

Analysis of antioxidant activity and total phenolic content of some homoeopathic mother tinctures

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Abstract

Background: Antioxidant compounds are widely used in health protection. Homoeopathic mother tinctures are commonly prescribed for prevention and cure of many illnesses. **Objective:** The present study focused to determine the antioxidant potential of six commonly prescribed mother tinctures. i.e. *Syzygium jambolanum*, *Damiana*, *Cinchona officinalis*, *Chelidonium majus*, *Convallaria majalis*, *Coca*. **Materials and Methods:** Antioxidant activity was estimated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) inhibition assay. A volume of 0.1 mM solution of DPPH was used while mother tinctures (5, 2.5, and 1.25 µl volumes) were used for estimating antioxidant activity. Quercetin was taken as a standard control in antioxidant activity. Total phenolic content was measured by Folin–Ciocalteu reagent assay. Total phenolic content of mother tinctures was measured in comparison to gallic acid. **Results:** Results of the study showed the significant antioxidant activity and high total phenolic content of all tested mother tinctures in the following order: *Syzygium jambolanum*, *Damiana*, *Cinchona officinalis*, *Chelidonium majus*, *Convallaria majalis*, *Coca*. **Conclusion:** Antioxidant potential of mother tinctures is related to total phenolic content present in them, and their role in prevention and cure of diseases may link through their antioxidant activity. Among six selected mother tinctures, *S. jambolanum* has highest antioxidant potential.

Keywords: *Syzygium jambolanum*, *Damiana*, *Cinchona officinalis*, *Chelidonium majus*, *Coca*, *Convallaria majalis*, *Turnera diffusa*

INTRODUCTION

Antioxidant compounds are an important factor in health protection. Moreover, they reduce the risk of chronic diseases such as heart diseases and cancer. Polyphenolic compounds have strong antioxidant activity, whereas monophenols are weak antioxidants. Antioxidants have the ability to trap free radicals. In the biological systems, highly reactive free radicals are present from many sources. These free radicals have the ability to cause degenerative diseases by oxidizing proteins, lipids, nucleic acids, and DNA. Polyphenols, flavonoids, and phenolic acids have the power of scavenging these free radicals and thus helpful in preventing degenerative disorders, aging, etc.^[1]

Homoeopathic mother tinctures are prescribed commonly in Homoeopathy^[2] as many mother tinctures can be used as antiseptics.^[3] Mother tincture or lower potencies of *Convallaria majalis* are used in heart failure. It is used as a heart tonic and increases the contractile power of heart.^[4] *Syzygium*

jambolanum mother tincture is useful against diabetes mellitus. It helps in managing the high blood sugar.^[4] Some studies also showed ameliorating effects of *S. jambolanum* Q in controlling streptozotocin-induced diabetes in rats.^[5,6] *Coca* is described in Homoeopathy as a useful remedy for aging and imbecility.^[6,7] *Chelidonium majus* is a prominent liver remedy in Homoeopathy and is useful for various diseases of this organ.^[4] *Cinchona officinalis* is beneficial for debility from exhaustive discharges of any kind.^[8] Tincture of *Damiana* is clinically indicated in sexual debility that is originated from nervous prostration and impotency.^[4]

The aim of the study is to estimate antioxidant activity and total phenolic content of six commonly used mother tinctures

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that is *Syzygium jambolanum*, *Damiana*, *Cinchona officinalis*, *Chelidonium majus*, *Convallaria majalis*, *Coca*.

MATERIALS AND METHODS

Chemicals and medicines procurement

All the homeopathic mother tinctures were purchased from Dr. Willmar Schwabe GmbH and Co., KG, Germany, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, Folin–Ciocalteu reagent (FCR), gallic acid, and quercetin were purchased from Sigma-Aldrich, Germany.

Apparatus used

BioTek® USA ELISA microplate reader, 96-well plates (Pyrex, Japan), and micropipette (HTL, Japan).

2,2-Diphenyl-1-picrylhydrazyl free radical scavenging assay

The scavenging activity of homeopathic mother tinctures was checked with DPPH radical. A volume of 0.1 mM solution of DPPH was prepared by adding 25 mg of DPPH in 100 ml of methanol. A microassay of antioxidant determination was used with some modifications.^[9] A volume of 90 µl of DPPH solution and different serial dilutions (5, 2.5, and 1.25 µl) of mother tincture were placed in wells of a 96-well microplate. The wells containing DPPH and 10 µl of ethanol were used as negative control. Quercetin was taken as standard control. The whole process was done in triplicate. The reaction mixture was placed for 30 min at 37°C. The absorbance was measured at 517 nm using Biotech ELISA microplate reader. The absorbance was taken as follows:

$$\text{Scavenging activity \%} = \left(\frac{[\text{Ac} - \text{Am.t}]}{\text{Ac}} \right) \times 100$$

Where,

Ac = Absorbance of negative control

Am.t = Absorbance of mother tincture

Total phenolic content estimation

Total phenolic content was estimated by FCR (Sigma-Aldrich, Germany) using the methodology of McDonald.^[10] A volume of 5 µl of mother tinctures and 50 µl of 1 mM sodium carbonate were added and then Folin–Ciocalteu 20% was added to make the volume up to 150 µl in 96-well plate, and after incubating at 40°C, the absorbance was checked at 765 nm.

Statistical analysis

The results were analyzed by Statistical Package of Social Sciences software (IBMSPSSV, 20) Armonk, NY: IBM Corp. One-way analysis of variance followed by *post hoc* test was applied for checking statistical significance of results. Pearson correlation coefficient test was used for checking any correlation between antioxidant activity by DPPH assay and total phenolic content of mother tinctures. Significance level was set at $P \leq 0.05$.

RESULTS AND DISCUSSION

In the present study, all the six mother tinctures were able to decolorize DPPH, and the free radical scavenging potentials

of the mother tinctures were found to be in the order of *S. jambolanum* < *Damiana* < *C. officinalis* < *C. majus* < *C. majalis* < *Coca* [Table 1]. *S. jambolanum* inhibits DPPH free radical, and the higher volume (5 µl) showed 87.2% inhibition and the lowest used volume (1.25 µl) showed 39.9% inhibition of DPPH. Similarly, all other mother tinctures showed volume-dependent inhibition of DPPH. *Damiana*, *C. officinalis*, *C. majus*, *C. majalis*, and *Coca* inhibited DPPH 81.6, 69, 68.5, 65.1, and 61, respectively, at 5 µl volume, and this percent inhibition was decreased with the decrease of volume and mentioned in detail in Table 1.

Different assays can be used to assess antioxidant activity. However, the use of free radical DPPH is the preferred method to measure antioxidant activity as it is a simple and rapid method. Moreover, it is not specific for particular antioxidant component, thus measure the total antioxidant capacity of sample.^[11] Hence, antioxidant activity of these mother tinctures is the estimate of their total antioxidant capacity.

DPPH free radical contains an odd electron and is purple in color. The color changes from purple to yellow when the odd electron of DPPH radical combined with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H.^[11]

The total phenolic contents of the mother tinctures are determined by Folin–Ciocalteu method are reported as gallic acid equivalents [Table 2].^[11] Among the six mother tinctures, *S. jambolanum* was containing the highest (94.5%±3.3%) amount of phenolic compounds, followed by *Damiana* (77.3%±3.3%), *C. officinalis* (75.5%±4.0%), *C. majus* (45.0%±5.0%), *C. majalis* (39.8%±2.4%), and *Coca* (37.4%±2.9%).

The content of total phenolic was carried out based on the absorbance values of the various mother tinctures, reacted with Folin–Ciocalteu reagent, and compared with the standard solutions of gallic equivalents. The way of measuring total phenolic content is based on chemical reducing capacity of a tested sample relative to gallic acid. It is not the absolute measurement of total phenols in a sample.^[12]

There is a positive relationship between antioxidant capacity and total phenolic content of the various mother tinctures. In this study, the results showed that mother tinctures contain a significant amount of phenolics, and it is the amount of phenolics present in this extract being responsible for its marked antioxidant activity. Pearson correlation coefficient showed a significant relationship between total phenolic content and DPPH inhibitory assay ($P = 0.004$) as described in Table 3 and Figure 1. Several reports have shown the close relationship between total phenolic content and antioxidant activity of the fruits, plants, and vegetables.^[13] Many phenolic derivatives' compounds are the vital antioxidants which exhibit scavenging efficacy on the free radicals and reactive oxygen species and commonly distributed in the plant kingdom.^[14] Hence, antioxidant activity of these mother tinctures may be due to their high phenolic content. Antioxidants are used to prevent aging, diabetes, heart diseases, cancer, and many other illnesses; strong potential of tested mother tinctures as

Table 1: Results of mother tinctures, quercetin, and alcohol against 2,2-diphenyl-1-picrylhydrazyl

Tested material	Percentage inhibition of DPPH, mean±SEM		
	5 µl	2.5 µl	1.25 µl
Quercetin	99.57±0.3 ^f	91.48±0.6 ^f	88.54±1.2 ^f
Alcohol	Nil	Nil	Nil
<i>Convallaria majalis</i>	65.1±0.57 ^d	50.9±0.64 ^c	38.94±1.18 ^a
<i>Syzygium jambolanum</i>	87.2±1.1 ^e	64.3±0.92 ^d	39.9±0.58 ^a
<i>Erythroxylon coca</i>	61±1.3 ^d	40.7±0.64 ^b	33.49±1.67 ^a
<i>Chelidonium majus</i>	68.5±0.6 ^d	52.2±1.22 ^c	38.44±1.18 ^a
<i>Cinchona officinalis</i>	69.9±0.6 ^d	56.8±0.68 ^c	45.8±1.1 ^b
<i>Damiana</i> (<i>Turnera diffusa</i>)	81.6±1.16 ^e	69.3±0.66 ^d	48.1±0.58 ^b

Results are expressed in terms of mean±SEM of DPPH percentage inhibition in experimental groups (n=3). Each value in the table is represented as mean±SEM (n=3). ANOVA followed by *post hoc* test was applied for group comparison. Values in the same column followed by a different letter (a-f) are significantly different (P<0.05) from each other. SEM: Standard error of mean; ANOVA: Analysis of variance; DPPH: 2,2-diphenyl-1-picrylhydrazyl

Table 2: Total phenolic content of mother tinctures and alcohol

Homoeopathic medicines (5 µl)	
Alcohol	Nil
<i>Convallaria majalis</i>	39.8±2.4 ^a
<i>Syzygium jambolanum</i>	94.5±3.3 ^d
<i>Coca</i>	37.4±2.9 ^a
<i>Chelidonium majus</i>	45.0±5.0 ^b
<i>Cinchona officinalis</i>	75.5±4.0 ^c
<i>Damiana</i>	77.3±3.3 ^c

Results are expressed as mean±SEM of percentage of TPC in comparison to gallic acid (n=3). Each value in the table is represented as mean±SEM (n=3). ANOVA followed by *post hoc* test was applied for group comparison. Values in the same column followed by a different letter (a-d) are significantly different (P<0.05) from each other. SEM: Standard error of mean; ANOVA: Analysis of variance; TPC: Total phenolic content

Table 3: Correlation between phenolic content and 2,2-diphenyl-1-picrylhydrazyl inhibition by mother tinctures

	DPPH inhibition by mother tinctures	TPC of mother tinctures
DPPH inhibition by mother tinctures		
Pearson correlation	1	0.916**
Significant (two-tailed)		0.004
n	7	7
TPC of mother tinctures		
Pearson correlation	0.916**	1
Significant (two-tailed)	0.004	
n	7	7

**Correlation is significant at the 0.01 level (two-tailed).

DPPH: 2,2-diphenyl-1-picrylhydrazyl; TPC: Total phenolic content

antioxidants in the present study suggests that effect of mother tinctures in various diseases' treatment may be due to their antioxidant activity.

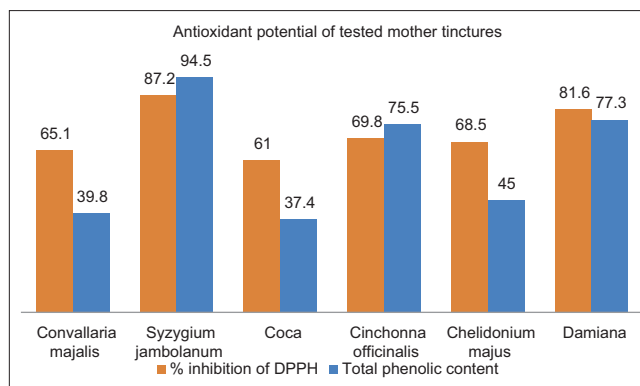


Figure 1: Representation of calculated values of total phenolic content and percentage inhibition of 2,2-diphenyl-1-picrylhydrazyl by mother tinctures (5 µl). Pearson correlation was used to estimate the relation between total phenolic content and 2,2-diphenyl-1-picrylhydrazyl inhibition (Sig [2-tailed] 0.004). Correlation was significant at the 0.01 level (2-tailed)

CONCLUSION

The results of the study showed antioxidant potential of all tested mother tinctures. These mother tinctures are being used in prevention and treatment of different diseases, and their use in clinical practice may have link through their antioxidant activity. The study emboldens the clinical use of these mother tinctures with more guarantee of their efficacy as all the selected mother tinctures showed antioxidant potential. Among six selected mother tinctures, *S. jambolanum* has highest antioxidant potential. The antioxidant activity is correspondingly related to concentration of total phenolic content, and it is perhaps the presence of phenolic content in mother tinctures that may be responsible for their antioxidant activity.

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Conflict of interest

None declared.

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Analyse der antioxidativen Wirkung und die Gesamtphenolkonzentration einiger homöopathischer Urtinkturen

Auszug

Hintergrund: Verbindungen von Antioxidantien sind in der Gesundheitsvorsorge weit verbreitet. Homöopathische Urtinkturen werden häufig zur Prävention und Heilung vieler Krankheiten verwendet.

Ziel: Die vorliegende Studie ist darauf ausgerichtet, das antioxidative Potential von sechs häufig verordneten Urtinkturen zu bestimmen. (*Syzygium jambolanum*, *Damiana*, *Cinchona officinalis*, *Chelidonium majus*, *Convallaria majalis*, *Coca*)

Materialien und Methoden: Die antioxidative Wirkung wurde mittels DPPH-Inhibitions-Prüfverfahren geschätzt. Eine 0,1 mM DPPH-Lösung und drei verschiedene Konzentrationen von Urtinkturen (5; 2,5; 1,25 µl) wurden zur Schätzung der antioxidativen Wirkung benutzt. Quercetin diente als Standardkontrolle. Der gesamte Phenolgehalt wurde mittels Folin Ciocalteu Reagenz gemessen. Die gesamte Phenolkonzentration der Urtinkturen wurde im Vergleich zu Gallussäure bestimmt.

Ergebnis: Die Ergebnisse der Studie zeigen eine signifikante antioxidative Wirkung und einen hohen Phenolgehalt aller getesteter Urtinkturen in der folgenden Reihenfolge: *Syzygium jambolanum* < *Damiana* < *Cinchona officinalis* < *Chelidonium majus* < *Convallaria majalis* < *Coca*.

Fazit: Das antioxidative Potential von Urtinkturen ist von ihrem Gesamtphenolgehalt abhängig. Ihre Rolle bei der Prävention und bei der Heilung ist möglicherweise an ihre antioxidative Wirkung gekoppelt. Von den ausgewählten sechs Urtinkturen zeigt *Syzygium jambolanum* die höchste antioxidative Wirkung.

Análisis de la actividad antioxidante y del contenido total en fenoles de algunas tinturas madre homeopáticas

RESUMEN

Fundamento: Los compuestos antioxidantes se utilizan ampliamente para la protección de la salud. Las tinturas madre homeopáticas se suelen prescribir para la prevención y la curación de muchas enfermedades.

Objetivos: El presente estudio se ha centrado en determinar el potencial antioxidante de seis tinturas madre habitualmente prescritas. Es decir, *Syzygium jambolanum*, *Damiana*, *Cinchona officinalis*, *Chelidonium majus*, *Convallaria majalis*, *Coca*

Materiales y métodos: La actividad antioxidante se estimó por el ensayo de inhibición del DPPH. Se utilizó una solución de 0,1 mM de DPPH para estimar la actividad antioxidante de las tinturas madre (5, 2,5 y 1,25 µl de volumen). La quercetina se empleó como control estándar de la actividad antioxidante. El contenido fenólico total se midió por ensayo del reactivo Folin-Ciocalteu. El contenido fenólico total de las tinturas madre se midió en comparación con el ácido gálico.

Resultados: Los resultados del estudio mostraron una actividad antioxidante significativa y un elevado contenido en fenoles de todas las tinturas madre examinadas en el siguiente orden: *Syzygium jambolanum* < *Damiana* < *Cinchona officinalis* < *Chelidonium majus* < *Convallaria majalis* < *Coca*.

Conclusiones: El potencial antioxidante de las tinturas madre está relacionado con el contenido fenólico total presente en las mismas. Su papel en la prevención y curación de enfermedades puede estar vinculado a su capacidad antioxidativa. Entre las seis tinturas madres, *Syzygium jambolanum* posee el máximo potencial antioxidante.

कुछ होम्योपैथिक मदर टिंचर की एंटीऑक्सीडेंट गतिविधि और कुल फेनोलिक सामग्री का विश्लेषण

सार

पृष्ठभूमि: स्वास्थ्य संरक्षण में एंटीऑक्सीडेंट यौगिकों का व्यापक उपयोग किया जाता है। कई रोगों की रोकथाम और इलाज के लिए होम्योपैथिक मदर टिंचर का सुझाव दिया जाता है।

उद्देश्य: वर्तमान अध्ययन का उद्देश्य सामान्य छः मदर टिंचरों साइज़ीजियम जैम्बोलैनम, डेमियाना, सिन्कोना ऑफिसनैलिस, चेलिडोनियम मेजस, कानवैलिरिया मैजेलिस, कोका की एंटीऑक्सीडेंट क्षमता के निर्धारण पर केंद्रित है।

सामग्री और विधि: एंटीऑक्सीडेंट गतिविधि का अनुमान डीपीपीएच निषेध परख द्वारा लगाया गया। डीपीपीएच के 0.1एमएच घोल का उपयोग किया गया, जबकि एंटीऑक्सीडेंट गतिविधि का आकलन करने के लिए मदर टिंचरों (5, 2.5 और 1.25 μ एल मात्रा) का इस्तेमाल किया गया। कुल फेनोलिक सामग्री का मापन फोलेन-सिओकेलट्यू अभिकर्मक परख द्वारा किया गया। मदर टिंचर की कुल फेनोलिक सामग्री का मापन गालिक अम्ल की तुलना में किया गया।

परिणाम: अध्ययन के परिणाम में जाँची गई सभी मदर टिंचर में महत्वपूर्ण एंटीऑक्सीडेंट गतिविधि तथा उच्च कुल फेनोलिक सामग्री प्रदर्शित की गई जो निम्न क्रम में है:—

साइज़ीजियम जैम्बोलैनम, डेमियाना, सिन्कोना ऑफिसनैलिस, चेलिडोनियम मेजस, कानवैलिरिया मैजेलिस, कोका

निष्कर्ष: मदर टिंचरों की एंटीऑक्सीडेंट क्षमता उनमें उपस्थित कुल फेनोलिक सामग्री से संबंधित है तथा रोगों की रोकथाम और इलाज में उनकी भूमिका को उनकी एंटीऑक्सीडेंट गतिविधियों से जोड़ा जा सकता है। चयनित छः मदर टिंचरों में से साइज़ीजियम जैम्बोलैनम उच्चतम एंटीऑक्सीडेंट क्षमता रखती है।

