment of infectious diseases in human being. *Tribulus terrestris (Gokhru)*, a medicinally important herb belongs to the family Zygophyllaceae. It is used for treating diseases like hypertension, in folk medicine as tonic, aphrodisiac, analgesic, astringent, stomachic, antihypertensive, diuretic, lithon-triptic and urinary antiinfectives. Phenols and flavonoids are active principles and stress releasing moieties so quantitative determination of it from various extracts of *T.terrestris* fruits was performed using spectrophotometric method. Catechol and quercetin reagents were used as the standards for calibration of the phenols and flavonoids respectively. Ethanol and acetone extracts were tested against phenols and flavonoids content. Ethanol extract was found to be more rich. The results indicates that *T. terrestris* extracts contain significant amount of phenols and flavonoids.
Key words: *Tribulus terrestris,* Phenols, Flavonoids.

Introduction:

Numerous studies have shown that aromatic and medicinal plants are sources of diverse nutrient and non nutrient molecules, many of which display antioxidant and antimicrobial properties and can protect the human body against both cellular oxidation reactions and pathogens. It is important to screen different types of medicinal plants for their antioxidant and antimicrobial potential[1]. Spices and herbs have been used for thousands of centuries by many cultures to enhance the flavor and aroma of foods. Since the late 19th century have documented the antioxidant properties of some spices, herbs by performing scientific experiments[2]. *T. Terrestris (Gokhru)*, a medicinally important herb belongs to the family Zygophyllaceae. It is used for treating diseases like hypertension, in folk medicine as tonic, aphrodisiac, analgesic, astringent,stomachic, antihypertensive, diuretic, lithon-triptic and urinary antiinfectives[3,4]. Quantitative determination of phenols and flavonoids in various extracts of

T.terrestris fruits was performed using spectrophotometric method. Phenols are one of the main secondary metabolites in the plant kingdom. They are commonly found in both edible and non edible plants. Flavonoids and other plant phenolics are especially common in leaves, flowering tissues and woody parts such as stems and bark have been reported to have multiple biological effects, including antioxidant activity[5]. Flavonoids, the most common groups of polyphenolic compounds, are found ubiquitously in plants. These secondary metabolites are widely distributed in plants fulfilling many functions. They are important in plant for normal growth development and defense against infection and injury[6]. Flavonoids are the most important pigments for flower coloration producing yellow or red/blue pigmentation in petals. They also protect plants from attcks by microes and insects. These are also shows anti-allergic, anti-inflammatory, anti-microbial and anti cancer activity[7]. There is well supported evidence that the phenolic compounds found in various plant materials possess free radical scavenging properties, it was reported that flavonoids were OH* scavengers[8]. Researchers have become interested in flavonoids and other phenolics for the medicinal property, especially their potential role in the prevention of cancer and heart diseases[9].This study presents quantitative estimation of total flavonoids and total phenolics contents from the fruits of *T.terrestris* by spectrophotometric method.

Experimental:

Plant Material was collected from Sangli district , Maharashtra, India. It was authenticated at Botanical Survey of India, Pune, Maharashtra, India. Its authentication number is BSI/ Wc/ Tech/ 20011/ NBP-1.The ethanol and acetone extracts of air shade dried material of *T.terrestris* fruits was taken for experiment. Folin-ciocalteau reagent and all other chemicals used were Merck products. UV-Vis S1700 Pharmaspectrophotometer, Schimadzu was used for absorbance measurements. Accurately weighed powder of sample was ground with a pestle and mortar in the measured volume of solvents (80:20 ethanol-water).The extract was filtered through Whatman(No.1) filter paper. Each extract was prepared freshly for the analysis to prevent any degradation.

Determination of total phenolics:

The total phenolics contents of ethanol and acetone extract of *T.terrestris* fruits were determined according to the method described by Malik and singh[10]. Aliquots of the extracts were taken in a 10ml glass tube(0.2ml) and made up to a volume of 3ml with distilled water. 0.5ml Folin ciocalteau reagent (1:1with water) and 2ml Na_2CO_3(20%) sequentially added in each tube. A blue color was developed in each tube because the phenol undergoes a complex redox reaction with phosphomolibdic acid in folin ciocalteau reagent in alkaline medium which resulted in a blue colored complex, molybdenum blue. The test solution were warmed for 1 minute, cooled and absorbance was measured at 650nm against the reagent used as a blank. A standard calibration plot was generated at 650nm using known concentrations of catechol.The concentrations of phenols in the test samples were calculated from the calibration plot and expressed as mg catechol equivalent of phenol/g of sample .

Determination of total flavonoids:

The aluminum chloride method was used for the determination of the total flavonoids content of the sample extracts[11]. Aliquots of extract solution (0.2ml) were taken in 10ml glass tube and made upto the volume 3ml with methanol. Then 0.1ml $AlCl_3$(10%), 0.1ml Na-k tartarate and 2.8ml distilled water were added sequentially. The test solution was vigorously shaken. Absorbance at 415nm was recorded after 30minutes of incubation. A standard calibration plot was generated **(Figure**-2**)** at 415nm using known concentrations of quercetin. The concentrations of flavonoids in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent/g of sample.

Results and Discussion:

Figure-1 and **Figure-2** presents the calibration plot for the determination of phenols and flavonoids ,respectively.

Figure-1: Calibration plot for phenolics determination

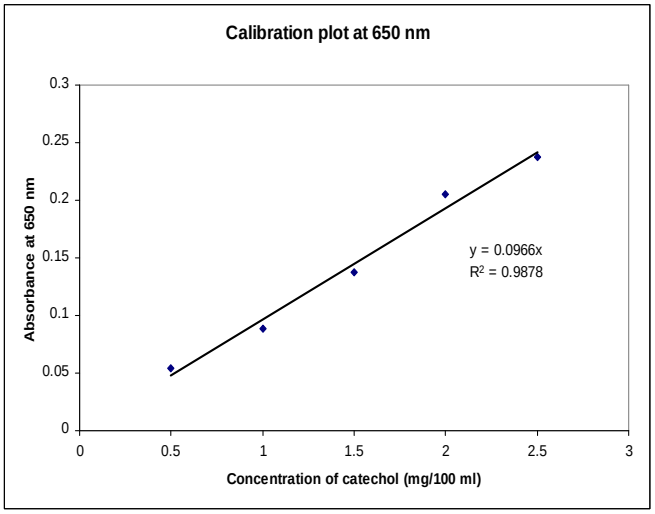

Figure-2: Calibration plot for flavonoids determination

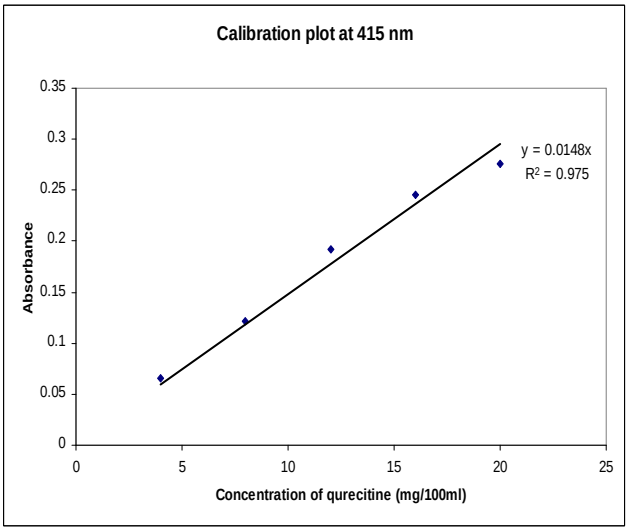

Table-1: Phenollics content

Extracts	Phenol content (mg catechol equivalent/g dry material)
Ethanol	41.2
Acetone	29.8

Table-2: Flavonoids content

Extracts	Flavonoid content (mg quercetin equivalent/g dry material)
Ethanol	601.3
Acetone	452.7

The present study reveals the phenolics contents of the fruits extracts of *T.terrestris* in terms of mg catechol equivalent/g of dry sample (standard plot: y=0.0966x, R^2=0.9878). The values are found between 41.2 to 29.8 mg catechol equivalent/g. The ethanol extract contains more phenolic compounds than acetone extract. Phenolics present in the ethanol and acetone extracts have received considerable attention because of their Polarity. Flavonoids as one of the most diverse and wide spread group of natural compounds are probably the most important natural phenols. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties. Using the standard plot of quercetin (y = 0.0148x, R^2 =0.975), the flavonoids contents of these extracts of *T.terrestris* are found ranging from 601.3 to 452.7 mg quercetin equivalent/g of dry sample. The flavonoids content of the ethanol extract is higher compared to that of the acetone extract.

Conclusion:

The present investigations revealed that the ethanol and acetone extracts of *T.terrestris* fruits contain significant amount of phenols and flavonoids. The objective of this study was to get information of the amount of phenolics and flavonoids in different extracts of *T.terrestis.* Further intention of this study is to correlate relationship of these secondary metabolites to possible biological activities and evaluate *T.terrestris* as a potential source of natural bioactive chemicals.

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