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Biological activity and phytochemical analysis of Acacia farnesiana extract against bacteria and antioxidant

[1]M. S. Al-Hamdani, [2]Sundus Hammed, [3]Saba Hadi, [4]Saja Mohammed

Abstract

Objective: *The interpretation and discussion of the conclusions of the scientific research depends mainly on the preliminary screening of phytochemicals of any plant known for its therapeutic effect of many common diseases.Therefore, the study was conducted to detect the essential compounds of acacia leaf extractAcacia farnesian leaves extract and their relationship to microbial growth, oxidation and its albumin inhibition.* ***Methods:*** *Phytochemical analysis of Acacia farnesianleafextract was studied for the bioactive group's detectionusing standard methods, GC-Ms and FTI. Types of pathogenic bacteria as Escherichia coli, Pseudomonas aeruginosa,Staphylococcus aureus, Klebsiellaspp was used for antibacterial inhibition. Then the extract was undergo to antioxidant and anti-inflammatory examined using DPPH and for inhibition of albumin denaturation.* ***Results:****The results of detection of phytochemical of plant extract showed the high content of reduced sugars and flavonoids, then tannins, glycosides, Emodin, Comarine and protein at the modiest level, then the phenols and terpenoid at lowest level. The value of* IC_{50} *forAcacia farnesian extract was 46.75µg/ml, whileit was38.42 µg/mlfor ascorbic acid that used as control. It was found the albumin denaturation inhibition 80.9% close to 85.6% for Asprin as standard and have scavenging activity for free radical produced by DPPH .* ***Conclusion:****The study concluded with the high content of the secondary components of the Acacia farnesianplant, which has antioxidant properties and anti-albumin denaturation comparable to that of ascorbic acid.*

Keywords: *Leaf extract,Secondary metabolites, GC-MS, FTIR, Anti-microbial*

I. Introduction

Since ancient times and the emergence of pathological injuries and human rights in the investigation and exploration of treatments with available resources and renewable over time and was an essential aspect with the development of creatures (Misar, et al., 2011). The wild and medicinal plants took a prominent position in

[1]*Market Research & Consumer Protection Center/University of Baghdad /Iraq*
[2]*University of al-Mustansiriyah/Biology Dep./Baghdad/Iraq*
[3]*University of al-Mustansiriyah/Biology Dep./Baghdad/Iraq*
[4]*University of al-Mustansiriyah/Biology Dep./Baghdad/Iraq*

natural remedies, especially after the discovery of biological compounds and their biological properties against microbes and asantioxidants, as well as their commercial and economic importance and the negative effects of the consumer to prove their safety (Finefrock,l et al., 2003**).**Plant extracts and phytochemical have pharmacological properties and gives a high significance in medicine field (AmmarAltemimi, et al., 2017). The phytochemical reveal the pharmacological property of secondary metabolites like phenolic compounds, tannins, essential oils etc. (Deciga-Campos, et al. 2007;Thaipong, 2006**)**. Dietary phytochemicals with antioxidant activity decrease the danger of death from numerous diseases like diabetes, acute hypertension, cancer, infectious diseases and cardiovascular diseases (Londonkar, R.; Kamble,2009).Researchers in the past years have paid much attention to phenolic compounds for their importance in removing harmful free radicals and their multiple benefits on human health (Manach et al., 2004;Tsimogiannis and Oreopoulou, 2004).*Acacia farnesian* are global for their wide spread throughout the temperate, hot and low cold areas, and their multiple types, which reaches 1350 species, and the genus *Acacia farnesian* belongs to the family(Maslin, et al. 2003). Its origin, native to North America (Edward, et, al. 1993).Acacia shrubs have been planted since ancient times for their beautiful yellow flowers as a decoration for the main gardens and streets in the cities as well as the use of flowers in the perfume industry, especially of Cassie perfume (Lapornik, 2005). There is a huge amount of previous scientific studies that proved the biological importance of *Acacia farnesian* aqueous and alcoholic extracts in the manufacture of many pharmaceuticals for the treatment of many ethnic diseases(Salfarina, et al., 2011**;**Ahmadi, et al., 2007). The valuable benefits of each part of the *Acacia farnesian* tree have proven in the field of medicine and treatments, including dermatological, neurological, antimicrobial and anti-oxidant (Hukkeri, et al., 2002). Previous and multiple studies have proven the effectiveness of the extract of *Acacia farnesian*and its various parts against the growth of various microbes and oxidative reactions (Krishnaraju, et, al. 2005; Shetty and Patil, 2015). The present study aimed at detecting the phytochemicals of the plant leaflets and its extraction using the alcohol extract and proving against the different types of microbes and the oxidation of the human blood albumin.

II. Material and Methods

Collection and Identification of Plant leaves

The fresh leaves of *Acacia farnesian* were collected from the orchard of Al-Mustansiriya University/Baghdad.Then, the taxonomy of the *Acacia* leaf was attested.

*Acacia's*leaf extractpreparation

The leaves of *Acacia farnesian*were collected and totally washed three times under running tap water to remove dust particles and sieved until semi-dried.Then, rinsed leaves were air-dried at room temperature under fan for 3 days. The dried leaves were grounded using electrical grinder until obtained fine powdered, then stored in sealed glass bottles and placed in the refrigerator at 4 ° C until use.10 g of leaf powder were immersed in distilled water and methanol (100, 200 ml) separately and left for 24 hours at room temperature. Then the aqueous and alcoholic extracts werefiltered through Watts's man paper and then concentrated the remaining to a volume of 50 ml and then the necessary phytochemical analyzes were carried out.

Phytochemical detection

Qualitative phytochemical analyses of the extracts were performed by following standard procedures of (Adetuyi and Popoola, 2001; Khandelwal, 2008; Farina, 2014).

Alkaloids

It was taken 0.5 mg of methanol extract then, drops of 1% HCl was added followed by 6 drops of Dragendroff's reagent. The appeared brownish-red precipitate was taken as evidence for the presence of alkaloids (Michielin, 2009).

Saponins

It was taken 5 mL of distilled water then,200 mg of plant extract was added in a test tube. Then it was taken 0.5 mL from filtrate then diluted to 5 mL with distilled water and shaken vigorously for 2 minutes. Formation of stable foam indicates the presence of saponin (Kokate, 2008).

Flavonoids

It *was* taken 4 ml of extract and then, 1.5 ml of methanol was added. Warm the mixture besides adding metallic magnesium followed added 4-5 droplets of hydrochloric acid. The formationof yellowishcolor indicates the presence of flavonoids (Michielin, 2009; Lapornik, 2005).

Phenoles

It was taken 2ml of alcoholicextractactionand then, a few drops of ferric chloride solution were added. The formation of bluish dark color indicates the presence of phenols (Michielin, 2009; Lapornik, 2005).

Glycoside

It was taken 2ml of alcoholic extract and then, a few drops of ferric chloride solution on concentrated sulfuric acid then added to the extract (in glacial acetic acid), until appeared two cleared layers.The reddish brown colorin the bottom, and the bluish green color in the upper layer, this indicates the presence of glycoside (Michielin, 2009).

Terpenoids and steroid

It was taken 2ml of alcoholic extract and thenadded 2 ml of chloroform ($CHCl_3$) carefully to 3 mL of concentrated sulphuric acid (H_2SO_4) to the extract until the reddish brown color appeared. The reddish brown colorindicates the presence of terpenoidsand steroid (Michielin, 2009).

Tannins

It was immersed 200 mg of plant material in 10 ml distilled water then, boiledand filtered. A few drops of $FeCl_3$ were added to the filtrate until it appeared a blue-black precipitate, and this indicates the presence of Tannins (Michielin, 2009).

Emodins

It was added 2 ml of NH_4OH and 3 ml of Benzene to the plant extract and shakedwell,then wait a few minutes untilred color appeared. Formation of the red color indicates the presence of emodins (Lapornik, 2005).

Fatty acid

It was mixed 0.5 ml of plant extract with 5 ml of ether. Then, the solution was heated and allowed for evaporation and filtered through Watts's man paper then dried. The appearance of ttransparent layeron filter paper indicates the presence of fatty acids (Farina, et al., 2014).

Iodine test

To the 2ml of Plant extract was mixed with 2ml of iodine solution gently until dark blue to purple color appeared. Formation of dark blue to purple color indicates the presence of the carbohydrate (Lapornik,2005).

FTIR-technique

Fourier Transform Infrared Spectroscopy (FTIR) is one of the high-precision analytical explorations of fast and nondestructive to atoms used as powder or as plant extracts.The FTIR technique work on pumping red radiation on the plant extract and thus heat will reach the molecules of the extract in the form of vibrations and those vibration oscillations that can analyze and determine the various chemical bonds such as (C–C, C=C, C–O, C=O, O–H, and N–H). Each bond can be identified by detecting the characteristic frequency absorption band in the infrared spectrum (Urbano, et al., 2006).

Antibacterial assay

Tablets of 6 mm diameter from filter paper were prepared using a special punching machine. The tablets were then sterilized in a dry heat sterilizer and placed in a sterile and airtight container and placed in the refrigerator until use.The lawn ofeach bacterial isolates used in this study was then prepared separately on MHA plates using a sterile cotton swab of vaccines showing growth of 0.5 Mac Ferland standard.MHA was then dried for 15 minutes in a laminated airflow cabinet. Then three pallets of filter paper were paved one over the other completely on the dried MHA platesand *Acassia* extract (20 µl) were added on each disc separately in consentrations (20,40,80 µg/ml).Bacterial Species *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus andKlebsiellaspp* were used for this test. Then, all plates were incubated at 37^0C for 18-24 hours and the zones of inhibition (diameter inmm) were measured on the agar surface.

Free radical scavenging activity (DPPH assay)

Free radical scavenging activity was determined as (Le, et al. 2007). It was drawn 100µl of DPPH in methanol (126µM)using pipette and placed into welldone microplate, and then added directly 100µl of alcoholic extract in five consentrations100,200,300,400µg/ml. then it was allowed to settled in an incubater for 30 minutes at room temperature. Then, the absorbance was measured at 517 nmusing microplatereader. It was used ethanol solution instead of plant extract for control treatment. The DPPH assay was calculatedby the following equation:

Scavenging activity%=(Absorbance control – Absorbance sample)/Absorbance control × 100. The scavenging activity of extract was expressed as theconcentration necessary to scavenge free radical by 50% (IC50). The IC_{50} values were calculated from calibration curve, IC50 values are defined asthe concentration of a test compound required to achieve half maximal inhibition, and lower IC_{50}value indicates greater antioxidant activity.

Denaturation inhibition of albumin

It was added alcoholic extract in different consentrations (100,200,400, µg/ml)to Human albumin (1%), accept control without extract addition, then it was incubated at 37 C^{o} for 20 minutes, heated at 51 ^{o}C and cooled. The turbidity was measured at 660 nm by UV Visible Spectrophotometer.. The percentage of denaturation inhibition calculated by this equation = (Abs Control –Abs Sample) X 100/ Abs control.

Statistical Analysis

All the measurements were done in triplicate and statistical analysis was performed by statistical software. All the data were expressed as±S.E.M. Statistical analysis weredetermined using one way analysis of variance (ANOVA).

III. Result and Discussion

Phytochemical screening

Secondary compounds (phytochemicals) of *Acacia farnesian*leaves extract are measured by the standard methods determined for each compound and by observing the apparent color intensity or by observing the sediment or surface foam after the various chemical reactions. The result of the phytochemical screening of *Acaciafarnesian*leaves extract revealed the presence of reducing sugar and flavonoids were highly, Tannins, glycosides, Emodin, Comarineand proteinwere fairly present. Phenols and Terpenoid were slightly present. While, Starch, Fatty acids, saponinsand alkaloid were missingas shown in Table 1.Studies have shown that flavonoids have a great importance and an important role in many antioxidants and antimicrobial for many pathogenic microbes and that cause food spoilage [Mongkolsilp, et, al. 2014].

Table 1: Qualitative phytochemical analysis of crude extract of *A. F*

No.	Name of active component	Result
1	Reducing sugar	+++
2	Tannins	++
3	Flavonoids	+++
4	Glycosides	++
5	Emodin	++
6	Starch	-
7	Comarine	++

8	Phenols	+
9	Proteins	++
10	Terpenoid	+
11	Fatty acid	-
12	Saponins	-
13	Alkaloids	-

Absorption Frequencies of FT-IR

Fourier Transform Infrared Spectroscopy (FTIR) was used to identify thechemical constituents and elucidate the structural compounds in different plants extract. Result Obtained from*Acacia farnesian*leaves extracts byFTIR analysis was shown in Fig. 1. Figure 1 was described the absorption band and the number of waves measured in (cm^{-1}) are described for each prominent peak occurring in this study.The peak at a frequency of 2850 cm^{-1}, 2920 cm^{-1} and 1750 cm^{-1} were strong while the others vary from medium to weak. The main and secondary components such as phytochemicals of plant extracts were detected by the infrared spectrum.Sharp peaks values within the limits from 1635 cm $^{-1}$ to 3430 cm $^{-1}$ were determined by FTIR technique, as shown in Figure 1.The high peaks3430 cm $^{-1}$,2850 cm^{-1} and 2920 $cm^{-1}$of the FTIR assay refers to the availability of stretching O-H bonds that means polyphenols presented with a high amount in the acacia plant extract. In contrast, the decreased peak at 1740 cm-1 and 1650 cm^{-1}referred to C=O stretching and C–O bending frequencies. Previous study showed, the C=Oand C–O stretching presence means that someflavones and terpenoids as carbonyl compounds in the leaf extracts (Jelly and Shauning 2007). Also, a previous study also showed that tracking the fingers of the peaks sites and their intensity and shape indicates the vital secondary components of the various plant extracts (S. Yallappa • J. Manjanna, 2013). Also, it was found the narrow strong peaks at intensity 2926 $cm^{-1}$and 2855 cm $^{-1}$ belong to CH2 (methylene) non-consistencyalkane. likewise the weak peak intensity at 1609 cm^{-1}, 1451cm $^{-1}$ and medium 1045 cm $^{-1}$ medium belong to C-N bond stretching amine werereferred toC=C skeletal stretching of alkene, to methylene CH_2 and to C-N bond stretching amine respectively. While, previous studies have shown that C=O, C-H, C=C and C-O, C-C and C-O bonding structures refer to alkyl groups, methyl groups,alcohols, ethers, carboxylic acid, anhydrides and deoxyriboserespectively (Urbano, et al., 2006).

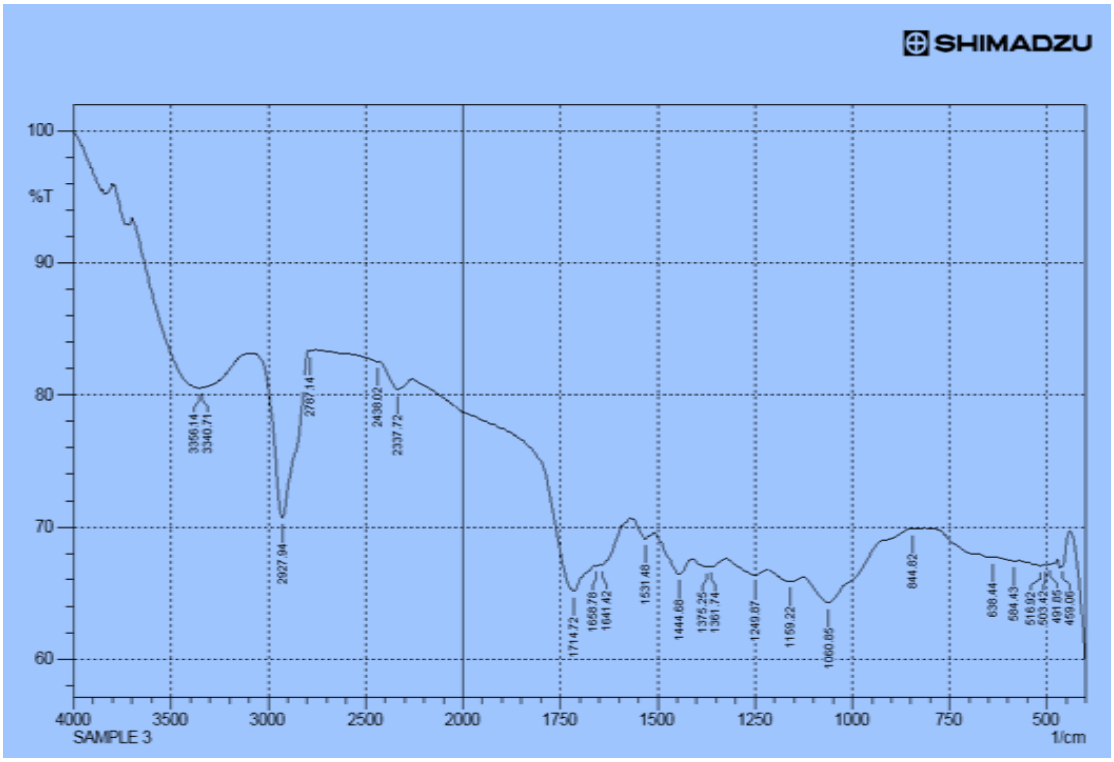


Figure 1.The FTIR Spectrum for the *Acacia farnesian* extract.

Anti -bacterial activity of *Acacia farnesian* extract

Previous studies were showed evaluated the anti -bacterial activity of *Acacia farnesian* extract was reported that ethanolic inhibited the growth of *Klebsiellaaerogenes, Proteus sp*., *Escherichia coli* and *Pseudomonas aeruginosa*.Togashi**,**(2008) determined the antimicrobial activities of ethanolic extracts showed an inhibition of microbial activity against both Gram positive and Gram negative (2).It was found that the extract at the highest concentration gave the highest anti microbial activity as shown :Staphylococcus aureus> Klebsiella spp. > Staphylococcus aureus> Pseudomonas aeruginosa > Escherichia coli as shown in fig (2).This finding comparable with (Mahesh and Satish 2008).The inhibition of these pathogenic microorganisms is due to the presence of phytochemical compounds of this acacia extract (Mamtha, et al.,2004). The synthesis complex compounds from tannins that combined with microbial cell,prolene rich protein, which inhibits the function of bacterial cells (Zablotowicz**,** et al., 1996).Also (**.**Hemandez, et al., 2000) explained, the saponine has the ability to leak proteins and certain enzymes from microbial cells, thereby impairing its harmful function.Terpenoid have proved effective against many in vitro microorganisms by forming complex complexes with cellular tissue proteins and dissolving the cellular wall proteins of bacteria and disrupting their function (Marjorie,1999).In addition,**(**Epand**,**et al., 2007), the presence of steroids has been shown to be anti-bacterial by binding to the lipid of microbial cell walls, leading to the leakage of the liposome enzymes, which inhibits the work of such harmful bacteria.

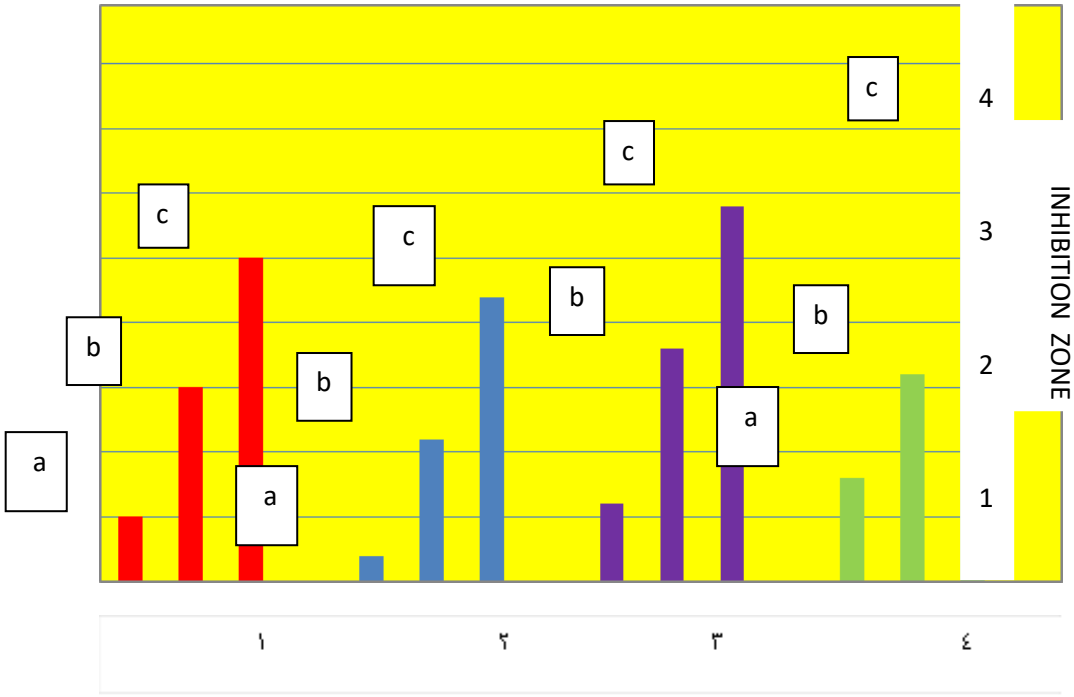
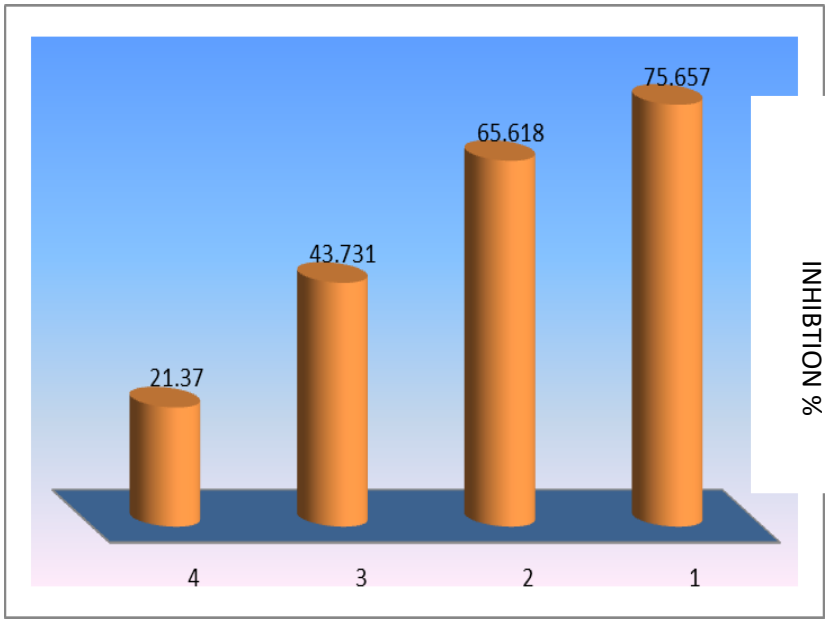


Fig 1: Antimicrobial activity of *Acacia farnesian album* against foodborne

Pathogen(1- *Pseudomonas aeruginosa, 2-Escherichia coli, 3-Klebsiella spp.,4- Staphylococcus aureus.* a ,b,c 20, 40, 80 µg/ml.

Antioxidant assay

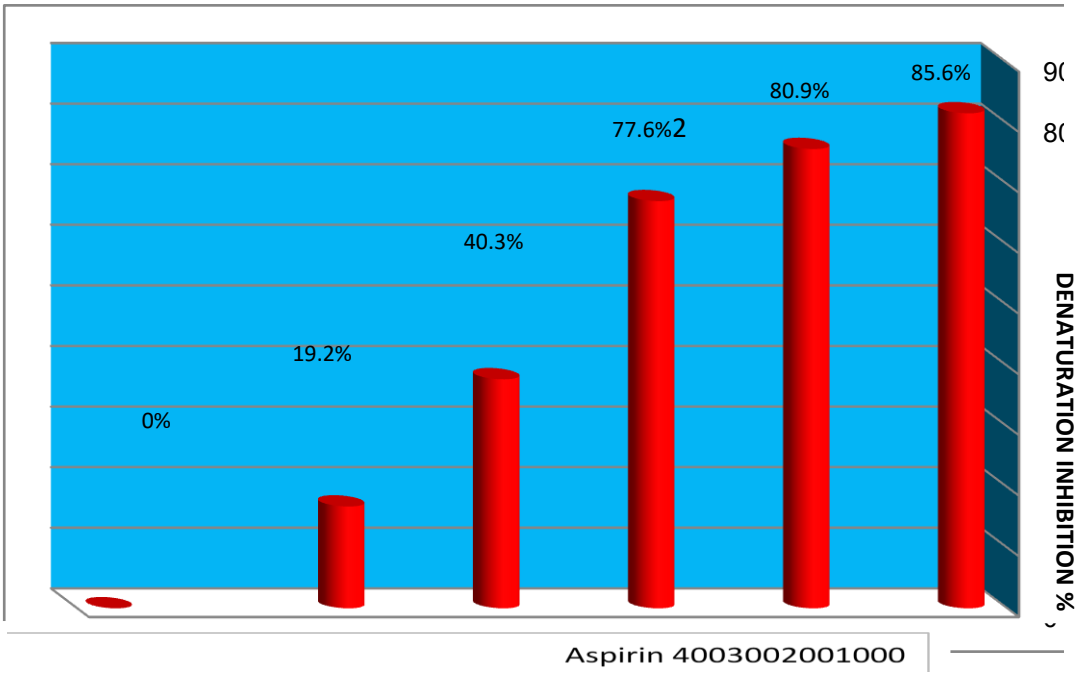
It has been scientifically proven since ages that the availability of natural or synthetic antioxidants is available in a food intake to sweep oxidized free radicals and free reactive oxygen species to inhibit and discontinue denaturation of proteins and lipid peroxide. In other words, this will inhibit and eliminate damage caused by oxidizing substances that occur in the body of the organism (Shetty and Patil, 2015).Recent scientific experiments have proven the importance of medicinal plants available in abundance and in very different types and in different parts of the world as a good source in their content of secondary compounds such as multiple phytochemicals and their benefits as antioxidants.Antioxidants for plant extracts are usually measured by DPPH examination.In order for the DPPH to be stable at room temperature, free radicals in the oxidation state can accept one electron or one free radical hydrogen to form a stable molecule. So, the increase or decrease in the ability of the root DPPH is measured by increasing or decreasing the absorbance at 517 nm, which is caused by the different antioxidants available in the extracts of different plants (Niknahad and O'Brien, 1996). It is visually noticeable as a change in color from purple to yellow. Result of this study showed the percentage of inhibition values of concentrated *Acacia* extract 1 and 2 gave the highest inhibition percentage 75.625 and 65.618 compared with the other concentration with IC_{50} value of 112 µg/ml. As the concentration of the extracts increased, the radical scavenging capacity tended to increase as well shown in Figure 3.



Fig(3): Inhibition of Free radical formation (DPPH) of *Acacia farnesian*extract concentrations (1, 2, 3 & 4 were represent 100, 200, 300 & 400µg/ml respectively) .

Albumin inhibition

In this study, the antioxidant potential of *Acacia farnesian* extract was evaluated with the help of *in-vitr*albumin denaturation Inhibition. It was found there were a high correlation ($p< 0.1$) between the extract concentration and the percent of inhibition of the albumin denaturation. The 400 ml concentration of *Acacia farnesian* extract had a high percent of albumin denaturation 80.9, which it was comparable to the artifician antioxidant ascorbic acid 85.6%, compared with control 0% of inhibition as shown clearly in Fig. 4.



Fig(4) Showed the results of of albumin denaturation Inhibition.

IV. CONCLUSIONS

This study revealed the great amount of phytochemicals such as alkaloids, flavonoids, saponins, steroids, glycosides and phenols etc.content. *Acacia farnesianleaf* extract exhibited antioxidant activity comparable with ascorbic acid. It was concluded that *Acacia farnesianleaf* extract was composed of comparatively great amount of phytochemicals such as alkaloids, flavonoids, saponins, steroids, glycosides and phenols etc. This study concluded with the high ability of *Acacia farnesian* extract to highly inhibit oxidation and microbes, so it is a good source of antioxidants and harmful organisms.Therefore, it is necessary to investigate and research more about the importance and explanatory of the structure and properties of the secondary bioactive components and their inhibitory effect of pathogenic macrophages and their properties against oxidation and inhibition of proteins and lipid peroxides of these medicinal and therapeutic plants and the manufacture of the necessary pharmaceuticals as natural substances free of side effects.

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