

In Vitro Evaluation of Antioxidant Activity of Homeopathic Mother Tincture and Total Phenolic Content

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ABSTRACT

Background: Antioxidants are those compounds which scavengers the free radicals, widely used in health protection and prescribed on large scale in biomedical health care units. In homeopathic system of medicine mother tinctures are prescribed. Mother tinctures of medicinal plants are having the potential to treat the various acute and chronic ailments.

Objectives: The present study was accompanied to determine the total phenolic content and the antioxidant activity of homeopathic mother tincture of plant origin i.e. *Baptisia tinctoria*, *Berberis aquifolium*, *Echinacea angustifolia*, *Hydrangea arborescens*, *Hydrastis canadensis*, *Kreosotum*, and *Thuja occidentalis* in comparison with ascorbic acid.

Methods: Antioxidant activity was evaluated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) inhibition assay method. A volume of 0.1 mM solution of DPPH was used while mother tinctures (1.25, 5, 2.5 µl volumes) were used for evaluation of antioxidant activity. Ascorbic acid was taken as a standard control and alcohol as negative control in antioxidant activity protocol. Total phenolic content was measured by Folin–Ciocalteu reagent assay. Total phenolic content of mother tinctures was measured in comparison with ascorbic acid.

Results: All tested homeopathic mother tinctures of plant origin showed significant antioxidant potential except *Hydrangea arborescens* and *Kreosotum*. Alcohol which was used as negative control exhibited no antioxidant activity. *Pulsatilla nigricans* possess highest antioxidant potential 85±0.3 among other tested mother tinctures against DPPH free radical and 98.8±2 maximum no of phenolic contents.

Conclusion: Homeopathic Mother Tinctures of plant origin showed antioxidant activity due to presence of phenolic content.

Key words: Antioxidant, Homeopathic tinctures, Total phenolic contents.

Authors' Contributions

- 1,3,6 Design the study
- 2 Generated the idea to consider this study
- 4,7 Help in data collection
- 5 Processed and executed the designed work
- 6 Supervised in data analysis

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INTRODUCTION

Free radicals are uncharged molecules they can damage the cell of organism by damaging the DNA. Free radicals also caused the oxidative stress by chain reactions [1]. Antioxidants are those substances which inhibit the process of oxidation [2, 3]. These are natural as well as the synthetic substances [4, 5]. Natural antioxidant have their potential to kill the free radicals due to presence of phenolic contents. In homeopathic system of medicine mother tinctures are prescribed. Homeopathic mother tinctures having potential to prevent oxidation present study was conducted to determine the antioxidant activity of homeopathic mother tinctures [6]. Homeopathic mother tinctures are in liquid form, prepared by the use of suitable concentration of alcohol treated with raw material (which may be fresh or dried). There are different methods for preparation of homeopathic mother tinctures depending upon nature of the raw material. Homeopathic mother tinctures are obtained by the methods of maceration, digestion, infusion, decoction and fermentation [7]. Homeopathic mother tinctures of plant origin carry the hydro-alcoholic extracts of medicinal plant. The major difference between the mother tincture and simple extract is the ratio of alcohol with medicinal substance. Homeopathic mother tinctures prepared by treating one part of medicinal substance with nine parts of alcohol where as simple extract comprises on one part of medicinal substance and 4 or 5 parts of alcohol. Theta Φ , or Q symbol is used to represent the homeopathic mother tincture [8-10]. Experimental mother tinctures were *Berberis aquifolium*, *Baptisia tinctoria*, *Echinacea angustifolia*, *Hydrastis canadensis*, *Hydrangea arborescens*, *Kreosotum*, and *Thuja occidentalis*.

METHODOLOGY

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

In current study, antioxidant activity of homeopathic mother tinctures was tested with free radical 2, 2 diphenyl 1-picrylhydrazyl method. In present study 2, 2 diphenyl 1-picrylhydrazyl is used in present study due to its certain advantage over other used methods. Evaluation of antioxidant activity through 2, 2 diphenyl 1-picrylhydrazyl (DPPH) inhibition assay is a simple and cost effective method [11]. The other advantage

of DPPH inhibition assay is lying in its ability to measure total antioxidant capacity of extract or mother tincture as it is not specific to any antioxidant constituent [12]. The scavenging activity of homeopathic mother tinctures was checked with DPPH radical technique. A volume of 0.1 mM solution of DPPH was prepared by adding 25 mg of DPPH in 100 ml of methanol. A micro assay of antioxidant evaluation was used with slight modifications [9]. A volume of 90 μ l of DPPH solution with subsequent different serial dilutions (5, 2.5, and 1.25 μ l) of mother tincture were placed in wells of a 96-well micro plate. The wells containing DPPH and 10 μ l of ethanol were used as negative control. Gallic acid was taken as standard control. The whole process was done in triplicate. The reaction mixture was placed for 30 min at 37°C [13, 14]. The absorbance was measured at 517 nm using Biotech ELISA micro plate reader. The absorbance was taken as follows.

Scavenging activity % = $([Ac - Am.t]/Ac) \times 100$
Where,

Ac = Absorbance of negative control

Am.t = Absorbance of mother tincture

Evaluation of total phenolic contents

Total phenolic content was estimated by FCR (Sigma-Aldrich, Germany) using the methodology of McDonald [15]. A volume of 5 μ l of homeopathic mother tinctures and 50 μ l of 1 mM sodium carbonate were added and then Folin-Ciocalteu 20% was added to make the volume up to 150 μ l in 96-well plate, and after incubating at 40°C, the absorbance was checked at 479 nm [16].

Statistical analysis

The results were analysed by Descriptive statistics was used to represent data in the form of Mean \pm S.E.M (Standard error of mean) between antioxidant activity by DPPH assay and total phenolic content of mother tinctures. Significance level was set at $P \leq 0.05$.

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

The scavenging activity of homeopathic mother tinctures was 2, 2-Diphenyl-1-picrylhydrazyl free radical scavenging assay
2,2-Diphenyl-1-picrylhydrazyl free radical scavenging

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

RESULTS AND DISCUSSION

Pulsatilla nigricans possess highest antioxidant potential among other tested mother tinctures against DPPH free radical. All the other mother tinctures also have good antioxidant activity except *Hydrangea arborescens* and *Kreosotum* that are weak antioxidants [17, 18] as described in Table 1. Flavonoids and phenolic compounds present in tinctures and extracts possess strong antioxidant potential. Poly phenolic compounds are more potent antioxidant as compared to mono-phenols [19].

Thuja occidentalis, *Hydrastis canadensis*, *Hypericum perforatum*, *Eucalyptus globulus*, *Baptisia tinctoria*, *Echinacea augustifolia* and *Berberis aquifolium* also showed marvelous antioxidant potential with inhibition of DPPH in descending order in range of 74 ± 0.03 to $84.8 \pm 0.6\%$. This study expressed 73.35% inhibition of DPPH by tested extracts at 75 µg/ml [20]) which is comparable with antioxidant result of present study regarding *Thuja occidentalis* mother tinctures, having higher (84.5%) inhibition of DPPH that may due to use of high concentration of medicinal part in the preparation of mother tincture i.e. 100 µg/ml [21] as shown in Figure 1..

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

Homeopathic mother tinctures	DPPH % inhibition (Mean ± S.E.M*)
Ascorbic acid	92.7±0.3
Alcohol	Nil
<i>Baptisia tinctoria</i>	83.6±0.3
<i>Berberis aquifolium</i>	74±0.03
<i>Echinacea augustifolia</i>	76.6±0.6
<i>Eucalyptus globulus</i>	84.2±0.4
<i>Hydrangea arborescens</i>	31.4±0.5
<i>Hydrastis canadensis</i>	83.03±0.03
<i>Hypericum perforatum</i>	82.5±0.4
<i>Kreosotum</i>	38.8±1.4
<i>Pulsatilla nigricans</i>	85±0.3
<i>Thuja occidentalis</i>	84.8±0.6

* S.E.M - Standard error of mean (n =3).

In a previous study, extracts of *Hydrastis canadensis* showed presence of phenolic compounds in it by using gas chromatography combined with mass spectrometry (GC-MS) Phenolic compounds have strong antioxidant potential and might be responsible for antioxidant potential of *Hydrastis canadensis* [22].

In a previous study it is documented that Liquid chromatography with mass selective (LC-MS) detected high content of flavonoids in *Hypericum perforatum*. *Hypericum perforatum* showed 79.2% inhibition of DPPH in a previous study [23]. The results are compareable to a present study that showed 82.5% inhibition of DPPH. Moreover, antioxidant capacity may be due to its flavonoid content. In another study, dose dependant effect of *Hypericum perforatum* against DPPH was detected and it inhibited 79% of DPPH free radical with an IC₅₀ of 109 µg/ml [24] In present study, it is concluded that *Pulsatilla nigricans* contains highest content of phenolic compounds i.e. 98.8±2 as shown in Table 2.

The results of present study are also compareable to a previous study, *Eucalyptus globulus* Labill leaves extracts were evaluated for their antioxidant potential and phenolic contents. HPLC/MS showed high concentration of phenolic contents, chiefly flavonoids. *E. globulus* expressed significant amount of hyperoside flavanoid (666.42 ± 5.02 µg/g dw plant material). *E. globulus* showed 61.1% inhibition of DPPH [25]. It is somewhat less than that showed in present study. Homeopathic mother tincture of *E. globulus* also contain gallic acid and protocatechuic acids in addition to hyperoside flavanoid [26] that might be responsible for more antioxidant potential of homeopathic mother tincture.

Table 2. Total phenolic content of homeopathic mother tinctures 5 µl and alcohol.

Tested Compounds	Phenolic Contents
Alcohol	NIL
<i>Thuja occidentalis</i>	97.5±3
<i>Hydrastis canadensis</i> ,	81.4±5
<i>Hypericum perforatum</i> ,	79.4±4
<i>Eucalyptus globulus</i> ,	77.6±3
<i>Baptisia tinctoria</i> ,	88.6±3
<i>Echinacea augustifolia</i>	69.7±2
<i>Berberis aquifolium</i>	93.1±5
<i>Kreosotum</i>	41.3±4
<i>Hydrangea arborescens</i>	94.9±1
<i>Pulsatilla nigricans</i>	98.8±2

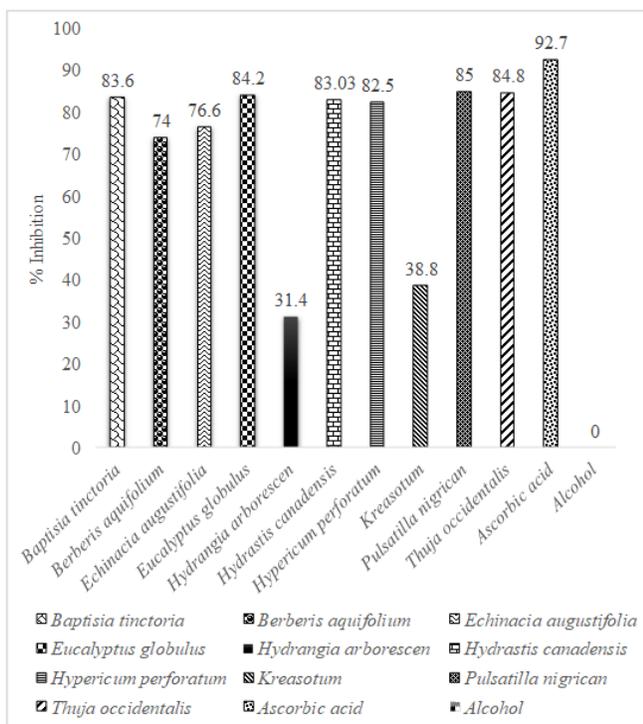


Figure 1. Graphical presentation of antioxidant activity of various homeopathic mother tinctures against DPPH.

Baptisia tinctoria contain alkaloids such as baptitoxine, glycosides like baptisin and baptin, isoflavones, flavonoids and polysaccharides. These active metabolites are responsible for its antioxidant potential [26].

In another study, Cichoric acid, echinacoside, cynarin, caffeic acid, chlorogenic acid and caftaric acid were identified and quantified in *Echinacea* roots. Analytical results revealed that roots of *E. angustifolia* contain 10.49 mg/g phenolic compounds. The current study showed 76.6% inhibition of DPPH. Echinacoside and cynarin are the pure compounds with marked antioxidant activity that are responsible for antioxidant activity of *Echinacea* [27].

The current study showed 74% inhibition of DPPH by *Berberis aquifolium*. In a previous study, berberine, jatrorrhizine, and magnoflorine in *Berberis aquifolium* (*Mahonia aquifolium*) have been studied that are responsible for its antioxidant potential [28-30].

CONCLUSION

Pulsatilla nigricans showed highest inhibition of DPPH (85%) among other tested plant mother tinctures. It

contains high content of flavonoids 98.8 ± 2 that might be responsible for its antioxidant activity.

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