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COMPARISON OF ANTI-OXIDANT ACTIVITY OF *ANDROGRAPHIS PANICULATA* AND *TINOSPORA CORDIFOLIA* LEAVES

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ABSTRACT

This work mainly focus on the anti-oxidant potency of aqueous, methanolic and ethanolic extracts of two Indian medicinal plants (*Andrographis paniculata and Tinospora cordifolia*). The methanolic extracts of leaves of *Andrographis paniculata* showed promising anti-oxidant activity followed by ethanolic extracts of leaves of *Tinospora cordifolia.* Results suggest that the active anti-oxidant compounds are better extracted in methanol for *Andrographis paniculata* and in ethanol for *Tinospora cordifolia.* Results also suggest that there is a direct co-relation between the total polyphenols extracted and anti-oxidant activity. Free radical scavenging potential of various extracts (methanol, ethanol and aqueous) of *A.paniculata and T. cordifolia* were evaluated by using reducing power. In this method, ascorbic acid was used as a standard of determining reducing power. The methanol extract of the leaves of *A. paniculata* exhibited appreciable activity as compared to the aqueous and ethanol extracts, indicating that *A. paniculata* has promising free radical scavenging activity.

Key words: Medicinal plants, *Andrographis paniculata, Tinospora cordifolia,* Anti-oxidant activity, Flavonoid.

INTRODUCTION

Over the past twenty years, interest in medicinal plants has grown enormously from the use of herbal products as natural cosmetics and for self-medication by the general public to the scientific investigations of plants for their biological effects in human beings. Beyond this pharmaceutical approach to plants, there is a wide tendency to utilize herbal products to supplement the diet, mainly with the intention of improving the quality of life and preventing the diseases of elderly people[1]. India has been identified as a major resourceful area in the traditional and alternative medicines globally.

Andrographis paniculata is an herbaceous plant of family Acanthaceae, genus *Andrographis* native to India and Srilanka. It is widely cultivated in southern Asia. Mostly the leaves and roots were used for medicinal purposes such as traditional siddha and ayurvedic systems of medicine in India and some other countries for multiple clinical applications. The plant extract exhibits anti-typhoid, antifungal activity, antioxidants[2], anti-inflammatory, anti-snake venom and antipyretic properties. It is also used as an immune stimulant agent[3]. The whole plant has variety of therapeutic values and it is useful in treatment of wounds, ulcers, leprosy, sore throat, hypertension, etc. The plant contains a number of diterpenoids. However, the major bitter constituent is andrographolide, which is diterpene lactone. Diterpenoids and flavonoids are the main chemical constituents of *Andrographis paniculata* which are believed to be responsible for the most

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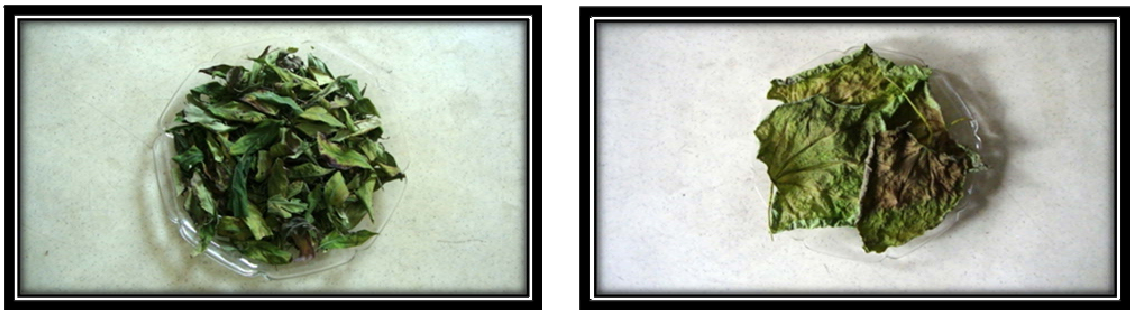
biological activities of this plant[4]. Two flavonoids, identified as 5, 7, 2', 3'- tetramethoxyflavonone and 5-hydroxy-7, 2', 3'-trimethoxyflavone, as well as several other flavonoids were obtained from the whole plant. The leaves and aerial parts of *Andrographis paniculata* are used in various formulations and used for the treatment of fever malaria and sore throat[5].

Tinospora cordifolia, which is known by the common name Guduchi, is an herbaceous vine of the family Menispermaceae indigenous to the tropical areas of India, Myanmar and Sri Lanka. The plant is a glabrous climbing shrub found throughout India, typically growing in deciduous and dry forests. The leaves are heart shaped. A variety of constituents have been isolated from *Tinospora cordifolia* plant and their structures were elucidated. They belong to different classes such as alkaloids, diterpenoids lactones, glycosides, steroids, sesquiterpenoid, phenolic, aliphatic compounds and polysaccharides. Leaves of this plant are rich in protein (11.2%) and are fairly rich in calcium and phosphorus[6]. The active adaptogenic constituents are diterpene compounds including tinosporone, tinosporic acid, cordifolisides A to E, syringen, the yellow alkaloid, berberine, giloin, crude giloininand, a glucosidal bitter principle as well as polysaccharides, including arabinogalactan polysaccharide (TSP)[7,8]. The active principles of *Tinospora cordifolia*, a traditional Indian medicinal plant were found to possess complementary and immunomodulatory activities. The role of medicinal plants in disease prevention or control has been attributed to antioxidant properties of their constituents[9]. The protective effect of plant products are due to the presence of several components such as enzymes, proteins, vitamins[10], carotenoids[11], flavonoids[12] and other phenolic compounds. It is reported to possess anti-spasmodic, anti-inflammatory, anti-allergic, anti-diabetic and anti-oxidant properties[13].

Andrographis paniculata and *Tinospora cordifolia* both have *some* common medicinal properties like anti-oxidant, anti-inflammatory, anti-malarial, hepatoprotective, immune stimulatory, anti-diabetic, etc. The objective of the present study was to compare the anti-oxidant activity of *Andrographis paniculata* leaves with *Tinospora cordifolia* in different solvents extracts using standard methods. The findings from this work may add to the overall value of the medicinal potential of *Andrographis paniculata*.

Andrographis paniculata ***Tinospora cordifolia***

Fig. 1: Plants growing in natural environment



Andrographis paniculata ***Tinospora cordifolia***

Fig. 2: Shaded dried leaves of plants

EXPERIMENTAL

- **Collection of plant material**

Fresh and healthy leaves of *Andrographis paniculata* and *Tinospora cordifolia* were collected at the same time and washed thoroughly with distilled water and dried in shade for seven days followed by grinding and then coarse powder was stored in air tight bottles.

- **Preparation of extracts**

The shade dried coarse powder of the leaves of A. *paniculata* and *T. cordifolia* (50 g each) was extracted using 250 mL of the extraction solvent using soxhlet apparatus. The solvents used for the extraction procedure were ethanol, methanol and distilled water. The extracts were concentrated to dryness to yield crude residues. The extracts were auto-claved and stored at 4°C, until further use.

- **Total phenolic content (TPC)**

Total soluble phenolic content of *A. paniculata and T. cordifolia* was estimated by Folin-Ciocalteu's reagent method[14] using gallic acid as a standard phenolic compound. Each solvent extracted solution (0.3 mL in triplicate) was mixed with 1.5 mL of 10% freshly prepared Folin-Ciocalteu's reagent and after 3 min, 1.2 mL of 7.5% (W/V) sodium carbonate was added and mixed thoroughly. The tubes were placed in boiling water for one minute, cooled and the absorbance was measured at 650 nm in a spectrophotometer against a reagent blank. The concentrations of the total phenolic compounds in the extracts were obtained by extrapolating the absorbance of gallic acid on standard gallic acid graph. The experiment was repeated thrice and concentration of total phenols was expressed as mg/g of dry extract.

- **Total flavonoid content**

The total soluble flavonoid content was estimated by aluminium chloride colorimetric method[15] for aqueous, methanolic and ethanolic extracts of *A. paniculata and T. cordifolia*. 0.5 mL of stock solution (1 g/mL) of the extract, 1.5 mL methanol and 0.1 mL potassium acetate (1M) was added to reaction test tubes and volume was made up to 5 mL with distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm and compared with quercetin, a positive control.

- **Determination of anti-oxidant activity**

Total reducing power

The reducing power of the extracts was performed as described by Yen and Duh[16]. Different concentration of methanol, ethanol and water extract of *A.paniculata and T.cordifolia* was prepared ranging from 0.1 to 0.9 mg/mL. An ascorbic acid stock solution was prepared, from which various concentrations were prepared. Various extracts (0.1-0.9 mg/mL) were mixed with phosphate buffer (500 µL, 20 mM, pH 6.6) and 1% potassium ferricyanide (500 µL), and incubated at 50°C for 20 min; 500 µL of 10% trichloro acetic acid (TCA) were added, and the mixture was centrifuged at 2500 rpm for 10 min. The supernatant was mixed with distilled water (1.5 mL), 0.1% ferric chloride (300 µL) and the absorbance were measured at 700 nm in ultra-violet visible spectrophotometer. Ascorbic acid was used as a standard solution. Phosphate buffer was used as blank solution. The experiment was repeated thrice. Increase in the absorbance of the reactions mixture indicated increase in the reducing power.

DPPH radical scavenging activity

The hydrogen donating ability of each extract in the presence of DPPH (2, 2-diphenyl-1-picrylhydrazyl) stable radical was examined according to method developed by Blois[17] and Cao et al.[18] All extracts were diluted with vehicle control to final extract concentration (0.1 to 0.9 mg/mL). 0.1 mL of test sample at different concentration (0.1-0.9 mg/mL) was mixed with 0.9 mL of Tris-HCl buffer (pH 7.4); then 1 mL of DPPH (500 μM in ethanol) was added. The mixture was shaken vigorously and left to stand for 30 min. The absorbance of ethanolic DPPH tincture from DPPH* (violet color) to DPPH (clear) was measured at 517 nm[19] in a spectrophotometer and compared with synthetic anti-oxidant butylated hydroxyl anisole (BHA). The experiment was repeated thrice. The percentage of DPPH scavenging was calculated using the following formula:

$$\% \text{ Scavenging} = [(A_{\text{control}} - (A_{\text{sample}} - A_{\text{sample blank}} / A_{\text{control}}))] \times 100 \quad \ldots(1)$$

RESULTS AND DISCUSSION

In this study, three crude extracts of leaves of *Andrographis paniculata and Tinospora cordifolia* have been investigated. Aqueous, methanolic and ethanolic extracts were tested for their total phenolic and total flavonoid contents. Anti-oxidant activities were performed by using assay of reducing power and DPPH radical scavenging activity.

Total phenolic content (TPC) and flavonoid content

Total phenolic content and flavonoid content of different extracts of both plants used in the present study is presented in Table 1. It is clear that level of polyphenols and flavonoid content in the methanolic extract of *Andrographis paniculata* leaves was higher when compared to ethanol and aqueous extracts of self and methanol, ethanol and aqueous extracts of *T. cordifolia* **(**Fig. 3 and Fig. 4**).** Aqueous extracts had the least polyphenols and flavonoid.

Table 1: Total phenolic content (TPC) and flavonoid content of *Andrographis paniculata and Tinospora cordifolia*

Solvents	Total phenols (mg/g)		Flavonoid content (mg/g)	
	A. paniculata	***T. cordifolia***	***A. paniculata***	***T. cordifolia***
Aqueous	17.52 ± 0.001	1.4 ± 0.5	0.16 ± 0.02	0.13 ± 0.02
Methanol	24.84 ± 0.002	4.3 ± 0.3	0.67 ± 0.02	0.41 ± 0.03
Ethanol	19.41 ± 0.006	5.6 ± 0.25	0.49 ± 0.02	0.54 ± 0.02

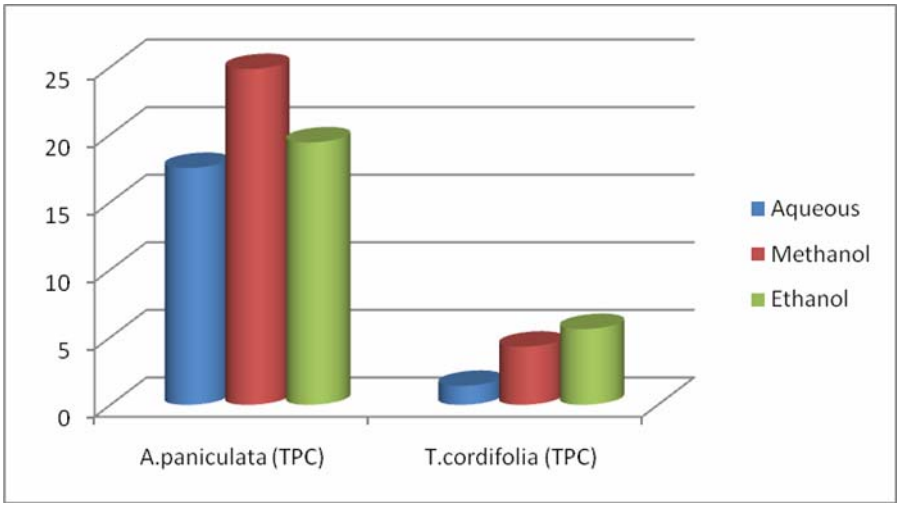


Fig. 3: Comparison of TPC of *A. paniculata and T. cordifolia.*

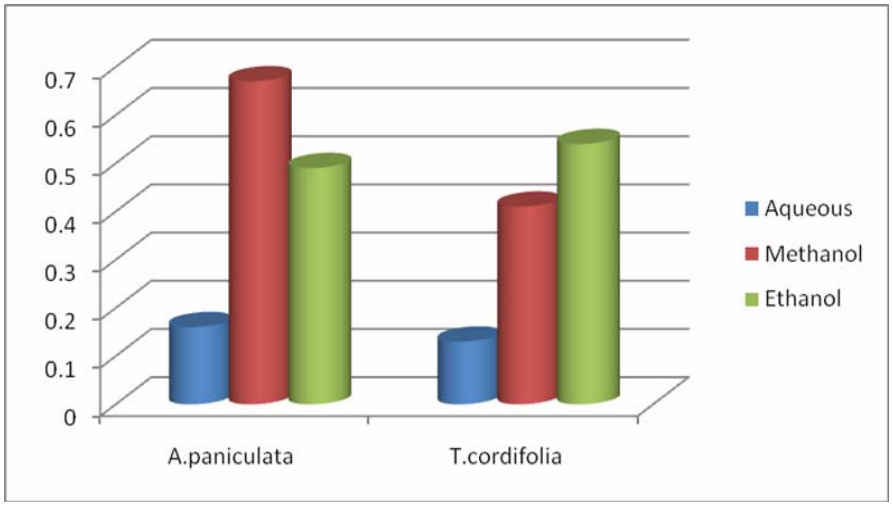


Fig. 4: Comparison of total flavonoid content of *A. paniculata and T. cordifolia*

Medicinal plants are an important source of antioxidants[20]. Polyphenols are the major plant compounds with anti-oxidant activity. Typical phenolics that possess anti-oxidant activity are known to be mainly phenolic acids and flavonoids[21]. It is reported that the phenolic are responsible for the variation in the anti-oxidant activity of the plant. Flavonoids are phenolic acids, which serve as an important source of anti-oxidants found in different medicinal plants and related phytomedicies[22]. The anti-oxidant activity of flavonoids is due to their ability to reduce free radical formation and to scavenge free radicals. The result of total phenolic and flavonoid content of leaves of *A. paniculata and T. cordifolia* clearly show that methanolic extract of *A. paniculata* had highest phenol and flavonoid content of 24.84 mg/g and 0.67 mg/g, respectively followed by ethanolic extract and lowest in aqueous extract.

Anti-oxidant activity

Total reducing power

The reducing power of different solvent extracts of *A. paniculata and T. cordifolia* using potassium ferricyanide method is shown in Fig. 5 and 6. The results indicate that the reducing ability of the extracts increased with the concentration. The reducing power of the aqueous, methanol and ethanol crude extracts of *A. paniculata and T. cordifolia* was also compared to the standard ascorbic acid. Among all the extracts

tested for their reducing abilities; methanolic extract of *A. paniculata* showed better reducing power than *T. cordifolia.*

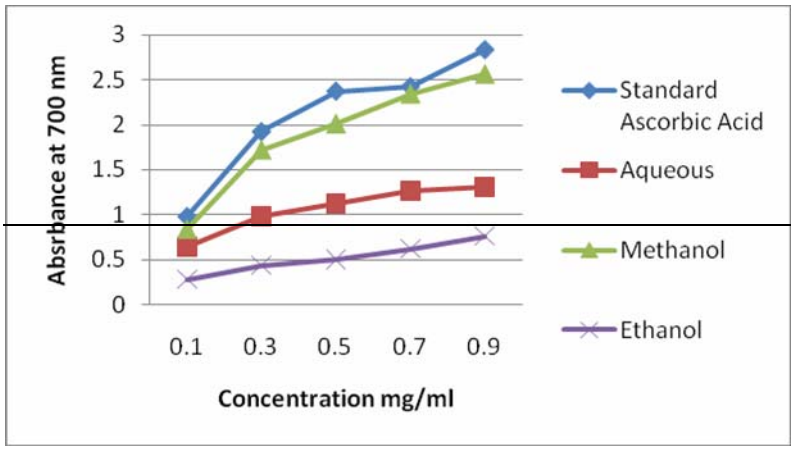


Fig. 5: Reducing power of *Andrographis paniculata* leaves extract at different concentration (mg/mL)

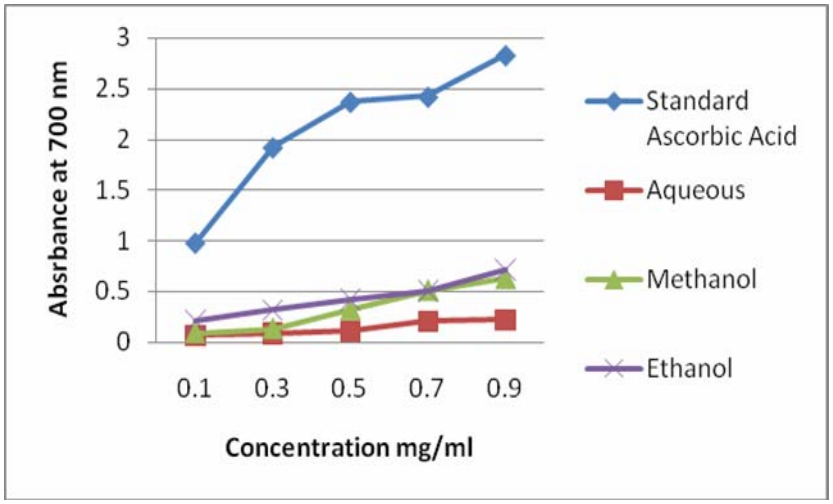


Fig. 6: Reducing power of *Tinospora cordifolia* leaves extract at different concentration (mg/mL)

Reducing power is associated with its anti-oxidant activity and may serve as a significant reflection of the anti-oxidant activity. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary anti-oxidants[23]. Reducing power of the methanolic extracts of the leaves of *A. paniculata* was found significant but less as compared to the standard selected ascorbic acid.

DPPH Radical scavenging activity

The DPPH radical scavenging activity of leaves of *A. paniculata and T. cordifolia* extracts is shown in Fig. 7 and 8. Among the extracts tested, methanol extract of *A. paniculata* had significant scavenging activity followed by ethanolic extract of *T. cordifolia.*

The stable radical DPPH has been used widely for the determination of primary anti-oxidant activity[24]. The DPPH anti-oxidant assay is based on the ability of DPPH, a stable free radical to decolorize in the presence of anti-oxidants. The methanol extracts of leaves of *A. paniculata* recorded the highest scavenging activity of 54% at 0.7 mg/mL and 52% at 0.9 mg/mL followed by ethanolic extract of leaves of

T. cordifolia consisting 51% at 0.9 mg/mL. Both have lower scavenging activity, when compared to synthetic anti-oxidant butylated hydroxyl anisole (BHA).

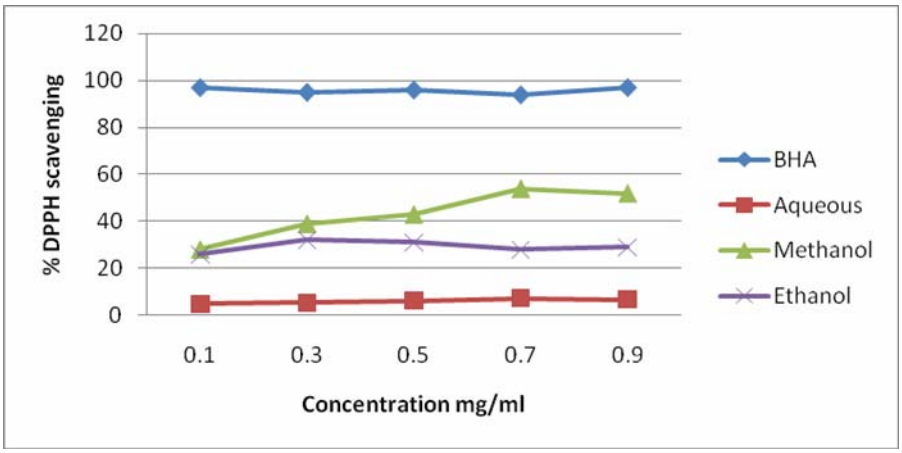


Fig 7: DPPH Radical scavenging activity of *A. paniculata.*

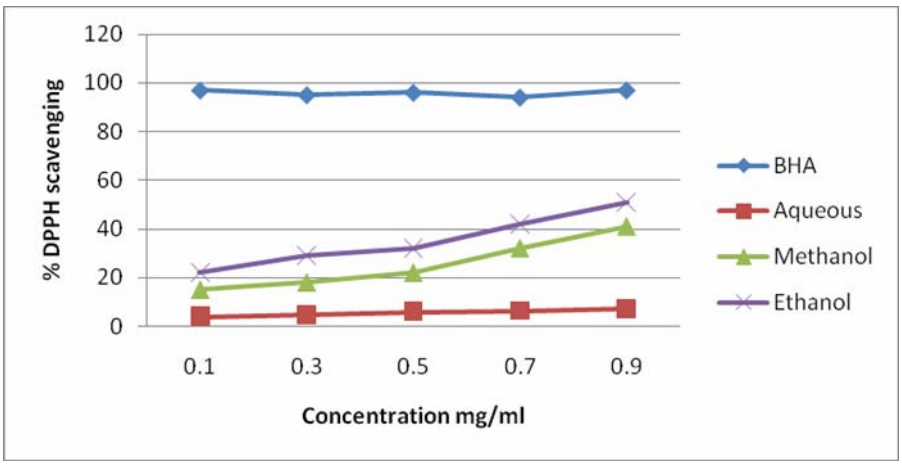


Fig. 8: DPPH Radical scavenging activity of *T. cordifolia.*

CONCLUSION

The result obtained from our study may vary from the earlier reports due to environmental differences within species, time of taking samples and determination methods. As methanolic extract of leaves of *A. paniculata* had good percentage of TPC and flavonoid content, the above findings recommend the further investigation of *A. paniculata* leaves to evaluate their chemical potential. Further studies are needed to isolate and characterize the active principles to elucidate their anti-oxidant mechanism.

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