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Antioxidant Activities of Methanolic Extract of Leaves of *Givotia rottlariformis* Linn.

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Abstract: The current study was aimed at evaluation of Antioxidant potential of the methanolic extract of *Givotia rottlariformis* leaves and to elucidate its phytochemical profile using TLC method. The methanol extract of leaves of *G. rottlariformis* was screened for its antioxidant potential by dot-plot assay, DPPH radical scavenging assay and hydroxyl radical scavenging assay. Since the results were quite encouraging, it stimulated the investigators to precede further to screen the phytochemical profiles by qualitative and quantitative methods which were further extended to thin layer chromatography. The result indicates that the methanol extract of leaves of *G. Rottlariform*is showed significant radical scavenging activity, potent toxicity to liver cancer cells. Since the results are promising, *G. rottlariformis* could be considered as a significant source of Antioxidant agent.
Key Words: *Antioxidant, DPPH, Nilgiri plant, TLC.*

Introduction

Latest trends have shown that there is an increasing demand for phyto-drugs and some medicinal herbs have proven potential to cure dreadful diseases like cancer, paralysis, bronchial asthma etc. Medicinal herbs and their extracts are widely used in the treatment of liver disorders like hepatitis, cirrhosis and loss of appetite[1]. The medicinal plants contains wide array of chemical compounds called as secondary metabolites which are responsible for curing various diseases and hence called as therapeutics agents[2]. Since the synthetic drugs possess a number of side effects currently, people developed confidence on herbal remedies to prevent or cure cancer. The side effects of synthetic drugs are remarkable because liver is the organ known to filter toxins and drug particle from blood. Hence it has become a great challenge for the synthetic drug manufacturers to find out an alternative and safe treatment for liver diseases, which resulted in exploring the herbal sources[3]. The Nilgiri district popularly known as "The Blue Mountains" is a vital place for medical, ethno-botanical as well as anthropological studies. It is located in the Western Ghats and the tribes of this district are Kotas, Kurumbas, Irulas, Paniyas, and Kattunayaks[4]. Regarding the potent tribal drugs, they remain unexplored due to lack of scientific knowledge. *G. rottlariformis* has been reported to be effective in curing indigestion, sunburn, skin diseases and external tumors. Hence the present study is aimed to explore the efficacy of *G. rottlariformis* as anti-oxidant compounds.

Materials and Methods

Materials

The plant leaves of *G. rottlariformis* was collected from Kotagiri zone of Nilgiri district and identified by S. Aroumougame, CAS in Botany University of Madras. Leaves of *G.rottlariformis* were washed with tap water, rinsed with distilled water and shade dried. The dried leaves were ground to obtain coarse powder and subjected to solvent extraction using methanol.[6]

Method

Qualitative Phytochemical Analysis

The phytochemical profile of methanol extract of leaves of *G. rottlariformis* (MELG) was studied by following standard procedures**.** [6]

Quantitative Phytochemical Estimations

Total Phenolic Content

The total phenol content was determined by Folin-Ciocalteu reagent method with slight modifications. 1 ml of MELG was mixed with 1 ml of Folin-Ciocalteu's phenol reagent. After 3 min, 1 ml of Na2CO3 (7%) was added to the mixture and it was made up to 10 ml by adding deionized water. The mixture was kept for 30 min at room temperature in dark. The absorbance was measured at 725 nm against the blank. The total phenolic content was expressed as mg of Gallic acid equivalents (GAE) per gram of dry extract.[8]

Total Flavonoids Content

The total flavonoid was determined by aluminium chloride reagent method.

1 ml of MELG was mixed with 100 µl of 5% NaNO2 solution and after 6 min, 500 µl of 10% AlCl3 and H2O were added. After 5 min, 0.5 ml of 1M NaOH was added. The absorbance was measured immediately against the prepared blank at 510nm.Quercetin was used as a standard and the results were expressed as mg of Quercetin equivalents (QE) per g of dry extract.[8]

Antioxidant Activity

The antioxidant activity of the MELG was assessed by dot-plot assay as well as radical scavenging assays.

Dot-Plot Rapid Assay

Aliquots of MELG (3µl) of various concentrations were spotted carefully on TLC plates and dried for 3 minutes. The sheet bearing the dry spots was placed upside down for 10 sec in a 0.4 mM DPPH methanol solution and the layer was dried. The stained silica gel plate containing active compounds was observed for any significant color change from purple to yellow.[7]

DPPH Scavenging Effects of Extracts

The DPPH Radical Scavenging was determined according to the method of Hossain *et al*, (2012).Various concentrations of MELG (1ml) were mixed with 0.1 mM of DPPH solution in methanol (1 ml). The mixture was shaken vigorously and left to stand for 30 min, at room temperature in dark and the absorbance at 517 nm. The percentage inhibition was calculated by the following formula.[8-10]

% RSA = {control – sample / control} X 100

Hydroxyl Radical Scavenging Assay

The hydroxyl radical scavenging activity the MELG was determined according to the method of Chang W. Choi*et al*, (2002). Various concentrations of the MELG were taken in different test tubes and evaporated to dryness. One milliliter of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml of EDTA (0.018%), and 1 ml of DMSO (0.85% v/v in 0.1 M phosphate buffer, pH 7.4) were added to these tubes,

and the reaction was initiated by adding 0.5 ml of 0.22% ascorbic acid. Test tubes were capped tightly and heated on a water bath at 80-90 °C for 15 min. The reaction was terminated by the addition of 1 ml of ice-cold TCA (17.5% w/v). Three milliliters of Nash reagent (75.0 g of ammonium acetate, 3 ml of glacial acetic acid, and 2 ml of acetyl acetone were mixed and raised to 1 L with distilled water) was added to all of the tubes and left at room temperature for 15 min for color development. The intensity of the yellow color formed was measured spectrophotometrically at 412 nm against reagent blank. The percentage hydroxyl radical scavenging was calculated by the following formula [8-9]

% HRSA = {control – sample / control} X 100

Thin Layer Chromatography

TLC plate of Slica gel 60 F254 (5x1.5cm) was used to carried out thin layer chromatography method. MELG was spotted on the TLC plate 0.5 cm above from the bottom. The solvents chloroform and methanol were used as the mobile phase. Slica gel supported aluminum sheet was used as the stationary phase. The spotted silica gel plate was placed upside down in the TLC chamber containing mobile phase. The mobile phase to move through the adsorbent of silica gel containing plant extract of the plate. The TLC plate was removed from the TLC chamber. After reaching the mobile phase up to 80%of the TLC plate. The eluted spots were observed under UV (250 nm) as well as in iodine. [11]

Rf = {Distance travelled by the solute/Distance travelled by the solvent}

Results

The qualitative phytochemical analysis of the MELG showed the presence of phenols, flavonoids, alkaloids, terpenoids, saponins and glycosides (Table- 1). The quantitative phytochemical analysis of MELG showed the total phenolic content was 382.23 mg of GAE equivalents per gram and the total flavonoids content was 77.73mg of QE equivalents per gram (Table- 2). The result of dot- plot assay showed (Figure- 1) that the MELG changed the purple background of DPPH to yellow, it indicates that the leaves of *G. rottlariformis* has antioxidant active compounds. The DPPH radical scavenging activity of MELG showed (Table- 3 & Figure- 2) maximum activity of 74.77% at 60 µg/ml concentration. The degree of discoloration indicates that the MELG showed free radical scavenging potentials due to the hydrogen donating ability. The $OH^{\cdot}$ radical scavenging activity of MELG showed (Table- 4 &Fig 3) maximum activity of 54.61% at 60 µg/ml concentration. The IC50 value of MELG for DPPH radical scavenging activity was 36.39µg/ml and $OH^{\cdot}$ radical scavenging activity was 50.62µg/ml as shown in Table 5. The cytotoxic activity of MELG on liver cancer cell line (HepG2) showed (Table- 6 & Figure- 4) maximum cell death of 69.37 % and the cell viability was 30.63% at 500 µg/ml. The IC50 of MELG was recorded as 300.84µg/ml against HepG2 (Table- 7). The Rf value of the eluted spots for the MELG were 0.73, 0.80 and 0.88 as exposed under UV at 254 nm, 0.47 and 0.60 as stained in iodine in the solvent ratio of 1:9 of methanol: chloroform showed in (Table- 8).

Table 1- Qualitative analysis of methanol extract of leaves of *G. rottlariformis*

S.No	Phytochemical Constituents	Test	Result
1.	Phenol	Ferric Chloride test	+
2.	Flavonoids	a) Mg filaments test b) NaoH test	+ +
3.	Alkaloids	Mayer's test	+
4.	Terpenoids	Salkowski test	+
5.	Saponins	Foam test	+
6.	Glycosides	Born trager's test	+

Table 2: Quantitative analysis of methanol extract of leaves of G. rottlariformis

S. No	Phenol GAE/g	Flavonoids QE/g
1	382.23	77.73

Figure 1: ot Plot analysis of methanol extract of leaves of *G. rottlariformis*

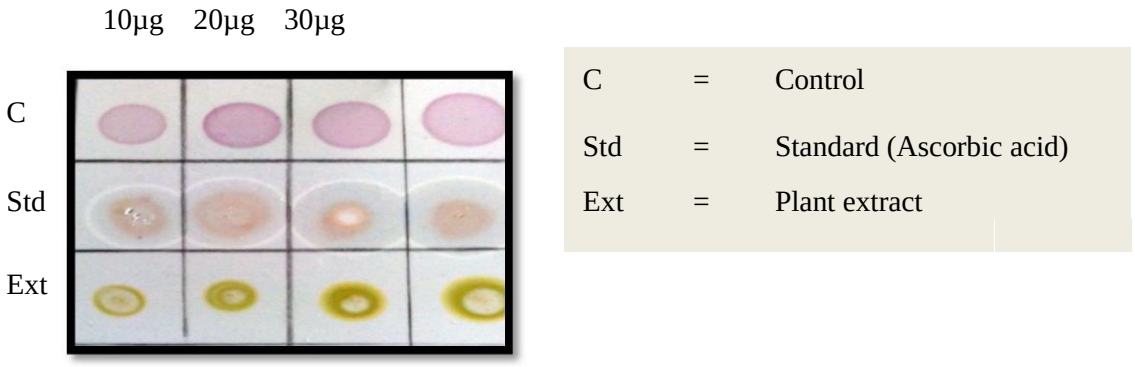

Table 3-DPPH radical scavenging assay of methanol extract of leaves of *G. rottlariformis*

S.No	Concentration (µg/ml)	% RSA
1.	10	19.72
2.	20	26.41
3.	30	34.25
4.	40	54.95
5.	50	67.52
6.	60	74.77

Table 4-Hydroxyl radical scavenging assay of methanol extract of leaves of *G. rottlariformis*

S. No	Concentration (µg/ml)	% HRSA
1.	10	16.09
2.	20	29.88
3.	30	33.33
4.	40	45.08
5.	50	49.38
6.	60	54.61

Figure 2: DPPH radical scavenging assay of methanol extract of leaves of *G. rottlariformis*

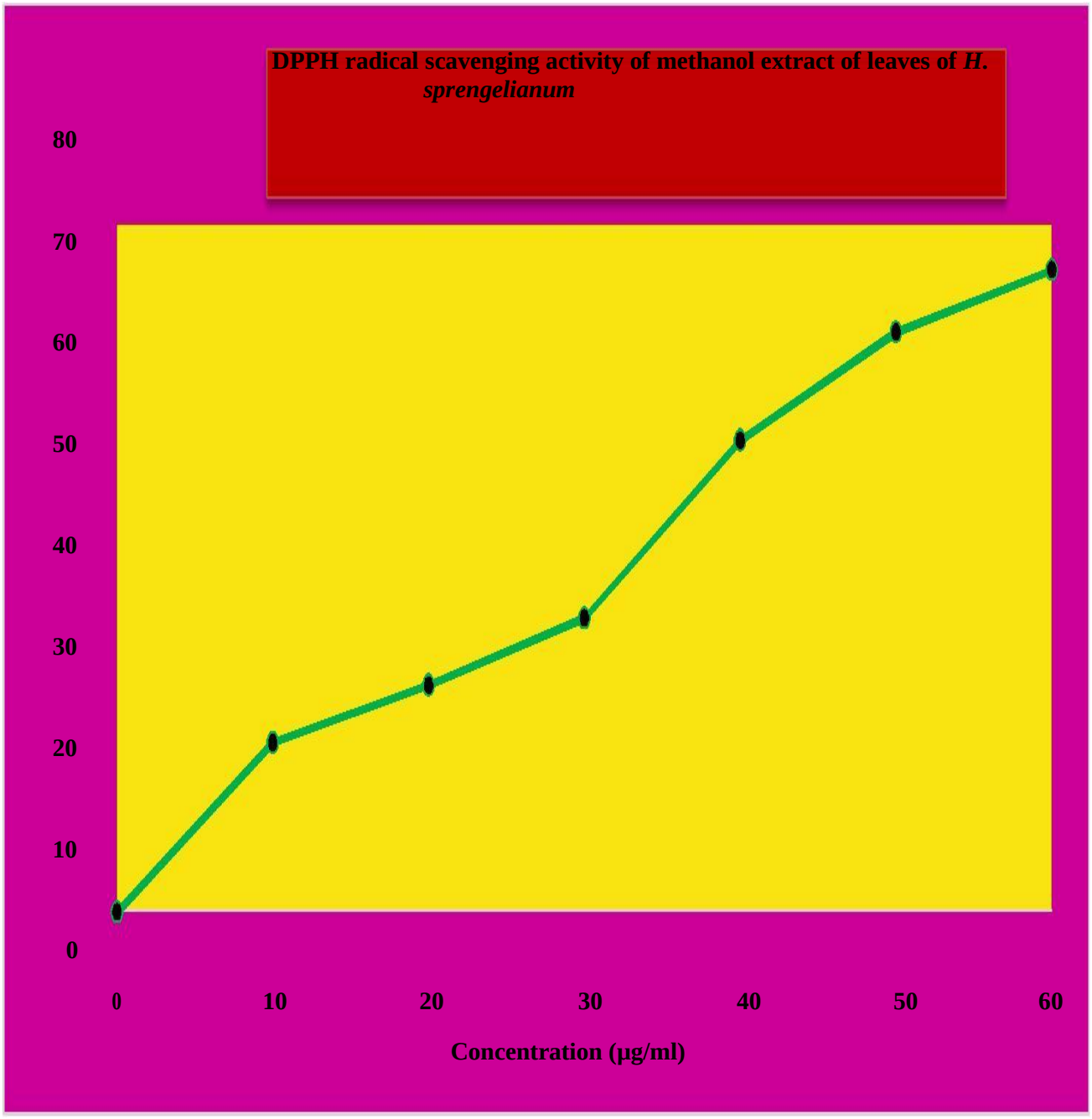

Table 5: IC50of methanol extract of leaves of *G. rottlariformis* on radical scavenging assay

S.No	Assay	IC50 (µg/ml)	
		G. rottlariformis	**Standard value**
1.	DPPH	36.39	4.58 (quercetin)
2.	OH·	50.62	5.14 (ascorbic acid)

Figure 3: Hydroxyl radical scavenging assay of methanol extract of leaves of *G. rottlariformis*

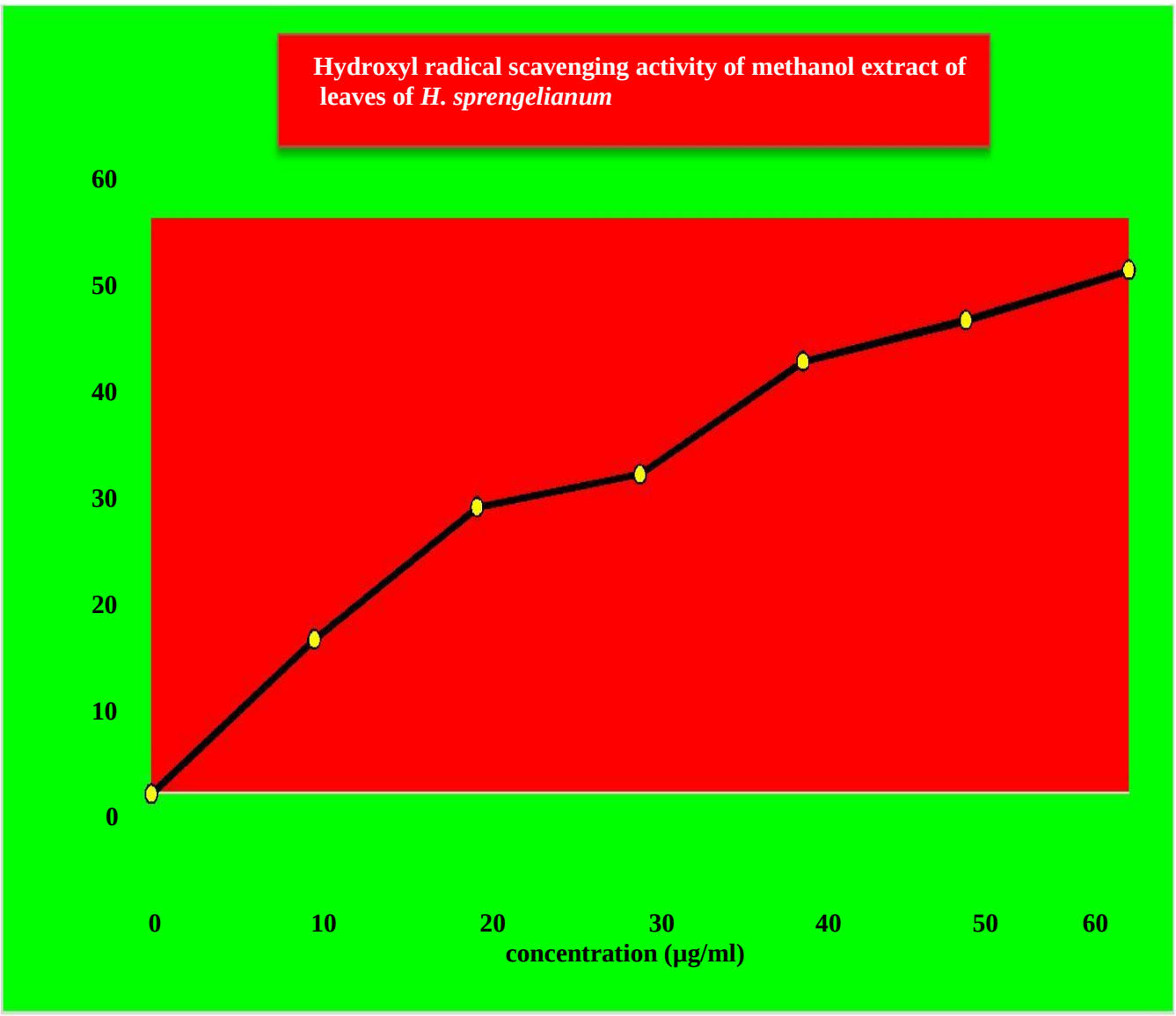

Table 6: Rf (Retention factor) value of methanol extract of leaves of *G. rottlariformis* by TLC method

S. No	Methanol : Chloroform (1:9)	
	UV	iodine
1. 2. 3.	0.73 0.80 0.88	0.47 0.60 -

Discussion

The methanol extract of leaves of *G. rottlariformis* have shown significant antioxidant property. Based on the above mentioned phytochemical results evident that the presence of phenols, flavonoids, alkaloids, terpenoids, saponins and glycosides.

Conclusion

Antioxidant studies of *G. rottlariformis* indicate its remarkable role in free radical scavenging activity also the possibilities of possessing anti-cancerous therapeutic property. The present study has provided a platform for further researches to probe scientifically on plant *Givotia rottlariformis* bring out potent drug for cancer.

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