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A Study of total phenolic content of *Tylophora indica* leaf extracts and it's correlation with *in vitro* DPPH scavenging activity

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ABSTRACT

Background: Oxidative stress is a life threatening problem of all ages, where the plant derived antioxidants are recommended in Ayurveda as a safer drug of antioxidation. In the present investigation the antioxidant activity of senescent leaf extracts of *Tylophora indica* was evaluated using DPPH free radical. **Methods:** The crude aqueous and organic solvent leaf extracts were prepared by cold maceration method. Total phenolic content (TPC) was determined and its correlation with free radical scavenging potential was evaluated using DPPH assay. **Results:** The methanolic leaf extracts showed higher phenolic content (58.8 mg GAE/g) compared to chloroform (44.9 mg GAE/g), ethanol (44.38 mg GAE/g) and aqueous extracts (19.38 mg GAE/g). Encouraging DPPH scavenging was observed in methanolic extract (IC_{50} 95.17 µg/mL) relating a strong correlation with TPC of the extract. The free radical scavenging evidenced the presence of antioxidant polyphenolics and other related metabolites in the extracts. **Conclusion:** The study confirmed a positive correlation with the phenolic content of the extracts in antioxidant activity. Our observation submits that *T.indica* leaves are the noble sources of antioxidant constituents paving a way for the future studies.

Keywords: Antioxidant, Free radicals, Polyphenols, Senescent leaves

1. INTRODUCTION

Civilization has made major changes in the routine lifestyle of ethnic populations. The unregulated practices of daily diet in the existence of communities have now been emerged with a great change in physiology and metabolism of the human body. In continuation with metabolic changes the mechanism of body fitness and healthy digestion was arrested, as in course communities were now prone to extensive diseases and disorders. Oxidative stress is discoursed as a major problem contributing to several disorders apart from infectious etiology. Experimental evidences and literature reviews states that free radicals (FR) and reactive oxygen species (ROS) can be involved in high number of lifestyle diseases such as atherosclerosis, heart attack, stroke, cancer, diabetes, senile cataracts and accelerated aging.[1-2] The World Health Organization (WHO) has estimated that about three quarters of the world's population still relies on plant derived medicines usually obtained from traditional healers, for their basic health care needs.[3-4] In developing countries like India an upsurge in phytotherapy is considered as an alternative medicine in treating diseases and disorders. Both edible and inedible plants in the nature produce antioxidants to scavenge the free radicals generated by sunbeams and oxygen.[5] The plant polyphenols could scavenge reactive chemical species minimizing oxidative damage from excessive light exposure. The hydroxyls groups of polyphenols plays an excellent role in antioxidation and are able to chelate transition metal ions, iron and copper, involved in inhibiting many enzymes participating in the formation of free radicals. [6-7]

Among many different herbs, *Tylophora indica* (Burm. F.) Merrill (Asclepiadaceae) is an important medicinal plant of India, reported to comprise variety of medicinal metabolites used in different ailments. The anticancer, antioxidant, antiasthmatics, antiallergic, hepatoprotective and immunomodulatory activities of *T.indica* extracts has been reported. [8-12] The present study was taken to evaluate the total phenolic content in the senescent leaf extracts of *T.indica* and its correlation with free radical quenching.

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2. MATERIALS AND METHODS

2.1. Collection of plant material

The plant was collected from Western Ghats, Coimbatore, Tamil Nadu, India. The herb was authenticated as *Tylophora indica* (Burm. F.) Merrill, Asclepiadaceae by Dr. G.V.S. Murthy, Scientist 'F' and Head, Botanical Survey of India, Southern Regional Centre, Coimbatore. The voucher specimen (No: BSI/SRC/5/23/2012-13/Tech.1194) was deposited in the Department of Microbiology, RVS College of Arts & Sciences, Coimbatore for future references. The plant leaves were

collected based on the information's obtained from tribal communities such as Malasars, Irulas and Konars living around the Western Ghats.

2.2. Plant extract preparation

Prior to solvent extraction, the fresh disease free powdered senescent leaf material was defatted with petroleum ether for 24 hours, filtered and allowed to dry for complete evaporation of solvent. About 200 gms of defatted plant powder was extracted with 800 mL of organic solvents *viz*. methanol, ethanol, chloroform, and distilled water by cold maceration method. After a week of socking, filtration was conducted with whatmann filter paper no.1, and concentrated *via*. rotary vacuum evaporator. The concentrated crude extracts were stored at 4°Cfor further analysis.

2.3. Qualitative and quantitative phytochemical analysis of plant extracts

The crude leaf extracts were subjected to qualitative phytochemical test.[13-15]The quantitative assays for alkaloids, tannins, saponins and flavonoids were accomplished by standard procedures.[16-18]The residue of each filtrate was calculated as percentage of the dried fraction.

2.4. Determination of total phenolic contents (TPC)

Total phenolic content was determined by the Folin-Ciocalteu method[19] calculated from a calibrated curve using gallic acid standard by recording the spectrophotometeric absorbance of solutions (10-180 µg/mL) at 765 nm. The results are expressed as mg gallic acid equivalent (mg GAE)/gram of extract.

2.5. DPPH free radical scavenging assay

Radical scavenging activity was executed by a standard procedure[20] with minor modification in the determination of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging potential of various factions. About 3 mL of test plant extract (5-150 µg/mL) was mixed with 1 mL of absorbance (<1.00 at 517 nm in spectrophotometer) adjusted DPPH solution and kept at room temperature in dark for 30 minutes for reaction to occur.

An absorbance adjusted DPPH solution and standard ascorbic acid was used as negative and positive controls respectively in the assay. Spectrophotometric absorbance of the mixture was measured at 517 nm and the percentage inhibition of DPPH radical by the sample was calculated as follows:

$$\text{DPPH Scavenging effect (\%)} = \frac{\text{OD of negative control - OD of plant sample treated}}{\text{OD of negative control}} \times 100$$

The radical scavenging ability was considered to be directly proportional to the quantity of antioxidants present in extracts. Concentrations yielding 50% inhibition (IC_{50}) was calculated by interpolation from linear regression analysis. The $1/IC_{50}$ coefficient was calculated to determine the correlation between antioxidant activity and the total phenolic content of the tested extracts.

2.6. STATISTICAL ANALYSIS

The data obtained in the present study are mean ±SEM of three replicate determinations. Statistical comparisons employed one way ANOVA, with $P<0.01$ was considered as statistically significant.

3. RESULTS AND DISCUSSION

3.1. Qualitative phytochemical screening

In the present study senescent leaf extracts of *T.indica* revealed the presence of medicinally active phytoconstituents (Table 1) such as alkaloids and flavonoids in TIMLE, TIELE and TICLE; tannins in TIALE, TIMLE and TIELE; triterpenoids in TIMLE and TIELE; steroids in TIMLE and TICLE; polyphenolics and saponins in all the four extracts.

Table 1 Phytochemical composition of *Tylophora indica* senescent leaf extracts

Tests for Alkaloids	TIALE		TIMLE		TIELE		TICLE	
Mayer's test	-	-	+	Cream color	+	Cream color	+	Cream color
Wagner's test	-	-	-	-	++	Red color	++	Reddish brown
Tannic acid test	-	-	++	Buff color	++	Buff color	-	-
Hager's test	-	-	-	-	++	Yellow color	++	Yellow color
Tests for Tannins								
Gelatin test	++	White precipitate	++	White precipitate	++	White precipitate	-	-
Tests for Phenolics								
$FeCl_2$ test	+	Pale blue	++	Bluish black	++	Bluish black	+	Sky blue
Tests for Flavonoids								
Lead acetate test	-	-	-	-	+	Yellow color	+	Yellow color
NaOH test	-	-	++	yellow color	+	Pale yellow color	++	Yellow color
Tests for glycosides								
Borntrager's test	-	-	-	-	-	-	-	-
Legal's test	-	-	-	-	-	-	-	-
Keller-Killiani test								
Carbohydrate test-								
Benedict's test	-	-	-	-	-	-	-	-
Molish's test	-	-	-	-	-	-	-	-
Test for Steroids (S) & Terpenoids (T)								
Salkowski test	-	-	++ (S)	Deep red color	+ (T)	Yellow color	+ (S)	Light red color
Libermann Buchard test	-	-	++(T)	Dark red	-	-	+ (S)	Pale green
Test for Saponins								
Froth test	*++ =Presence of persistent foam ;*		***S:Steroids; T: Triterpenoids; ++:Quantitative; +:Positive; -:Negative***					

3.1.1. Quantitative analyses of phytoconstituents

The leaf extracts were executed for quantitative assay and the results from Table 2 indicates the percentage phytoconstituents. The presence of alkaloid was higher in TIMLE (0.708±0.047%) followed by TIELE (0.55±0.002%), TICLE (0.044±0%) and TIALE (0.036±0.001%). The pattern of tannin was higher in TICLE (0.56±0%), followed by TIALE (0.25±0.006%), TIMLE (0.112±0%) and TIELE (0.041±0%). A gradual decline in the phytoconstituent tagging was observed, when the polarity of organic solvent was decreased. Saponins recorded higher in TIELE (0.66±0.049%) trailed by TIALE (0.466±0.015%), TIMLE (0.412±0.025%), and TICLE (0.3±0.013%). Appreciable flavonoid content was predicted in TIMLE (0.826±0.03%) compared to TIELE (0.616±0.004%), TICLE (0.533±0.02%) and TIALE (0.025±0%).

Table 2 Percentage phytochemical analysis of *Tylophora indica* senescent leaf extracts

Plant extract	TIALE	TIMLE	TIELE	TICLE
Alkaloids	0.036±0.001^{d}	0.708±0.047^{a}	0.55±0.002^{b}	0.044±0^{c}
Flavonoids	0.025±0^{d}	0.826±0.03^{a}	0.616±0.004^{b}	0.533±0.02^{c}
Tannins	0.25±0.006^{c}	0.112±0^{b}	0.041±0^{d}	0.56±0^{a}
Saponins	0.466±0.015^{b}	0.412±0.025^{c}	0.66±0.049^{a}	0.3±0.013^{d}

Data expressed as mean±SEM. Difference in superscript letters ranging from a to d indicate least significant level at p≤0.05

3.2. Estimation of total phenolic content

The total polyphenolic content of *T.indica* leaf extracts were determined by Folin-Ciocalteu method and the variations in mean concentration of polyphenolics were present in Table 3. Higher polyphenolics were observed in TIMLE with 58.83±0.24 mg GAE/g of extract, whereas the other extracts TICLE, TIELE, and TIALE showed 44.94±1.20, 44.38±0.26, and 19.38±1.01 mg GAE/g of extract respectively.

Table 3. Total phenolic content of *T. indica* senescent leaf extracts and its correlation in DPPH radical scavenging activity

Plant Extract	Total polyphenol content (mg GAE /gm of extract)	DPPH scavenging (IC_{50} µg/mL)	$1/IC_{50}$
TIALE	19.38±1.01^{d}	>140	-
TIMLE	58.83±0.24^{a}	95.17	0.0105
TIELE	44.38±0.26^{c}	173.16	0.0058
TICLE	44.94±1.20^{b}	125.3	0.008
Ascorbic acid	-	68	0.0147

Datas were expressed mean±SEM (n= 3). Difference superscript letters ranging from a to d indicate least significant level at p<0.01

3.2.1.Correlation of polyphenolic content with antioxidation

In the present investigation DPPH free radical scavenging ability of *T.indica* leaf extracts with IC_{50} values were determined. As cited in Table 3, solvents used in the extraction differed in tagging the phenolic compounds they yielded from the *T.indica* leaf extracts, and consequently the scavenging ability of extracts differed with the polarity of solvents used. The $1/IC_{50}$ was calculated to determine the correlation between antioxidation and total polyphenolics in *T.indica* leaf extracts. The IC_{50} of standard ascorbic acid was 68 µg/mL and a significant DPPH haunting was observed in methanol, chloroform and ethanolic leaf extracts with IC_{50} values of 95 µg/mL, 125 µg/mL and 173 µg/mL respectively. The aqueous leaf extracts in our study showed least scavenging of DPPH and no IC_{50} was predicted.

Literature reviews correlates the linear relation between TPC and antioxidant activity. As polyphenolics are the major contributors of antioxidant activity, a focus on phenolic content, its quantification and relation with antioxidation was studied. The quantitative analysis examined the correlation between antioxidation and phenolic contents in the leaf extracts of *T.indica*. The $1/IC_{50}$ values were recorded for antioxidation and its correlation coefficients was calculated (R^2 = 0.8088, y = 0.0003x-0.0047), and the correlations ($1/IC_{50}$ vs. total phenolics) are shown in Figure 1. Higher antioxidant activity corresponded to total phenolics in the tested extracts. Our results suggest that the antioxidant activity of TIMLE with 74.5%, TICLE with 67.2% and TIELE with 51.8% at 140 µg/mL is due to the influence of phenolic compounds in the extracts. We also conclude that the antioxidant activity of *T.indica* extracts are not limited to phenolic compounds but may also be related to the presence of other antioxidant secondary metabolites that are present in *T.indica* leaves.

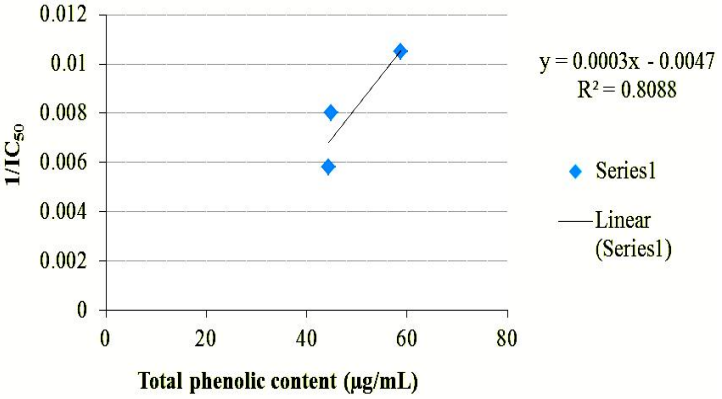

Fig.1. Correlation of $1/IC_{50}$ value with total phenolics of *Tylophora indica* senscent leaf extracts.

4. CONCLUSION

The tested leaf extracts of *T.indica* were similar in its total phenolic content and antioxidant activity. The quality of the extracts depends on the solvent used in extraction procedure. Methanol was found to be the best solvent in the preparation of herbal extracts influencing strongest antioxidant activity to the extract. Our study confirms a strong correlation between antioxidant activity and phenolic content in the subjected extracts. Hence, we conclude that the *T.indica* leaves are good sources of antioxidants and their antioxidant properties are encouraging comparable to the reports in other research studies.[21-25]

CONFLICTS OF INTEREST

We declare that, we all authors have no conflict of interest.

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5. REFERENCES

1. Harman D. Ageing: a theory based on free radical and radiation chemistry, *J Gerontol*. 1956;11: 298-300.
2. Halliwell B. Antioxidants and human disease: a general introduction. *Nutrition Rev*. 1997; 55: 44–52.
3. Kuruvilla A. Herbal formulations as pharmacotherapeutic agents. *Indian J Exp Biol*. 2002; 40:7-11.
4. Kulkarnia M, Wakadea A, Ambayea R Juvekara A. Phytochemical and pharmacological studies on the leaves of *Couroupita guiaesis* aubl. *Pharmacologyonline*. 2011; 3: 809-814.
5. Pods-edek A. Natural antioxidants and antioxidant capacity of brassica vegetables: A review. *LWT- Food Sci and Technol*. 2007; 40: 1-11.
6. Villańo D, Fernández-Pachón MS, Moyá ML, Troncoso AM, García-Parrilla MC. Radical scavenging ability of polyphenolic compounds towards DPPH free radical *Talanta*. 2007;71: 230–235.
7. Li H, Wong C, Cheng K, Chen F. Antioxidant properties *in vitro* and total phenolic contents in methanol extracts from medicinal plants. *LWT – Food Sci and Technology*. 2008; 41: 385–390.
8. Mujeeb M, Aeri V, Bagri P, Khan SA. Hepatoprotective activity of the methanolic extract of *Tylophora indica* (Burm. f.) Merill. Leaves. *Int J Green Pharm*. 2009; 3(2): 125-127.
9. Ganguly T, Badheka P, Sainis KB, Immunomodulatoy effect of *Tylophora indica* on Con A induced lymph proliferation. *Phytomedicine*, *Proquest Health and Medical complete*. 2001; 8(6): 431-437.
10. Gupta M, Mukhtar HM, Ahmad S. *In-vitro* evaluation of antioxidant activity and total phenolic content of *Tylophora indica* (burm f.) merill. *International Journal of Pharmaceutical Sciences and Research*. 2011; 2(1): 121-126.
11. Malathi R, Ahamed John S, Cholarajan A. Antioxidant activity of extract from the leaves of *Tylophora asthmatica*. *Journal of Microbiology and Antimicrobials.* 2012; 4(4):70-73.
12. Bhatia A, Anand M, Singla R, Sharma A. *In-vitro* evaluation of antioxidant activity and total phenolic content of *Tylophora indica* (Burm f.) Merill. *J Nat Prod Plant Resour*. 2013; 3 (1):1-7
13. Trease GE, Evans WC. A Textbook of Pharmacognosy, 15th ed, Balliere Tindall, London, 2002; 176-80.
14. Harborne JB. Phytochemical methods. 2nd ed, Chapman and Hall publications, London, NewYork 1984; 288.
15. Ramakrishnan S, Rajan R. In: Text book of Medical Biochemistry 2nd ed. Orient Longman, New Delhi, India 1994.
16. Van Buren JP, Robinson WB. Formation of complexes between protein and tannic acid. *J Agric Food Chem*. 1981; 1: 77.
17. Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. *Global J Pure Appl Sci*. 2001; 8: 203-208.
18. Boham AB, Kocipai AC. Flavonoid and condensed tannins from Leaves of *Hawaiian vaccininum vaticulum* and *vicalycinium*. *Pacific Sci*. 1994; 48:458-463.
19. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *Am J Enol Viticul*. 1965; 16: 144-158.
20. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J Sci Technol*. 2004; 26: 211–219.
21. Chitnis MP, Khandalekar DD, Adwamkar MK, Sahasrabudhe MB. Anti-tumor activity of the extracts of stem and leaf of *Tylophora indica. Indian J Med Res*. 1972; 60:359-62.
22. Dhananjayan R, Gopalakrishnan C, Kameswaran L. Studies on the pharmacological effects of extracts and total alkaloids of *Tylophora indica*. *Ind J Pharmacol*. 1975; 7:13-20.
23. Shah NC, Kapoor LD. Ethnobotany of *Tylophora indica* (Burm.f.) Merr. *Quarterly J Crude Drug Res*. 1976; 14:27-34.
24. Jagetia GC, Baliga MS. Evaluation of nitrogen oxide scavenging activity in medicinal plants. *J Med Food*. 2004; 7: 343-8.
25. Asdaq SB, Sowmya SK. *Iranian Journal of Pharmacology and Therapeutics*. 2010; 9: 15.

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