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Studying of Phytochemical, Nutritive values and Antioxidant ability of Commiphora myrrha.

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Abstract:
The present study deals with the phytochemical, nutritional, mineral contents and in vitro antioxidant activity of Commiphora myrrha. Preliminary phytochemical result indicate that the plant contain phenolic compounds, flavonoids, tannins, glycosides, alkaloids, terpenoids and quinines. Secondary Metabolites has been estimate quantitatively, the highest concentration of tannins 3677.1 ±2.15 mg/100g and then for alkaloids 1880 mg/100g, sterols 155.215 ±1.00 mg/100g, and Flavonoids 47.266 ±0.013 mg/100g and phenolic compounds 30.647 ±2.481 mg/100g. Nutritional Profiling, minerals and antioxidant activity were determined. Flavonoids and glycosides isolated were exhibited lower reducing power and scavenging ability than ascorbic acid

Key words: Secondary metabolites, Nutritional, Antioxidant activity, Commiphora myrrha

مفاتيح الكلمات: دراسة المركبات الأيضية الثانوية والقيم الغذائية وقابلية مضادات الأكسدة للمر Commiphora myrrha

الخلاصة:

شملت الدراسة الحالية المركبات الكيميائية النباتية والغذائية ومحتوى المعادن وفعالية مضادات الأكسدة في المختبر للمر. تشير نتائج الاختبارات النباتية الأولية للمر احتوائه على مركبات فينولية، فلافونويدات، عفصيات، كلايكوسيدات، قلويدات، تربينات وكوينينات. قدرت المركبات الايضية الثانوية كمياً، أظهر العفص أعلى تركيز 3677.1 ± 2.15 ملغم/ 100غم، والقلويدات 1880 ملغم/ 100غم، الستيرولات 155.215 ± 1.00 ملغم/ 100غم، والفلافونويد 47.266 ± 0.013 ملغم/ 100غم والمركبات الفينولية 30.647 ± 2.481 ملغم/ 100غم. قدرت القيم الغذائية والمعادن وفعالية مضادات للأكسدة. أظهرت الفلافونيدات والكلايكوسيدات المعزولة انخفاض القوة الاختزالية وقابلية الاقتناص مقارنة بحامض الاسكوربيك.

Introduction:

Utilization of plants as herbal therapy has been gaining popularity among clinicians and represents a good source of therapeutic agents due to their beneficial effects with minimum toxicity, natural origins, lower side effects and relatively lower costs as compared to synthetic drugs [1, 2].

Medicinal plants have been used for a wide variety of purposes such as food preservation, alternative medicine, pharmaceutical and natural therapies for thousands of years [3]. Medicinal plants represent a safer of drugs chemically synthesized drugs, which produce harmful or toxic side effects [4] .Medicinal plants are the richest bio-resource on drugs of traditional systems of medicine, modern medicines, food supplements, pharmaceutical intermediates, nutraceuticals, folk medicines and chemical entities for synthetic drugs [5].

Plant secondary metabolites are also of interest because of their use as dyes, fibers, glues, oils, waxes, flavoring agents, drugs and perfumes, and they are viewed as potential sources of new natural drugs, antibiotics, insecticides and herbicides [6, 7], also are can produce anti-hyperglycemic

drugs [8], and are vital in new drugs development due to their content of bioactive compounds that have plentiful of biological activities [8]. The antioxidants may be either natural or synthetic. Natural antioxidants such as α-tocopherol and ascorbic acid are widely used because they are regarded as safer and causing fewer adverse reactions. While synthetic antioxidants like butylated hydroxyl toluene (BHT), butylated hydroxyanisole (BHA), propylgallate (PG) and tertiary butyl-hydroquinone (TBHQ) are known to amend oxidative damages, but they have restricted use in foods, as they are carcinogenic [9]. Natural antioxidants are considered as safer and cause fewer adverse health effects than synthetic antioxidants [10].

Commiphora myrrha (Burseraceae family), known in folklore medicine as myrrh, itself is an aromatic oleo gum resin obtained as an exudate from the stem of several species of Commiphora, small trees that thrive in arid/semi-arid regions of East Africa, Arabia, and the Indian subcontinent (11,12). The name "myrrh" is probably derived from the Arabic or Hebrew word "mur", which means bitter [13].

The genus Commiphora with more than 150 plant species is distributed in the tropical and subtropical regions, especially occurring in north eastern Africa, southern Arabia and India (14). Myrrh consists of water-soluble gum (40-60%), alcohol-soluble resins (23-40%), volatile oils (2-8%) and a bitter principle (10-25%), and has a characteristic odour ascribed to the presence of furanosesquiterpenes (15). Myrrh increased glucose tolerance in vivo under both normal and diabetic conditions (16). It is an effective antimicrobial agent used in the treatment of mouth ulcers, gingivitis, sinusitis, glandular fever, brucellosis and as an anti-parasitic agent [17,18]. Moreover, myrrh volatile oils and their crude extracts exhibited diverse biological activities such as cytotoxic, anesthetic, antimicrobial effects [19,20], Cardioprotective effects [21], anti-bacterial [22,23], anti-inflammatory, reduces body weight gain and improves blood lipids profile [24].

The aim of the present study is to investigate phytochemical constituents of C. myrrha with nutritional values, mineral composition and antioxidant ability.

Materials and Methods:

All chemicals in the present study are of analytical grade and highly pure, products of Aldrich, Sigma and BDH. C. myrrha was purchased from market in Samarra city in Salahaddin-Iraq. Dried in hot air oven at 40 °C for 1 hr. The dried plant was then coarsely powdered using a mixer grinder and stored in an airtight container

Methods:

A. Preliminary Phytochemical screening

Two extracts were prepararted by macerated 10 g of C. myrrha in 100 ml of water and 100 ml ethanol. The mixture was then shaking in a magnetic bar at 25 °C for 24 hr. After that, the extracts were filtered by using filter paper and Buchner. The filtrates obtained were concentrated at 60 °C in hot air oven and stored at 4 °C until chemical analysis. While petroleum ether extract (b.p. 40-60 °C) was preparated by soxhlet for 10 hr. Phytochemical tests to identify the phytochemical constituents in the three extracts of C. myrrha, were carried out using standard procedures (25-31). The extracts were tested qualitatively for the presence of primary and secondary metabolites like carbohydrates, proteins, Fats, glycosides, flavonoids, phenolic compounds, tannins, alkaloids, phytosterols, terpenoids, saponins, coumarins, lignins and quinines.

B. Quantitative Estimation of some Secondary Metabolites by colomitric methods.

The total phenolic compounds of C. myrrha was estimated by Folin-Ciocalteu reagent (32). The total phenols were calculated by using the formula; TPC = C × V/m; Where, TPC =Total phenols compounds in mg/g sample; C = concentration of gallic acid established from the calibration curve in mg/ml; V = volume of extract in ml; m= weight of plant extracting in g; GAE = gallic acid.

Total Flavonoids content

The total flavonoids content of C. myrrha was measured by the method of the aluminum chloride calorimetric method(33). The total flavonoids were calculated by using the formula; TFC = C × V/m; Where, TFC =Total flavonoids content in mg/g sample; C = concentration of quercetin established from the calibration curve in mg/ml; V = volume of extract in ml; m = weight of plant extract in g; QUE = quercetin.

Total Tannin content

The total Tannin of *C. myrrha* was measured by the method [34]. The total tannin was calculated by using the formula; TTC = C × V/m; Where, TTC =Total tannin content in mg/g sample; C = concentration of tannic acid established from the calibration curve in mg/ml; V = volume of extract in ml; m = weight of plant extract in g; TA = tannic acid.

Crude alkaloids content

Crude alkaloid content was determined by using Harborne method [30]. The amount of crude alkaloid was determined in percentage by using the following formula:

$$\%\ \text{alkaloids} = \frac{\text{Final weight} - \text{Inital weight}}{\text{Inital weight}} \times 100$$

C. Proximate nutritive values of C. myrrha

The Moisture content

The moisture content was determined by measuring the mass of the sample before and after the water is removed by heating

Total Phenolic compounds

Total Tannin content

The total Tannin of C. myrrha was measured by the method (34). The total tannin was calculated by using the formula; TTC = C × V/m; Where, TTC =Total tannin content in mg/g sample; C = concentration of tannic acid established from the calibration curve in mg/ml; V = volume of extract in ml; m = weight of plant extract in g; TA = tannic acid.

Total Sterols content

Estimation of sterols in C. myrrha was carried out by using a calorimetric method by Liberman-Burchard reagent (35). The total sterols were calculated by using the formula; TSC = C × V/m; Where, TSC =Total sterols content in mg/g sample; C = concentration of β-sitosterol established from the calibration curve in mg/ml; V = volume of extract in ml; m = weight of plant extract in g; β-S = sitosterol.

in an oven at 105 °C, according to the author procedure [41]. The moisture percentage was calculated according to the following formula

$$\%\ \text{Moisture} = \frac{\text{W1} - \text{W2}}{\text{W1}} \times 100$$

W1= sample weight before drying (g)

W2= sample weight after drying (g)

The Ash content

The total ash content (in %) was calculated by the formula given below [37]:

Crude Fiber content

Crude fiber was estimated by acid-base digestion known as Coarse fiber determination according to the following procedure [37]. The Crude fiber content in the samples by using the following formula:

$$\% \text{ Crude fiber } = \frac{\text{residue weight of sample}}{\text{sample weight}} \times 100$$

Crude Fat content

The crude fat content was determined according to the following procedure [37]. The percentage was calculated according to the following equations:

$$\% \text{ Fat } = \frac{\text{fat weight}}{\text{sample weight}} \times 100$$

Crude Carbohydrate content

The total carbohydrate content was determined according to the method (38).

Crude Protein content

The protein content was estimated by Burette method (39).

D. Determination of minerals components

The analyses of the minerals were done using the experimental method protocol proposed by Sadzawka(40).

E. Isolation of glycosides and flavonoids

Glycosides isolation

100 ml of 80% ethanol was added to 10 g of the sample; leave the mixture for 24 hr. The solution was filtered and focused in half by a rotary evaporator, and added to 50 ml of ether and 5 ml of lead acetate solution 0.3 M, concluded in separating funnel. Dried water layer at 30 °C until completely dry.

Flavonoids isolation

100 ml of 70% ethanol was added to 10 g of the sample. The mixture is heated to the boiling water bath with stirring for 2 hr. then filtered hot solution and the solvent is evaporated to dryness to obtain precipitate

F Antioxidant activity of C.myrrha

Reducing power assay

The reducing power of isolated glycosides and flavonoids was determined according to the method of Oyaizu (41).

Hydrogen peroxide scavenging

The ability of isolated glycosides and flavonoids to scavenge hydrogen peroxide were determined according to the method (42):

Phosphomolybdenum Method

The phosphomolybdenum method is based on the reduction of Mo6+ to Mo5+ in presence of antioxidant compound and subsequent formation of a green phosphate Mo5+ complex at acidic pH and at a higher temperature with a maximum absorption at 695 nm (43).

3. Results & Discussion:

Phytochemical screening

Preliminary phytochemical screening results of the three extracts of C. myrrha were illustrated in the Table 1.

Phytochemical constituent	Water extract	Extract Ethanol	Extract Pet. Ether
Glycosides	+	+	+
Flavonoids	+	+	+
Phenolic comp.	+	+	+
Tannins	+	+	+
Alkaloids	+	+	-
Phytosterols	-	-	-
Terpenoids	-	+	+
Saponins	-	-	-
Coumarins	-	-	-
Lignins	-	-	-
Quinines	+	+	-
Carbohydrates	-	-	-
Proteins	-	-	-

+ = indicates present, - = indicates absent

Estimation of some Secondary Metabolites
The quantitative contents of phenolic compounds, flavonoids, tannins, sterols and alkaloids are shown in table 2, which indicate the highest concentration of tannins 3677.1 ±2.15 mg/100g and then for alkaloids 1880 mg/100g, sterols 155.215 ±1.00 mg/100g, and Flavonoids 47.266 ±0.013 mg/100 g and phenolic compounds 30.647 ±2.481 mg/100g.

Table-2: Quantitative estimation of some secondary in C. myrrha

Phenolic compounds mg/100g	Flavonoids mg/100g	Tannins mg/100g	sterols mg/100g	alkaloids mg/100g
30.647±2.481	47.266±0.013	3677.1±2.15	155.215±1.00	1880

The presence of phenolic compounds and flavonoid components in the extract of C. myrrha were found to be low, but the tannins and alkaloids were found to be high when compared to (44). Plant phenolic compounds and flavonoids are highly effective free radical scavengers and antioxidants. They are used for the prevention and cure of various diseases which are mainly associated with free radicals [45].
Flavonoids have been reported to exert a wide range of biological activities. These include: Anti-inflammatory, antibacterial, antiviral, anti-allergic(46-48).
Tannins have also shown potential antibacterial and antiviral effects (49,50). Alkaloids protect against chronic diseases and earlier recorded that bitter leaf contains an alkaloid that is capable of reducing the headaches associated with hypertension (51).

Nutritional Profiling
Nutritional analysis has revealed that dried of C. myrrha has moisture content 8%, fiber content 8%, ash content 12.73%, fat content 18.68%, protein content 10.3%, carbohydrate content 55.07%, constituents of nutritive values as shown in table 3.

Table 3: Proximate Nutritive Values of C. myrrha

Moisture %	Fiber %	Ash %	Fat %	Protein %	Carbohydrate %
8±0.21	8±0.0	12.73±0.08	18.68	10.3	55.07

Result show that C. myrrha contain a percentage of ash 12.73% and fiber 8% high when compared to (44), but percentages of moisture 8% low when compared to (44). Soluble dietary fiber lowers total cholesterol, has the ability to relieve or prevent constipation, lowers the risk of diabetes by slowing down the absorption of sugar, and also helps in achieving healthy weight (52, 53). Result show protein contain 10.3% these values show consistency with (44, 54).

Mineral components
The mineral components of C. myrrha were shown in table 4. The C. myrrha contains high amount of magnesium, iron, sodium and Selenium.

Table 4: Mineral Components of C. myrrha

Fe(ppm)	Ca(ppm)	Mg(ppm)	Mn(ppm)	Na(ppm)	Cd(ppm)
141.643	21.857	365.304	2.648	43	0.186
Cr(ppm)	Pb(ppm)	Se(ppm)	Cu(ppm)	Zn(ppm)	Ni(ppm)
0.775	0.7515	18.777	0.661	0.6651	0.498

The concentrations of Fe^{+2}, Ca^{+2}, Mg^{+2}, Mn^{+2}, Cd^{+2}, Cr^{+3}, Pb^{+2}, Cu^{+3}, Zn^{+2} and Ni^{+3} found low when compared with(44).
The elements Fe, Ca, Mg, Mn, Na, Ni, Cu and Zn have been classified as essential elements, while Cd, Pb and Cr are non-essential elements for the human body [55]. The Manganese, which also are essential for normal functioning of central nervous system and are a good anti-oxidant [56]. *C. myrrha* may protect the body from Iron deficiency because it contains high amount of iron, in present of Mo, which helps the body to regulate iron stores [57] and Cu [58]. The presence of Ca^{+2}, Mg^{+2},Na^{+}, K^{+}, Co^{+3}, Cr^{+3}, Cu^{+3}, Fe^{+2}, Mn^{+2}, Ni^{+3} and Zn^{+2} reflects their function as essential nutrient elements, often as co-factor activators in metal-ligand enzyme complexes [55].

Antioxidant activity

Reducing power assay

Reducing power of both the flavonoids and glycosides isolated, and ascorbic acid as a reference antioxidant increase with the increase of sample and standard concentrations Figure 1. In this assay, flavonoids and glycosides isolated were exhibited lower reducing power than ascorbic acid. Flavonoids exhibited the most powerful effect in comparison to glycosides.

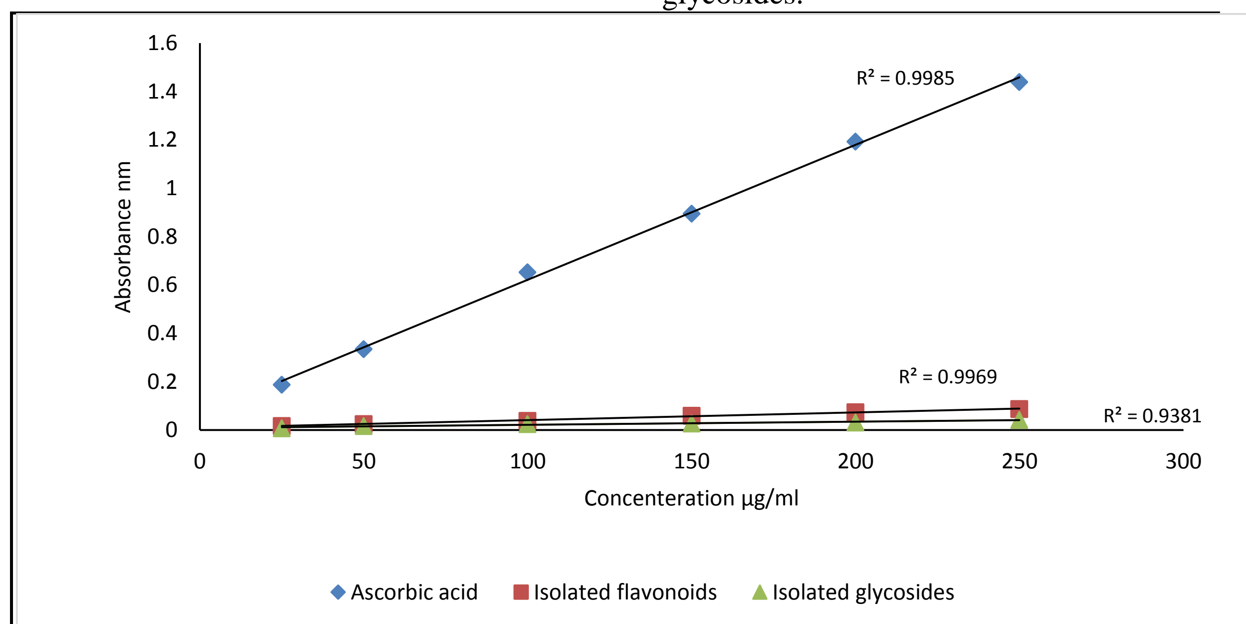

Figure 1:Reducing power assay of flavonoids and glycosides isolated

In this assay, Fe3+ reduction is often used as a significant indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action [59], and is correlated with the presence of reductones which exhibits its antioxidant

action by breaking the radical chain by donating a hydrogen atom [60]. The antioxidant capability of a plant is reportedly responsible for their polyphenolic content, which have potential reducing power due to presence of reductones (phenyl -OH group) that have the ability to donate a hydrogen atom to highly reactive free radicals resulting breaking of free radical chain reaction [61]. Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants. In this assay, the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of each compound [62].

Hydrogen peroxide scavenging assay

The flavonoids and glycosides isolated were exhibited higher absorbance in comparison to that of standard antioxidant ascorbic acid Figure 2. Flavonoids exhibited the most powerful effect in comparison to glycosides. Flavonoids and glycosides isolated were exhibited lower scavenging ability than ascorbic acid**.**

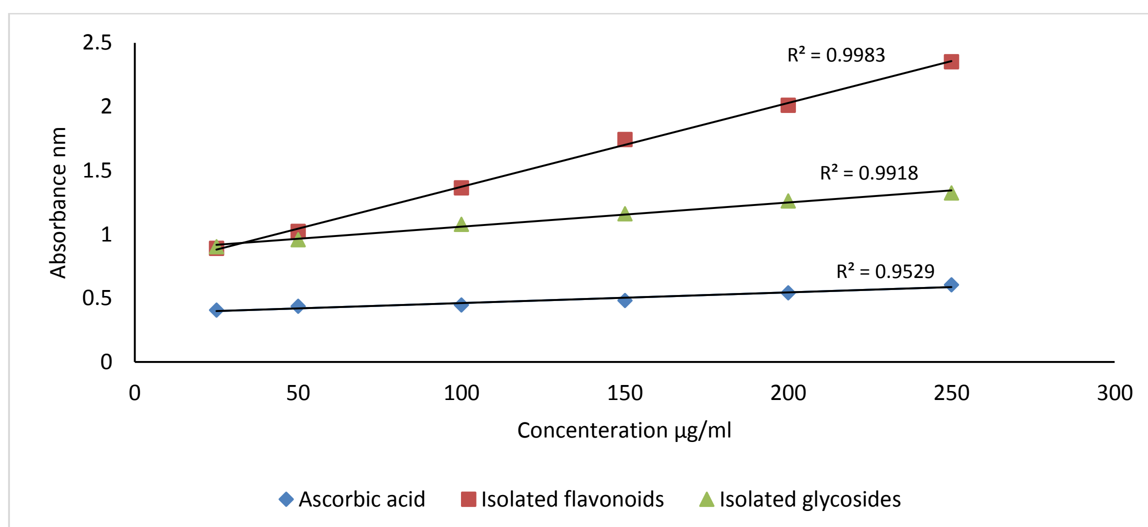

Figure 2: Hydrogen peroxide scavenging of flavonoids and glycosides isolated

The hydrogen peroxide scavenging abilities of the extracts were evaluated by determining their capability to convert hydrogen peroxide in to water. Polyphenolic and other compounds capable of donating electron might accelerate the conversion of H_2O_2 in to H_2O. The absorbance of H_2O_2 in presence of extract at different concentration was taken as a measure of scavenging activity [63].

Free radical especially reactive oxygen species (ROS), such as superoxide (O_2^-), hydroxyl (OH) and hydrogen peroxide (H_2O_2) have a greater brunt of human both from within the body and from their surroundings. If the body fails to eliminate, ROS can attack on biomolecules such as lipids, proteins, enzymes, DNA and RNA. Though, human body possesses many defense mechanisms through antioxidant enzymes and non-enzymatic compounds against these oxidative stresses. But when these free radicals go out of control, the organism becomes incapable to scavenge all ROS, which may lead to the development of chronic diseases, such as cancer, arteriosclerosis, nephritis, diabetes mellitus, liver injury, rheumatism, ischemia, cardiovascular and neurodegenerative disorders such as Alzheimer's and Parkinson's disease [64].

Chemical agents, radiation, toxins, deep fried foods and environmental factors such as pollution, radiation, cigarette smoke and herbicides can generate these reactive free radicals (65). Phenolic compounds and flavonoids are important plant secondary metabolites, that's having conjugated ring structures and hydroxyl groups, that may have the potential to function as antioxidants by scavenging the free radicals which are involved in oxidative processes via hydrogenation or complexation with oxidizing species and may resist many oxidative stresses and diseases [65].

The plant metabolites such as vitamins, like E and C, carotenoids or enzymes involved in the antioxidant mechanisms, shows their biochemical effects via several mechanisms, including hindrance of chain initiation, chelation of metal ions, breakdown of peroxides, sustained hydrogen abstraction, reductive ability and radical scavenging [66]. The free radicals like hydroxyl, nitric oxide, superoxide & lipid peroxyl and non-free radicals mostly include singlet oxygen and hydrogen peroxide, can be scavenged by natural antioxidants, that may be beneficial in various physiological and neurodegenerative disorders [67]. Though in all living organism there is a protective antioxidant system that protect the body systems from the consequences of free radical formations [66].

Phosphomolybdenum Method

The present study show that the flavonoids and glycosides isolated exhibited the lower antioxidant capacity for phosphomolybdate reduction, figure 3. Flavonoids exhibited the most powerful effect in comparison to glycosides.

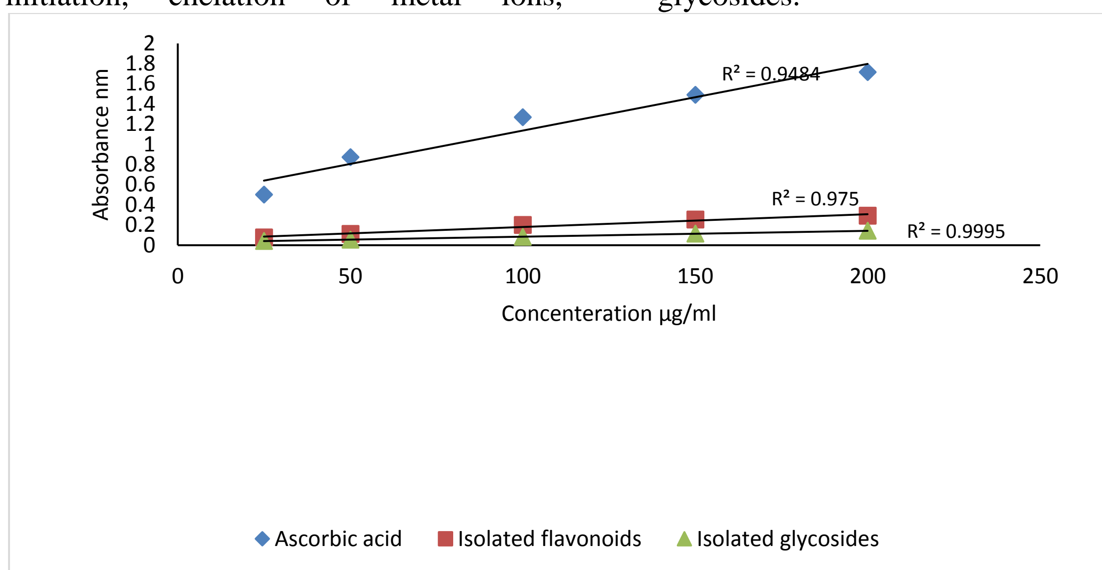

Figure 3: Total Antioxidant Capacity of flavonoids and glycosides isolated

Recent studies have shown that many flavonoids and related polyphenols contribute significantly to the phosphomolybdate scavenging activity of medicinal plants [68].

This activity is believed to be mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides [69].

Conclusion

The phytochemistry of myrrh showed the presence of bioactive compounds such as phenolic compounds, tannins, flavonoids, glycosides, quinine and terpenoids, and contain moisture, fiber, ash, fat, with logical amounts of Fe, Ca, Mg, Mn, Na, Cd, Cr, Pb, Se, Cu, Zn and Ni

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