

**Anti-inflammatory effect of turmeric plant ( *Curcuma longa* L. ) rhizomes and myrrh ( *Commiphora myrrha* L.) gums and ginkgo *Ginkgo biloba* L. leaves ( tablets ) extracts**

**Assist. Prof. Dr. Ahmed Abies Motar\* , Assist. Prof. Dr. Rajaa Ali Hussein\*\* & Assist. Prof. Dr. Meison Abdulbary\*\***

\*Department of Biology /College of Sciences /University of Kufa , Republic of Iraq.

\*\* Department of Pharmacognosy and Medicinal Plant / College of Pharmacy /University of Kufa , Republic of Iraq.

**Key words:** *Curcuma longa* , *Commiphora myrrha* , *Ginkgo biloba* , Anti-inflammatory effect.

**Abstract:**

**Back ground:** the plants importance resulting from their uses as a nutrition source and for their therapeutic effect. These plants include turmeric , myrrh and ginkgo.

**Aim of study:** this study was conducted to test the anti-inflammatory effectiveness of turmeric plant ( *Curcuma longa* L. ) rhizomes and myrrh ( *Commiphora myrrha* L.) gums and ginkgo , *Ginkgo biloba* L. leaves ( tablets ) extracts.

**Method:** plants under study were extracted by soxhlet using methanol as a solvent, and then was fractioned by water, chloroform, ethyl acetate and hexane. The extracts tested to determine the anti-inflammatory effect through testing their ability to maintain the stability of the membranes of red blood cells , prevent protein denaturation and heat induced hemolysis.

**Results:** the result showed the effectiveness of all the plants under study in resisting inflammation with compared to standard medicines (Aspirin 100µg / ml and Diclofenac sodium 100µg / ml).

**Conclusion:** It can be concluded that the presence of secondary metabolites as alkaloid, polyphenolic compounds and phenolic acid in the plants under study would be marked a good anti-inflammatory effect.

التأثير المضاد للالتهابات لمستخلصات رايزومات نبات الكركم *Curcuma longa* وصمغ نبات المر  
*Ginkgo biloba* واوراق نبات الجنكو *Commiphora myrrha*

أ.م.د. ميسون عبد الباري

أ.م.د. رجاء علي حسين\*\*  
عبد الله\*\*

أ.م.د. احمد عبيس مطر\*

\*\* كلية الصيدلة / جامعة الكوفة

\* كلية العلوم / جامعة الكوفة

الخلاصة

أجريت هذه الدراسة لاختبار الفعالية المضادة للالتهابات لمستخلصات رايزومات نبات الكركم *Curcuma longa* L. وصمغ نبات المر *Commiphora myrrha* L. , و أوراق نبات الجنكو *Ginkgo biloba* L. والتي استخلصت بواسطة جهاز السكسوليت باستعمال الميثانول كمذيب ، ثم تم تجزئتها بواسطة الماء والكلورفورم وخلات الاثيل والهكسان ، وذلك من خلال اختبار قدرتها في الحفاظ على استقرار أغشية كريات الدم الحمراء ومنع تمسخ البروتين وانحلال الدم الناجم عن الحرارة وأظهرت النتائج فعالية جميع النباتات تحت الدراسة في مقاومة النشاط الالتهابي بالمقارنة مع الأدوية القياسية ( الاسبرين 100µg/ml و ديكلوفينك صوديوم 100µg/ml ).

**Introduction:**

The plants importance resulting from their uses as a nutrition source and for their therapeutic effect. These plants include turmeric , myrrh and ginkgo. The powder of turmeric plant ( *Curcuma longa* L. ) is a major ingredient of curry powder as food flavoring called the ‘Kitchen Queen’, the main spice of kitchen and as a dye in addition to use it in the marriage ceremonies (1). The turmeric rhizome well known popularly used in treatment of many diseases like dyspepsia , gastric ulcer , scabies, snake bite, smallpox ,swellings, and sprains (2). The major components of turmeric are called curcumin and many researches on laboratory animals and cell cultures showed that curcumin could have a potential role in the treatment of inflammatory bowel disease, pancreatitis, arthritis, and chronic anterior uveitis (3).

*Commiphora myrrha* is well known traditional herb which it may be define as the yellow fragrant oleo-gum resin obtained from the stems and branches (4). It has different Phytochemicals metabolites including terpenoids, steroids, flavonoids, glycoside , alkaloids and lignans (5). It represented as home-grown remedy for the treatment of soreness, diarrhea,

coughing , chest pain parasitic infection , gastrointestinal diseases , arthritis , wounds , and gingivitis <sup>(6)</sup>.

*Ginkgo biloba* L. It is monotypic genus illustrate the sole survivor of the family Ginkgoaceae which is found now in the wild small area in China , that it was found in the garden of holy place of worship and prayer <sup>(7)</sup> and become popular as an ornamental tree in parks, gardens and city streets in many states for instance Northern America <sup>(8)</sup>. The major bioactive constituents found in the leaves of Ginkgo are reported to be flavonoids and glycosides <sup>(9)</sup> . Ginkgo leaf extract has shown the potential role in the prevention of Alzheimer's disease ( AD ) and treatment of neurodegenerative diseases like stress, memory loss, tinnitus <sup>(10)</sup> , in addition to extract beneficial effects in treating cardiovascular diseases particularly ischemic cardiac syndrome , that it may obstruct the various effect of cardiovascular disorder as blood curdling and platelet accumulation <sup>(11)</sup>.

## 2. Methods:

### 2.1 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 manufacturing in the United Kingdom by FSC Food Supplement Company, while Plants *Commiphora myrrha* L. gum and *Curcuma longa* L. rhizome were collected from 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 show two layers , the lower layer ( chloroform layer ) mixed immediately in separatory funnel with 1:1 rate of chloroform and hexane solution , the result show 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 show 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 the biological activity of

plants. Chemical detection of the active components in alcoholic plant extracts of studied plants were chemically tested by treatment with precipitation reagents<sup>(12)</sup>.

## 2.2 Evaluation of the anti-inflammatory effects of plants extracts:

### A. Albumin Denaturation Inhibition :

The plant solutions ( extract and fractions ) action against inflammation could be prepared as Sakat *et. al.*( 2010 )<sup>(13)</sup> work pursued by negligible modification. A combination of 1 ml plant solutions ( extract or fractions ) and 1 ml bovine serum albumin ( 1% aqueous solution ).

The plant solutions combinations placed in incubation about 20 min at 37°C and adjust to ( 20 ) minutes the solutions would be got warmth to 51°C. Next , the plant solutions combinations would be got cool, the haziness had been determined at 660 nm with spectrophotometer , the work was executed in triplicate. Inhibition rate of albumin protein denaturation was estimated as below:

Inhibition percentage = ( Control absorbance – sample absorbance ) x 100 / control absorbance.

### B. Preparation of red blood cells ( RBCs) suspension:

An entirely unsullied blood ( 10 ml ) obtained from a hale and hearty unpaid assistant which is in support of 2 weeks earlier to the experimental work did not receive drugs for inflammation treatments ( Non Steroidal Drugs) relocated to the tubes of centrifuge to centrifuge about ( 10 minutes ) at 3000 round / minute and were diluted three times with equal volume of Alsever's solution<sup>(14)</sup>.

### C. Hemolysis stimulated by heating :

A combination of 1 ml from plant solutions ( extract or fractions ) of varying concentrations (100 - 1000 µg/ml) with ( 1 ml ) of 10% RBCs suspension, the negative control represented by test tube including saline's solution only while the positive control represented by standard drug ( Aspirin , 100µg/ml) and all these tubes ( centrifuge tubes ) were kept warm at 56 °C adjust to 1/2 hour in water bath , then with current tap water , the centrifuge tubes be less warm. Finally , at 2500 round / minute for 5 minutes , the combinations of plant solutions with RBCs were centrifuged and the upper floating absorbance was measured at 560 nm. The work was executed in triplicate<sup>(15)</sup>. The Hemolysis inhibition rate was estimated as below:

Inhibition rate = ( control absorbance – sample absorbance ) X 100 / control absorbance.

#### D- The membrane stabilization test:

In this experimental work , Diclofenac sodium (100µg/ml) standard drug represented the positive control while the plant solutions ( extract and fractions ) with diverse concentration ranges (100 µg/ml – 1000 µg/ml ) were blended with ( 1ml ) of phosphate buffer saline ( PBS ), 2ml of hypotonic saline (0.25% w/v of sodium chloride NaCl ) and these solutions were mixed with RBCs suspension in amount of ( 0.5 ml ).

Next , the samples of this work were exposed to heat by incubation for 30 min next to 37 °C then centrifuged at 3000 round / minute. The top layer of centrifuging solutions was poured , at the same time as , the content estimation of hemoglobin was occurred with a spectrophotometer by the side of 560nm<sup>(16)</sup>. The hemolysis rate calculated approximately with 100% Hemolysis in the negative control supposed that be produced as below equation:

$$\text{Protection Rate} = 100 - [ ( \text{sample absorbency} / \text{control absorbency} ) \times 100 ]$$

#### Results and discussion:

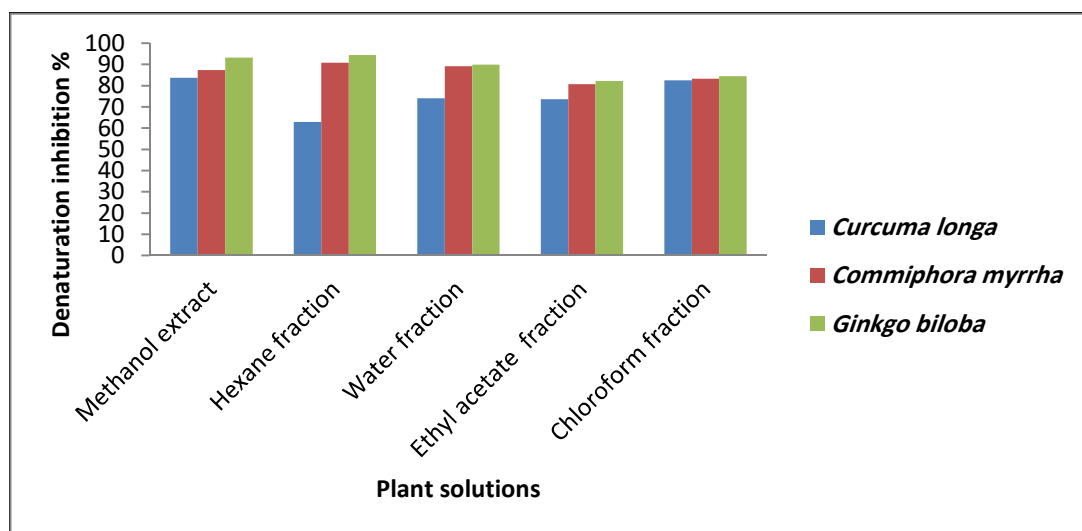
Phytochemical screening of methanol alcoholic extracts of *Curcuma longa* L. rhizomes , *Commiphora myrrha* L. gums and *Ginko biloba* L. leaves by using precipitation reagents reveal that many of active compounds are found in the extracts as tannins , alkaloids , glycosides and phenols and this is in agreement with many previous studies<sup>(17, 18, 19)</sup>.

Despite phylogenic differences , Human red blood corpuscle (HRBC) membrane is similar to lysosomal membrane components<sup>(20)</sup>, HRBC were selected for investigating the toxic substances effects on its membrane because of their ready availability and relative simplicity<sup>(21)</sup>.

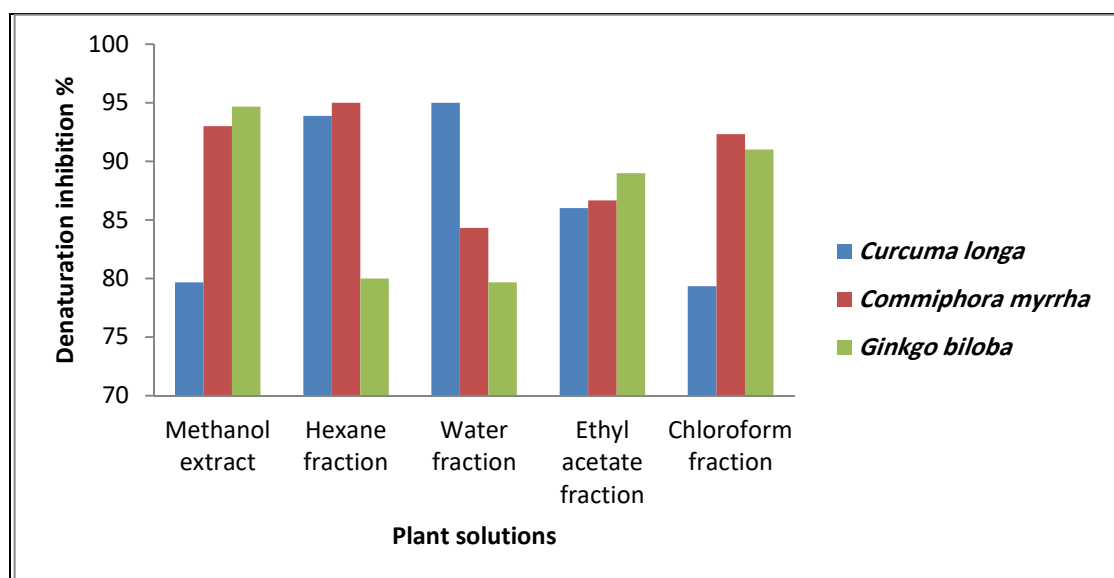
Throughout inflammation the lysosomal enzymes ( neutrophil lysosomal constituents include bactericidal enzymes and protease ) released and caused cell membrane lysis and damaging of macromolecules and HRBC membranes with cations leakage from the cell membranes , thus the stabilization of lysosomal membrane will prevent the inflammation and lysosomal enzymes releasing<sup>(21)</sup>. The protein lose its tertiary structure and secondary structure by denaturation which is predictable of inflammation<sup>(22)</sup>.

The nonsteroidal drugs ( Diclofenac sodium and Aspirin ) anti-inflammatory action is well correlate with biological activity in animals and humans by stabilizing lysosomal membrane or by inhibition the lysosomal enzymes actions<sup>(23)</sup>.

The ability of extract to inhibit protein denaturation was studied and the HRBC membrane stabilization has been used as a method to study the *in vitro* anti inflammatory activity and the heat induced haemolysis and the results illustrated by figures ( 1 to 6).



**Figure ( 1 ):** Albumin protein denaturation inhibition effects with low concentration ( 100 µg/ml).



**Figure ( 2 ):** Albumin protein denaturation inhibition effects with high concentration ( 1000 µg/ml).

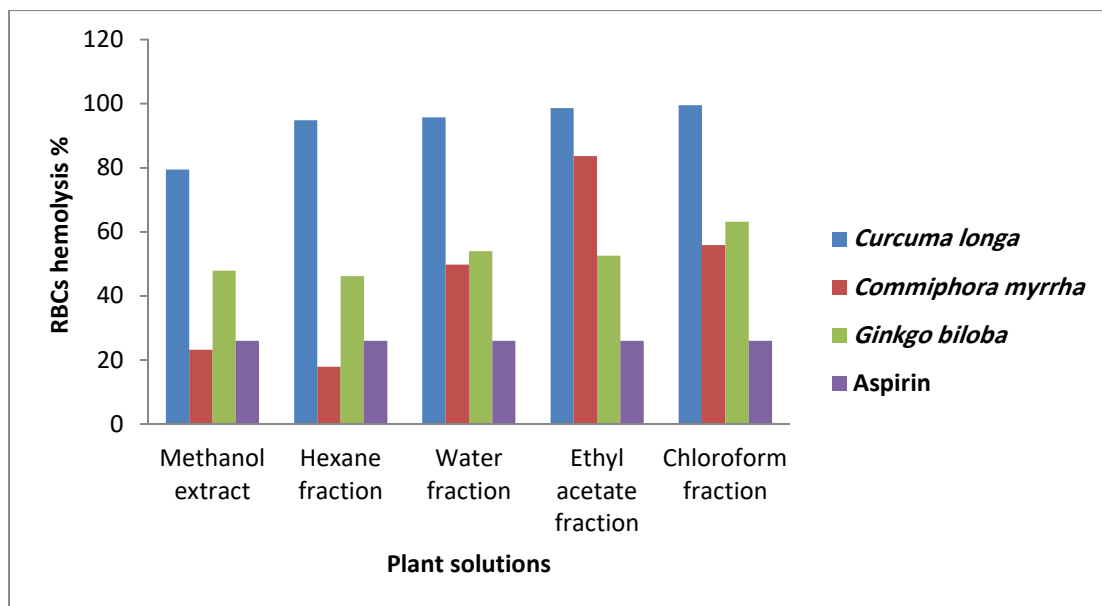


Figure ( 3): Heat induced hemolysis effect on erythrocytes with low concentration ( 100 µg/ml).

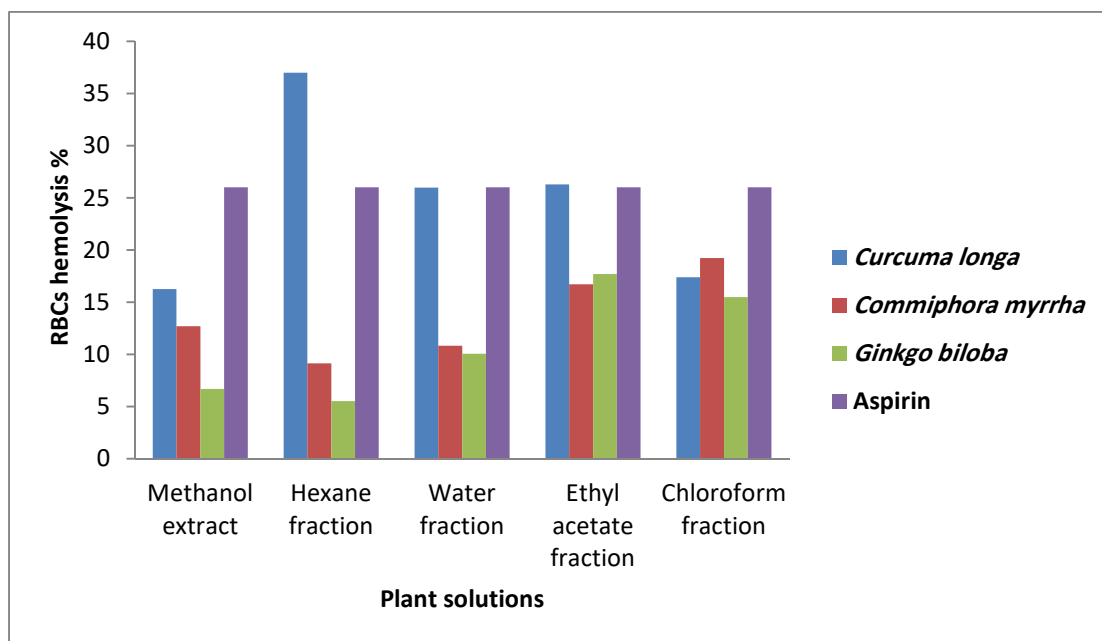
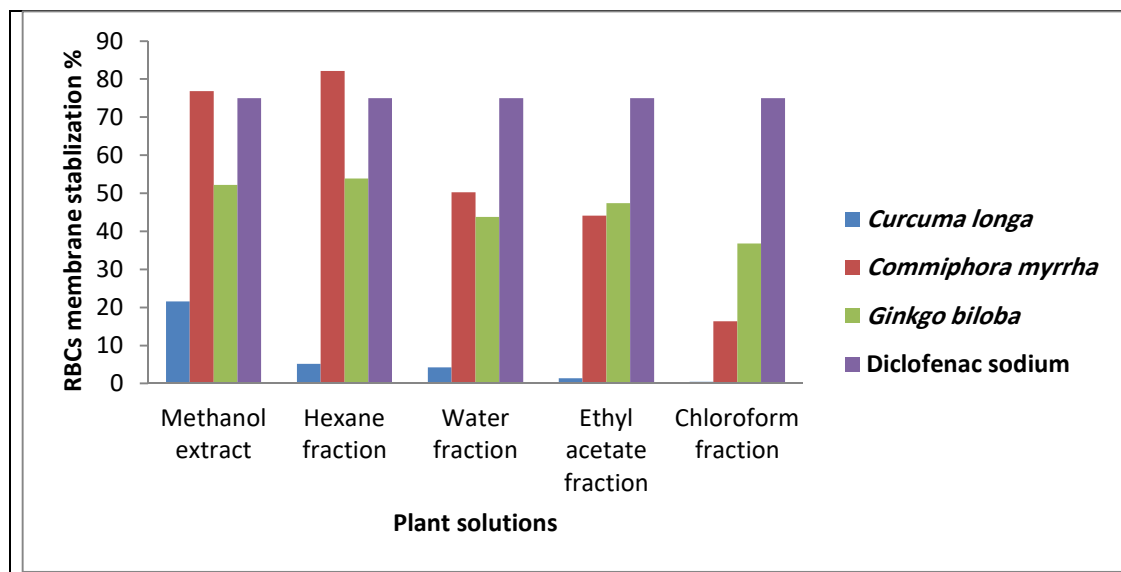
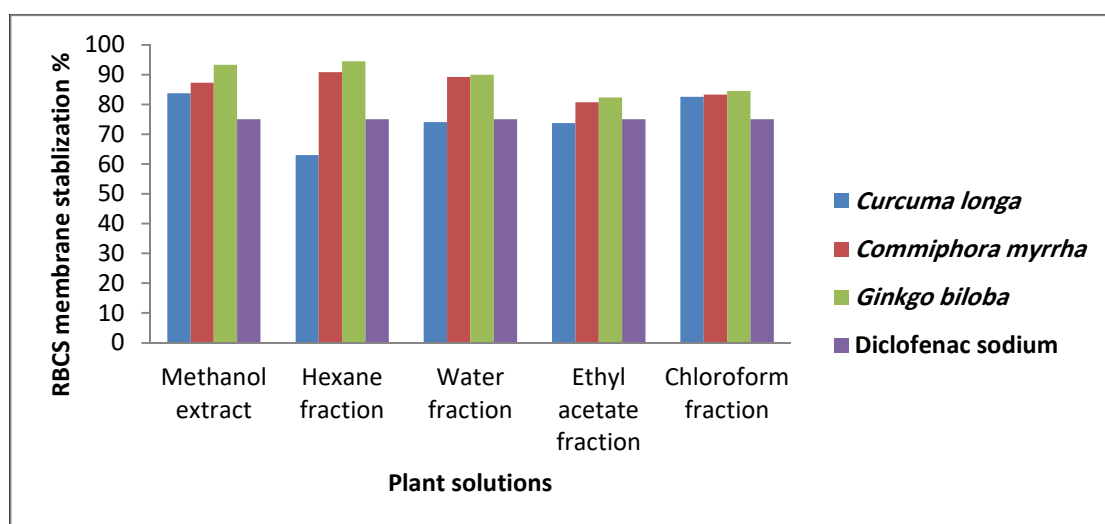


Figure ( 4 ): Heat induced hemolysis effect on erythrocytes with high concentration ( 1000 µg/ml).



**Figure ( 5 ): Membrane stabilization effect on erythrocytes with low concentration ( 100 µg/ml).**



**Figure ( 6 ): Membrane stabilization effect on erythrocytes with high concentration ( 1000 µg/ml).**

The results showed that all studied plants extracts have significant membrane stabilizing activity which was comparable to the standard diclofenac sodium especially *C. myrrha* and *G. biloba*. The (*C. myrrha*) solutions were effective in inhibiting the heat induced hemolysis of erythrocyte membrane at different effective concentrations which was comparable to the standard aspirin.

Also all studied plants extracts are effective in inhibiting albumin protein denaturation at different effective concentrations. The results revealed that methanolic extracts and all



fractions contained principles that protected the erythrocytes membranes effectively. Also the activities of the extracts/fractions were higher than that of the standard drugs even at lower concentration ranges especially in *C. myrrha* solutions.

These extracts possibly inhibit the release of lysosomal content and enhance membrane stabilizing activity because the saponins , flavonoids and tannins are involved in the stabilization effects on lysosome membrane and stabilizing erythrocyte membrane by binding with divalent cations like  $Ca^{+2}$  and  $Mg^{+2}$  (24). It can be concluded that alkaloid, polyphenolic compounds like tannins and phenolic acid possessed marked *in vitro* anti-inflammatory effect against the denaturation of protein (25).

**Conclusion:** It can be concluded that the presence of secondary metabolites as alkaloid, polyphenolic compounds and phenolic acid in the plants under study would be marked a good anti-inflammatory effect.

#### References:

1. Sangeeta and Sharma A, ( 2014 ) The amazing pharmacological properties of haridra- a short review, **An International Journal of Research in AYUSH and Allied Systems**, 1(1):27-31.
2. Ugochukwu SC, Bob SE, Ozioma O, Odii EB, Ijeoma IC and Olanike O, (2013 ) Shoot Proliferation of In vitro Turmeric ( *C. longa* L.) Affected by Different Concentrations of Benzylaminopurine (BAP) , **World Journal of Agricultural Sciences**, 9 (3): 227-230.
3. Funk JL, Oyarzo JN, Frye JB, Chen G, Lantz RC, Jolad SD, Solyom AM, Kiela PR and Timmermann BN, ( 2006 ) Turmeric extracts containing curcuminoids prevent experimental rheumatoid arthritis, **J. Nat. Prod.** 69: 351-355.
4. Brendler T, Eloff JN, Gurib-Fakim A and Phillips LD, ( 2010 ) *C. myrrha* In: African Herbal Pharmacopoeia, **Port Louis, Mauritius: Association of African Medicinal Plant Standards**. pp88-93.
5. Su SL, Duan JA, Tang YP, Zhang X, Yu L, Jiang FR, Zhou W, Luo D and Ding AW, ( 2009 ) (Jiangsu Key Laboratory to TCM Formulae Research, Nanjing University of Chinese Medicine, Nanjing, P.R. China), **Planta Medica** , 75(4): 351-355.
6. Abdul-Ghani RA, Loutfy N and Hassan A, (2009) Myrrh and trematodoses in Egypt: an overview of safety, efficacy and effectiveness profiles, **Parasitol. Inter.** 58: 210 - 214.
7. Shepperd WD, (2008).Ginkgoaceae — Ginkgo family *G. biloba* L. ginkgo, **Woody Plant Seed Manual**. pp 559 – 561.
8. Zhou Z, Quan C and Liu YSC, ( 2012 ) Tertiary Ginkgo ovulate organs with associated leaves from North Dakota, U.S.A., and their evolutionary significance, **Int. J. Plant Sci.** 173(1):67–80.

9. Xie J, Ding C, Ge Q, Zhou Z and Zhi X, (2008) Simultaneous determination of ginkgolides A, B, C and bilobalide in plasma by LC-MS/MS and its application to the pharmacokinetic study of *G. biloba* extract in rats, **J Chromatogr B Analyt Technol Biomed Life Sci.** 864: 87–94.
10. Gold PE, Cahill L and Wenk GL, (2002 ) *Ginkgo Biloba*: A Cognitive Enhancer? **Psychological Science in the Public Interest**, 3(1):2-11
11. Mahady GB, (2002) *G. biloba* for the prevention and treatment of cardiovascular disease: a review of the literature, **J Cardiovasc Nurs**, 16:21–32.
12. Harborne JB, (1984) Phytochemical methods ; A guide to modern techniques of plant analysis , 2nd ed. **Chapman and Hall , London**, pp 307.
13. Sakat S, Juvekar AR and Gambhire MN, ( 2010) In vitro antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. **International Journal of Pharma and Pharmacological Sciences**, 2(1):146-155.
14. Shinde UA, Kulkarni KR, Phadke AS, Nair AM, Dikshit VJ, Mungantiwar and Saraf MN, ( 1999 ) Mast cell stabilizing and lipoxygenase inhibitory activity of *Cedrus deodara* (Roxb.) Loud. Wood Oil, **Indian J Exp Biol**, 37(3): 258-261.
15. Azeem AK, Dilip C, Prasanth SS, Junise V and Shahima H, ( 2010 ) Anti-inflammatory activity of the glandular extracts of *Thunus alalunga*. **Asia Pac J for Med.** 3(10): 412-20.
16. Hashim FJ, ( 2011 ) The effect of ethanolic extract of *Curcuma longa* rhizomes on two types of leukemic cell lines and karyotyping of human lymphocytes treated by Nickel, **M.Sc. thesis. University of Baghdad/ College of Science Department of Biotechnology**.
17. Ahmed IS, El Tohami MS, Almagboul AZ and Verpoorte R (2013 ) Quercetin and  $\beta$ -sitosterol from *C. myrrha* Fum Extracts, **University of Africa Journal of Sciences**, 3: 57 – 70.
18. El-Beltagi HS, Ahmed OK and Hegazy AE, ( 2015 ) Molecular Role of Nitric Oxide in Secondary Products Production in *Ginkgo biloba* Cell Suspension Culture, **Not Bot Horti Agrobo.** 43(1):12-18.
19. Manukumar GHM and Umesha S, (2015) Assessment of membrane stabilizing activity from honey, An in-vitro approach, **Acta Sci. Pol. Technol. Aliment.** 14(1): 85–90.
20. Priya M, ( 2001 ) Biochemical investigations on the stability of biological membranes. **Ph.D. Thesis in Biochemistry. Department Of Marine Biology, Microbiology And Biochemistry, Cochin University Of Science And Technology**, Chapter 8, pp 88 .
21. Chippada SC, Volluri SS, Bammidi SR and Vangalapati M, ( 2011 ) In vitro anti inflammatory activity of methanolic extract of *Centella asiatica* by HRBC membrane stabilization, **Rasayan J. Chem.** 4(2): 457-460.
22. Leelaprakash G and Dass SM, ( 2011 ) Invitro anti-inflammatory activity of methanol extract of *Enicostemma axillare* , **Int. J. Drug Dev. & Res.** 3(3): 189-196.
23. Vadivu R and Lakshmi KS, (2008) In vitro and in vivo anti inflammatory activity of leaves of *Symplocos cochinchinensis* (Lour) Moore ssp Laurina, **Bangladesh J Pharmacol.** 3: 121-124.

24. Oyedapo OO, Akinpelu BA, Akinwunmi KF, Adeyinka MO and Sipeolu FO, ( 2010 ) Red blood cell membrane stabilizing potentials of extracts of *Lantana camara* and its fractions, **International Journal of Plant Physiology and Biochemistry**, 2(4): 46-51.

25. Chandra S, Chatterjee P, Dey P and Bhattacharya S,( 2012 ) Evaluation of in vitro anti-inflammatory activity of coffee against the denaturation of protein, **Asian Pacific Journal of Tropical Biomedicine**, S178-S180.