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In vitro investigation of the antioxidant effect of some medical plants by(DPPH methods)

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Abstract:

The use of plant extracts is very important and significant in therapeutic treatments that are employed to many functions in view of the fact that primeval epochs. The present study was conducted to investigate antioxidant effect of extracts from some medicinal plants, extracted by soxhlet with methanol alcohol and fractionation of active constituents from *Curcuma longa* L. rhizomes , *Commiphora myrrha* L. gums and *Ginkgo biloba* L. leaves with water , chloroform , ethyl acetate and hexane. Investigation of the antioxidant activity carried out by DPPH Free Radical Scavenging Assay and showed that all plants were significant ($p \leq 0.05$) antioxidant agents compared to ascorbic acid.

Key words: antioxidant agents , DPPH , *Curcuma longa* , *Commiphora myrrha* , *Ginkgo biloba*.

Part of Ph.D. dissertation of third author

Introduction

Plants are valuable sources to individual verdure care[10] because there are a high rate of phytochemical compounds which can be extracted from plants which are used as pure compounds or as extracts[12] which exhibit a large potential role in clinical pathology and they are useful in the treatment of many diseases[5]. The use of the extracts is very important and significant in therapeutic treatments that are employed to many functions in view of the fact that primeval epochs[17]. Since most of medicinal plants and their biological and therapeutical activities have not been studied yet, it is very important to search and work in order to decline the plants properties and activities and establishing their roles in the promotes the present disease. Thus , the present study efforts have been made to study the active components and their antioxidant effect of alcoholic extracts and (chloroform , hexane , ethyl acetate and water) fractions of *Curcuma longa* L. rhizomes , *Commiphora myrrha* L. gums and *Ginkgo biloba* L. leaves.

Material and Methods:

The plant preparing:

This employment was passed in the Department of Biology , Faculty of Science , Kufa University (January 2016 – April 2016). *Ginkgo biloba* L. plants obtained from pharmacies as 500 mg food supplement tablets manufactured in the United Kingdom by FSC Food Supplement Company, while plants *Commiphora myrrha* L. gum and *Curcuma longa* L. rhizome were collected from local Najaf city markets .

The plant parts powders were extracted by Soxhlet by putting twenty five grams of desiccated plants powder in filter paper and dissolved with 250 ml of solvent (methanol 95%) for 24 hours, the resultant dried and then mixed in separatory funnel with 1:1 rate of

water and chloroform solution and shaking well. The result showed two layers , the lower layer (chloroform layer) mixed immediately in separatory funnel with 1:1 rate of chloroform and hexane solution , the result showed two layers , the upper layer(hexane layer) and the lower layer(chloroform layer).

The upper layer (water layer) mixed immediately in separatory funnel with 1:1 rate of water and ethyl acetate solution , the result showed two layers , the upper layer(ethyl acetate layer) and the lower layer(water layer). All the layers were dried and stored until used. Then , the methanolic extract and four fractions (chloroform layer , hexane layer , water layer and ethyl acetate layer) were used for investigation about biological activity of plants. Chemical detection of the active components in alcoholic plant extracts of studied plants were chemically tested for the presence or absence of the following active compounds by treatment with precipitation reagents[7].

The antioxidant effect investigation

DPPH applied to occupy as a reagent toward estimation of free radical scavenging effect of test samples. In the methanolic alcohol solution , DPPH show evidence of steady purple color[13]. DPPH method attempted to find out the radical scavenging effect of the plant solutions (extract and fractions) which was achieved according to Alothman *et al.* 2009 work[4]. The dried plants samples thawed with DMSO were positioned in 96-well plate at different concentration (100 mg / ml – 1000 mg / ml) in triplicate and the DPPH solution (methanolic solution) was laid in 96-well plate jointly with plant samples , also methanol alcohol represented as control placed on plates, in the lasting wells. Then for (2) minutes gently wavering movement for plates with in murk incubation at 37˚C for 20 minutes.

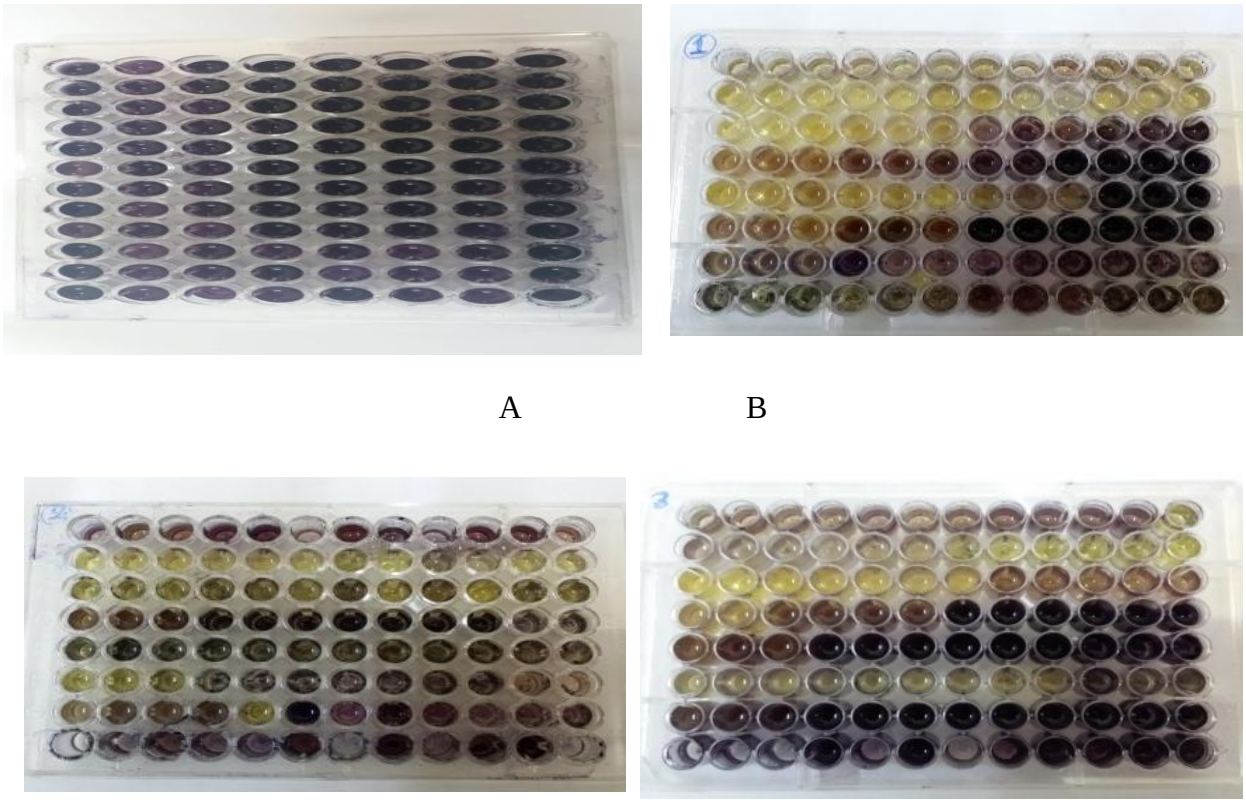
The inhibition rate of oxidation was measured at 517 nm.

The inhibition rate of oxidation was plotted adjacent to the plant sample concentration, then half maximal inhibitory concentration (IC_{50}) rate was found out. Vitamin C (L-ascorbic acid) represents as positive control because it is standard antioxidant. The antioxidant effect was calculated with the inhibition rate of oxidation by DPPH free radical scavenged method as the equation: Inhibition % = [(control absorbency – sample absorbency) / control absorbency] x 100%

Results and discussion:

Phytochemical screening of methanol alcoholic extracts of *Curcuma longa* L. rhizomes , *Commiphora myrrha* L. gums and *Ginkgo biloba* L. leaves by using precipitation reagents reveal that many of active compounds are found in the extracts as tannins , alkaloids , glycosides and phenols and this is in agreement with many previous studies[8,1and 6].

The inflammation usually leads to produce free and non free radical by direct or indirect oxidation which follows by membrane destruction , so any factor can hunt these reactive oxygen speci es can be useful in the management of inflammatory disorders. Thus , the plant with anti – oxidant activity will reduce danger of occurrence of chronic diseases and certain types of cancer[4]. In the DPPH radical scavenging assay, antioxidants react with DPPH, and convert it to the yellow color, the degree of yellowing indicates the radical-scavenging potential of the sample[11] and parallel to examination of the antioxidant activity of plant extracts, the values for standard compound Vitamin C (L-ascorbic acid) (which is water soluble compound produced in mammalian except human by liver from glucose[16]),



A B

C D

Figure (1) : The DPPH radical scavenging assay with 96-well microtiter plate ; A : before incubation , B : *Curcuma longa* after incubation , C : *Commiphora myrrha* after incubation and D : *Ginkgo biloba* after incubation.

were obtained and compared to the values of the antioxidant activity.

The examination of antioxidant activities of plants extracts showed different values. The results showed that all the studied plants extracts have significant antioxidant activity which is comparable to the standard compound Vitamin C (L-ascorbic acid) especially *Commiphora myrrha* solutions then *Curcuma*

longa methanol extract and fractions showed highly inhibition in contrast with the methanol extract and fractions of *Ginkgo biloba*. The effective concentration of sample required to scavenge DPPH radical by 50% (IC_{50} value) is obtained by linear regression analysis of dose-response curve plotting between %inhibition and concentrations[9]. The reducing power of the plant extracts is associated with their antioxidant latent , the reducing power of the extracts increased with concentration increasing and the smaller value of IC_{50} represents a better antioxidant activity[14].

The plant extract proceed as free radicals receptors and arrested their action because of the presence of phenols , flavonoids and tannins which may be found in all plants parts[3].

The plant extract anti – oxidation activity is not related with the phenol and flavonoids total quantity or concentration but related to phenolic compounds form and quality[2]. Phenolic compounds are forming stabilized phenoxy radicals because they bear one or more hydroxyl groups which have able to put out free radicals[15], as a result , the anti-oxidant ability of plants may correspond to their phenolic content[19]. Also flavonoids compounds play a major role in the antioxidant action and chelating properties which rely on the structure and replacement outline of hydroxyl groups and reduce the free radical formation and hence exhibit several biological activities[18].

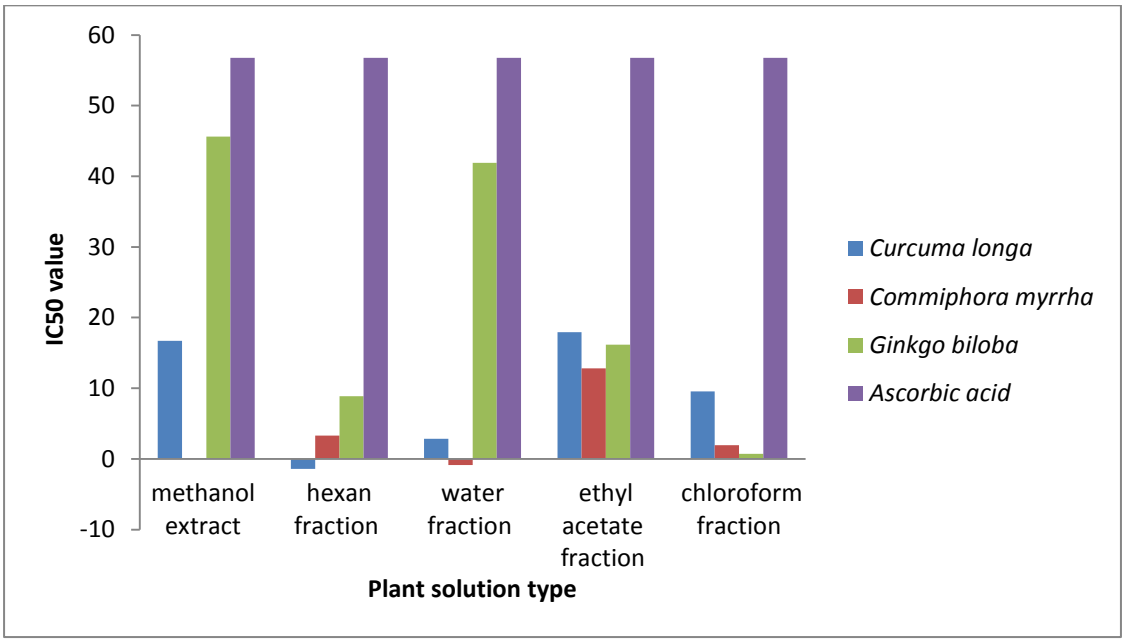


Figure (2) : IC_{50} ^{DPPH} Values for the studied plants *Curcuma longa* , *Commiphora myrrha* and *Ginkgo biloba* solutions in contrast to Ascorbic acid ; (lower value is better).

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التحري عن الفعالية المضادة للأكسدة لبعض النباتات الطبية بطريقة DDPH خارج الجسم الحي

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المستخلص:

ان استعمال المستخلصات النباتية في العلاج مهما جدا وذو دلالة على حقيقة قدم استعمال النبات في المعالجة. الدراسة الحالية وضعت للتحقق من التأثير المضاد للأكسدة لمستخلصات بعض النباتات الطبية (الكركم والمر والجنكو) المستخلصة بالسكسوليت مع كحول الميثانول وأجزائه في الماء ، الكلورفورم ، الاثيل اسيتيت و الهكسان وذلك باستخدام طريقة DDPH لسحب الجذور الحرة وأظهرت النتائج ان جميع النباتات لها هذه الفعالية بالمقارنة مع حامض الاسكوربك بمعنوية ($p \leq 0.05$).

كلمات مفتاحية : عوامل مضادة للأكسدة ، DPPH ، الكركم ، المر ، الجنكو

البحث جزء من أطروحة دكتوراه للباحث الثالث