TO EVALUATE THE ANTI-OXIDANT POTENTIAL OF ANTI-DIABETIC AYURVEDIC MEDICINES USING ABTS AND DPPH ASSAY

Dissertation/Project report submitted in partial fulfillment of the requirement for the degree of

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By

KANIKA BAJPAI

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DR. UDAYABANU M.

DEPARTMENT OF BIOTECHNOLOGY AND BIOINFORMATICS

JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY

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DECLARATION

I hereby declare that the work reported in the B-Tech thesis entitled **"TO EVALUATE THE ANTI-OXIDANT POTENTIAL OF ANTI-DIABETIC AYURVEDIC MEDICINES USING ABTS AND DPPH ASSAY"** submitted at **Jaypee University of Information Technology, Waknaghat, India,** is an authentic record of my work carried out under the supervision of **Dr. Udayabanu M.**. I have not submitted this work elsewhere for any other degree or diploma.

Kanika Bajpai (141827)

Department of Biotechnology and Bioinformatics

Jaypee University of Information Technology (JUIT), Waknaghat, India

Date

CERTIFICATE

This is to certify that the work entitled **"To evaluate the anti-oxidant potential of anti-diabetic Ayurvedic medicines using ABTS and DPPH assay"** pursued by **"Ms. Kanika Bajpai"** in partial fulfillment for the award of degree of Bachelor of Technology in Biotechnology from Jaypee University of Information Technology, Waknaghat, has been carried out under my supervision. This work has not been submitted partially or wholly to any other university or institute for the award of any degree or appreciation.

Dr. Udayabanu M.

Associate Professor

Department of Biotechnology and Bioinformatics

Jaypee University of Information Technology, Waknaghat

Dist – Solan, Himachal Pradesh- 173234

Email: udayabanu.m@juit.ac.in

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Kanika Bajpai

141827

LIST OF ABBREVIATIONS

- **DM :** Diabetes Mellitus
- **T2DM :** Type 2 Diabetes Mellitus
- **ABTS :** 2,2′-azino-bis(3-ethylbenzothiazoline)-6-sulphonic acid
- **DPPH :** 2,2-diphenyl-1-picrylhydrazyl
- **OD :** Optical Density
- **BHT :** 2,6-di-tert-butyl-4-hydroxytoluene

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SUMMARY

Diabetes Mellitus is a condition that is to a great degree genuine from both clinical and general wellbeing outlooks. The accustomed human services arrangement of India, Ayurveda offers an adjusted way to deal with treating this issue. Negative effect of radicals on people and creatures is in charge of developing examination enthusiasm for cancer prevention agent properties of substances, which shield living beings from the harming impact of these receptive species. Among various strategies for cancer forestalling agent action estimation, DPPH and ABTS are the most well known and usually utilized ones because of their simplicity, speed, impressionability and the utilization of stabile radicals. In the present examination, the counter oxidant activiity of ayurvedic solutions i.e. Madhunashini vati by Patanjali, Hyponidd by Charak Pharma, and Diabecon by Himalaya has been considered, to trap free radicals is estimated by the response energy between the inspected cell brace and the radical.

CHAPTER 1

INTRODUCTION

1.1 Diabetes Mellitus

Diabetes is a gathering of metabolic diseases delineated by hyperglycemia occurring by virtue of mutilations in insulin surge, insulin development, or both. The predictable hyperglycemia of diabetes is associated with entire arrangement hurt, brokenness, and dissatisfaction of various organs, particularly the eyes, kidneys, nerves, heart, and veins.

1.2 Types of Diabetes Mellitus

1.2.1 Type 1 Diabetes

Type 1 diabetes (ahead of time known as insulin-penniless, immature or youth starting) is portrayed by lacking insulin creation and requires well ordered relationship of insulin. The clarification behind type 1 diabetes isn't known and it isn't preventable with current information. Signs combine unbelievable arrival of pee (polyuria), thirst (polydipsia), determined requiring, weight diminishment, vision changes, and fatigue. These indications may occur all of a sudden.

1.2.2 Type 2 Diabetes

Type 2 diabetes happens as intended because of the body's deficiency with regards to utilization of insulin. Type 2 diabetes contains the dominant part of individuals with diabetes around the globe, and is, everything viewed as, the deferred result of abundance body weight and physical inaction. Signs may take after those of sort 1 diabetes, yet are a significant part of the time less stamped. Along these lines, the suffering might be explored quite a while ensuing to beginning, once pesters have as of late created. Beginning in the generally later past, this sort of diabetes was seen just in grown-ups in any case it is eventually in addition happening constantly routinely in kids.

1.2.3 Gestational Diabetes

Gestational diabetes makes in two a few women when they are pregnant. When in doubt, this sort of diabetes leaves after the child is born. Notwithstanding, on the off chance that you've had gestational diabetes, you have a more unmistakable shot of making compose 2 diabetes sooner or later further not far off. A bit of the time diabetes analyzed amidst pregnancy is really type 2 diabetes.

1.3 Epidemiology of Diabetes Mellitus

Diabetes is a general issue and tolerably standard in each made and moreover making nation. No longer a defilement of phenomenally rich countries, the inevitability of diabetes is perseveringly developing all around, most remarkably on the planet's inside wage nations. Exhaustive, an ordinary 422 million grown-ups were living with diabetes in 2014, emerged from 108 million of each 1980. Diabetes caused 1.5 million passings in 2012.

1.4 Antioxidants

A growth anticipation specialist is a molecule that curbs the oxidation of various particles. Oxidation is a compound reaction that can convey free radicals, provoking chain reactions that may hurt cells. Tumor counteractive action specialists, for instance, thiols or ascorbic destructive (vitamin C) end these chain reactions.

1.5 ABTS assay

In natural science, 2,2′-azino-bis(3-ethylbenzothiazoline)-6-sulphonic acid) or ABTS is a substance compound used to watch the reaction vitality of specific proteins. An average use for it is in the impetus associated immunosorbent look at (ELISA) to recognize for definitive of molecules to each other.

It is consistently used as a substrate with hydrogen peroxide for a peroxidase compound, (for instance, horseradish peroxidase) or alone with blue multicopper oxidase chemicals, (for instance, laccase or bilirubin oxidase). Its usage allows the reaction vitality of peroxidases themselves to be taken after. Hence it moreover can be used to roundaboutly take after the reaction vitality of any hydrogen peroxide-making protein, or to simply assess the measure of hydrogen peroxide in an illustration.

ABTS is moreover as frequently as conceivable used by the sustenance business and cultivating researchers to measure the cell support breaking points of foods. In this measure, ABTS is changed over to its radical cation by extension of sodium persulfate. This radical cation is blue in shading and acclimatizes light at 734 nm. The ABTS radical cation is responsive towards most growth avoidance specialists including phenolics, thiols and Vitamin C. In the midst of this reaction, the blue ABTS radical

cation is changed over back to its bleak fair-minded shape. The reaction may be watched spectrophotometrically.

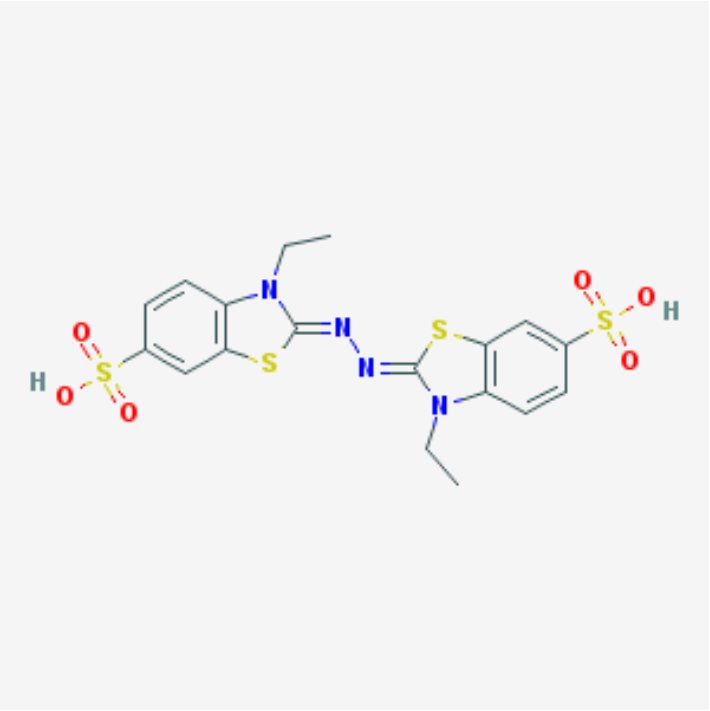

Figure1.1 Structure of ABTS

1.6 DPPH assay

DPPH is a typical condensing for the natural synthetic compound 2,2-diphenyl-1-picrylhydrazyl. It is a dim shaded crystalline powder made out of stable free-radical atoms. DPPH has two noteworthy applications, both in lab look into: one is a screen of substance responses including radicals, most quite it is a typical cell reinforcement measure, and another is a standard of the position and power of electron paramagnetic reverberation signals.

DPPH is an outstanding radical and a trap ("scavenger") for various radicals. In this way, rate decline of an invention interminable supply of DPPH is used as a marker of the radical thought of that reaction. By virtue of a strong absorption band centered at around 520 nm, the DPPH radical has a significant violet shading in course of action, and it ends up dreary or light yellow when executed.

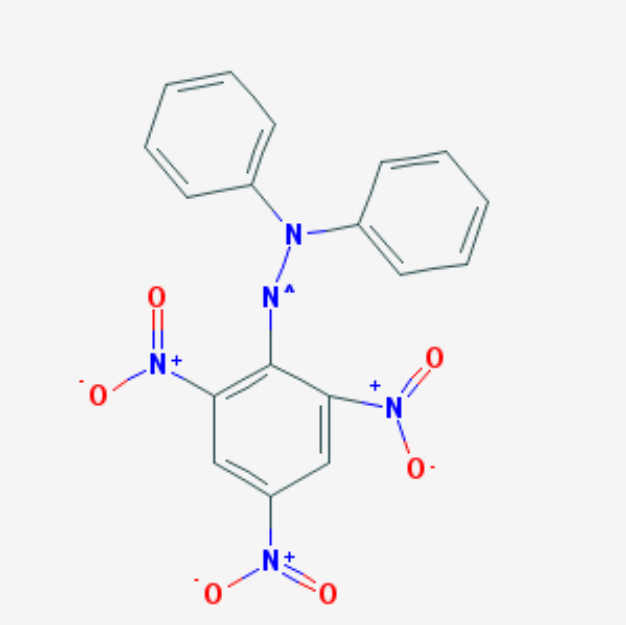

Figure 1.2 Structure of DPPH

CHAPTER 2

LITERATURE REVIEW

2.1 Diabetes Mellitus

Diabetes suggests a metabolic issue portrayed by relative or total deficiency of insulin discharge or possibly insulin protection.[2] The distress shows an imperative restorative issue that beginning at now impacts 382 million individuals the world over combining 316 million patients with blocked glucose obstacle. This masses may two fold by 2030. Diabetes is known to be one of the vital purposes behind mortality and horridness on the planet. It impacts the possibility of patient's essence with an assortment of indications which intertwine torment, insufficiency, ataxia, weakness, and unmistakable difficulty.[6]

There are three key flaws in the start of hyperglycemia in DM, specifically extended hepatic glucose creation, lessened insulin emanation, and crippled insulin action. Consistent prescriptions treat diabetes by improving insulin affectability, growing insulin age and moreover decreasing the measure of glucose in blood.

Diabetes has been known as an oxidative weight issue caused by disproportion between free radical plan and the limit of the body's ordinary growth counteractive action operators. Various examinations have prescribed that oxidative weight expect a section in foundational disturbance, endothelial brokenness, impaired release of pancreatic cells and blocked glucose use in periphery tissues.[7]

In patients with type 2 diabetes mellitus (T2DM) who are in risk, concentrated intervention with different prescription mixes and lifestyle changes exhibited a beneficial effect on vascular troubles and reduce passing rate as a result of cardiovascular affliction and diverse causes. There has moreover been shown that adjust of responsive particles would altogether be able to limit the progression of endothelial brokenness, cardiomyopathy, retinopathy, nephropathy, and neuropathy in patients with DM. Starting late the use of growth counteractive action operators still remains a dialog, anyway its use as a treatment for DM can be considered in light of the way that its showed feasibility in cutting down the peril of making diabetes and its perplexities. Diverse disease anticipation operators have been delivered for oxidative weight treatment in DM, including the use of vitamins and supplements and furthermore the usage of a couple of parts of plants and fresh characteristic items which have shown cell fortification" effect in DM patients. In some present examinations, a couple of

prescriptions routinely used as a piece of the treatment of DM also displayed growth counteractive action operator impacts. [7]

2.2 Antioxidant activity by ABTS assay and DPPH assay

A system for the screening of cell fortification development is represented as a decolorization measure significant to both lipophilic and hydrophilic disease counteractive action specialists, including flavonoids, hydroxycinnamates, carotenoids, and plasma cell fortifications. The pre-formed radical monocation of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic destructive) (ABTS•+) is delivered by oxidation of ABTS with potassium persulfate and is diminished inside seeing such hydrogen-giving tumor counteractive action operators. The effects of both the union of growth avoidance specialist and length of reaction on the limitation of the radical cation osmosis are considered while choosing the cell support activity.

The purpose of this examination was to look into the free radical scrounging utmost of darker sugars in view of the staying small film covering on the sugar valuable stone. ABTS and DPPH tests were associated with dull hued sugar liquid courses of action and dichloromethane expels. The particles that might be responsible for the activity, phenolic blends, and Maillard reaction things were perceived by GC-MS and LC-MS. [7]

To assess the equivalence of the two most normal radical rummaging examines utilizing 2,20-azino-bis-3-ethylbenzthiazoline-6-sulphonic corrosive (ABTS) and 1,1-diphenyl-2 picrylhydrazyl (DPPH) radical, the 50 most well known cell reinforcement rich organic products, vegetables and drinks in the US eating regimen were distinguished and broke down for their cancer prevention agent limits, add up to phenolics and flavonoids content.[8] Spearmans– Rho connection coefficients were figured keeping in mind the end goal to describe the connection between cell reinforcement limits, add up to phenolics and flavonoids content. Cancer prevention agent limit demonstrated a solid positive relationship contrasting both measures ($r = 0.949$, $p < 0.001$).[9] Antioxidant limit distinguished by ABTS test was more grounded decidedly connected with the oxygen radical absorbance limit (ORAC) from USDA database and flavonoids content. Cell reinforcement limit recognized by ABTS test was altogether higher for natural

products, vegetables and refreshments contrasted with that by DPPH test. The high-pigmented and hydrophilic cancer prevention agents were preferable reflected by ABTS measure over DPPH examine. These information propose that ABTS examine might be more helpful than DPPH test for recognizing cell reinforcement limit in an assortment of foods. [9]

The inspiration driving this examination was to study the cell support activity of carotenes and xanthophylls evaluated by various procedures, appeared differently in relation to a-tocopherol, BHA and BHT. Four inspects were refined a broad assortment of specific benchmarks. Among them, the two are ABTS radical cation, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) seeking measure. Most of the blends demonstrated critical differences in their activity of looking radicals depending upon the test used. Of the 22 blends attempted, only two or three, for instance, lutein, zeaxanthin and capsanthin gave equivalent results in the distinctive looks at. Shockingly, as opposed to a-tocopherol, BHA and BHT, carotenoids did not exhibit any DPPH looking activity. This procedure was used to study the cell fortification cutoff of a couple of crushes and oil tests. The most hoisted lipophilic malignancy counteractive action specialist restrain in all looks at was looked for sea buckthorn berry juice, trailed by tomato juice, carrot squeeze and crushed orange. Inside the oil tests, the demand of cell fortification point of confinement depended upon the measure used. [12]

CHAPTER 3

AIM AND OBJECTIVE

3. AIM AND OBJECTIVE

3.1 To evaluate the anti-oxidant potential of anti-diabetic Ayurvedic medicines using ABTS and DPPH assay.

CHAPTER 4

MATERIALS AND METHOD

4. Materials and Method

4.1 Screening Antioxidant activities of different anti-diabetic ayurvedic medicines

4.1.1 Materials

- Conical flask
- Weighing balance
- Cylindrical flask
- Methanol
- Distilled water
- ABTS salt
- Potassium permagnate or Potassium persulphate
- DPPH
- 80% ethanol
- Sample
- Distilled water test tube
- Eppendorf (2ml)
- Pipette
- Tips for pipette
- Microtitre well plate
- Spectrophotometer
- **Anti-diabetic medicines**: (Madhunashini vati by Patanjali, Hyponidd by Charak pharma, Diabecon by Himalaya)

4.1.2 Experimental procedure

Solvent extraction of the medicines:

- 10 tablets of each medicine were taken and weighed.
- Using mortar and pestle, medicines in tablet form were converted into powdered form.
- After converting into powdered form, each medicine was mixed with methanol (100mg/100ml) in 3 different conical flasks respectively **(by organic solvent extraction).**
- Each medicine was mixed with distilled water (100mg/100ml) in 3 different conical flasks respectively **(by aqueous solvent extraction).**
- All the flasks were covered with aluminium foil and then they were kept for maceration for 3 days at room temperature.
- After 3 days, the solvent was filtered (fig. 4.1) and then their antioxidant activities were checked by ABTS and DPPH assay.

Figure 4.1 After maceration, solvent was filtered

ABTS assay:

- ABTS was prepared by mixing a stock solution with potassium persulphate and dissolved in 80% ethanol.
- The reagent was then incubated overnight in dark.
- ABTS solution was diluted with 80% ethanol to an absorbance of 0.8±0.05 at 734nm.
- 50µL of sample was mixed with 140µL of ABTS solution and was allowed to stand at room temperature for 30 minutes in dark condition.
- The absorbance was determined at 734 nm.
    - 1450µL ABTS + 50µL sample [10 dilutions]
    - 1450µL ABTS + 50µL sample [100 dilutions]
    - Reaction volume= 1500µL= 1.5 mL

DPPH assay:

- DPPH reagent was prepared by adding 0.01183g in 100mL of 80% ethanol and incubated overnight in dark.
- 50µL of diluted sample were taken in micro centrifuge tubes.
- Absorbance of the reagent was brought down to 0.8 using 80% Ethanol.
- 1250µL of this reagent was added to each sample.
- Samples were incubated for 20 minutes in dark conditions.
- Absorbance of each sample was taken at 515nm.

CHAPTER 5

RESULTS AND DISCUSSION

5. RESULTS AND DISCUSSION

The results shows the antioxidant activities of the anti-diabetic ayurvedic medicines i.e. Madhinashini Vati by Patanjali, Hyponidd by Charak Pharma, and Diabecon by Himalaya, which have been studied using the ABTS assay and DPPH assay.

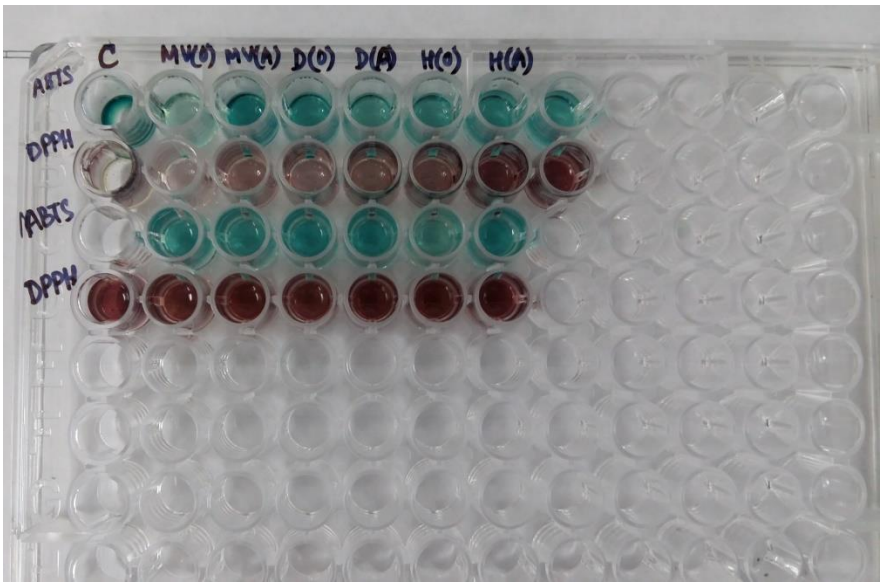

Figure 5.1: The changing color of samples containing ABTS and DPPH in different concentrations

Formula used for calculating the scavenging percentage is:

$$\%\ \boldsymbol{scavenging} = \frac{\textbf{OD of the control} - \textbf{OD of the sample}}{\textbf{OD of the control}} \times \mathbf{100}$$

Table 5.1: OD observed at 734nm and 515nm for ABTS and DPPH assay respectively.

Name of medicine	ABTS assay (734nm)	DPPH assay (515nm)	Antioxidant activity	Antioxidant activity
control	0.799	1.208	ABTS	DPPH
Madhunashini Vati				
organic	0.775	0.91	3.003	24.66
aqueous	0.703	1.049	12.01	13.16
Diabecon				
organic	0.779	0.9	2.503	25.49
aqueous	0.786	1.105	1.627	8.52
Hyponidd				
organic	0.485	1.076	39.29	10.92
aqueous	0.79	1.151	1.12	4.71

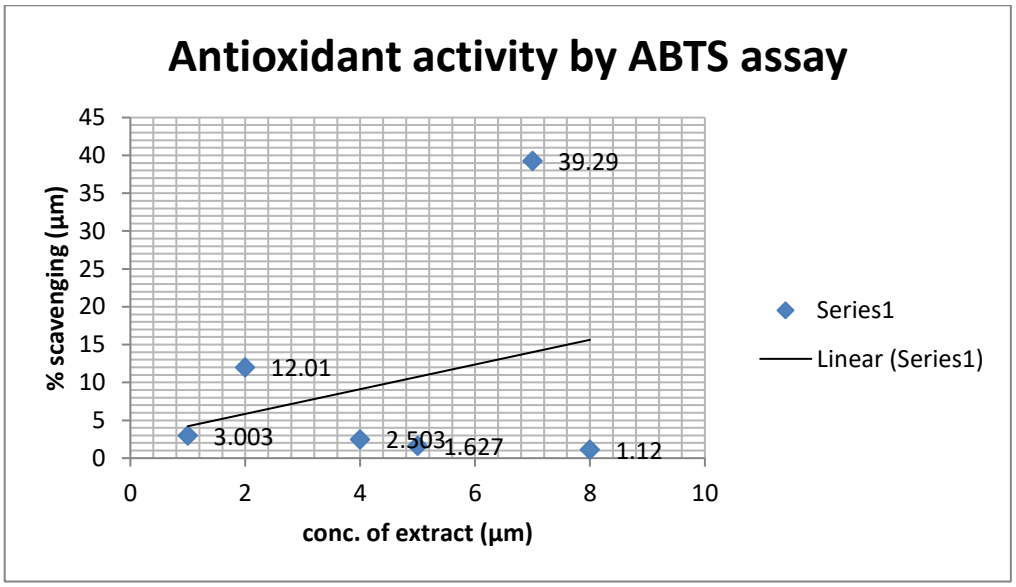

Figure 5.2: This graph shows the Antioxidant activity by ABTS assay

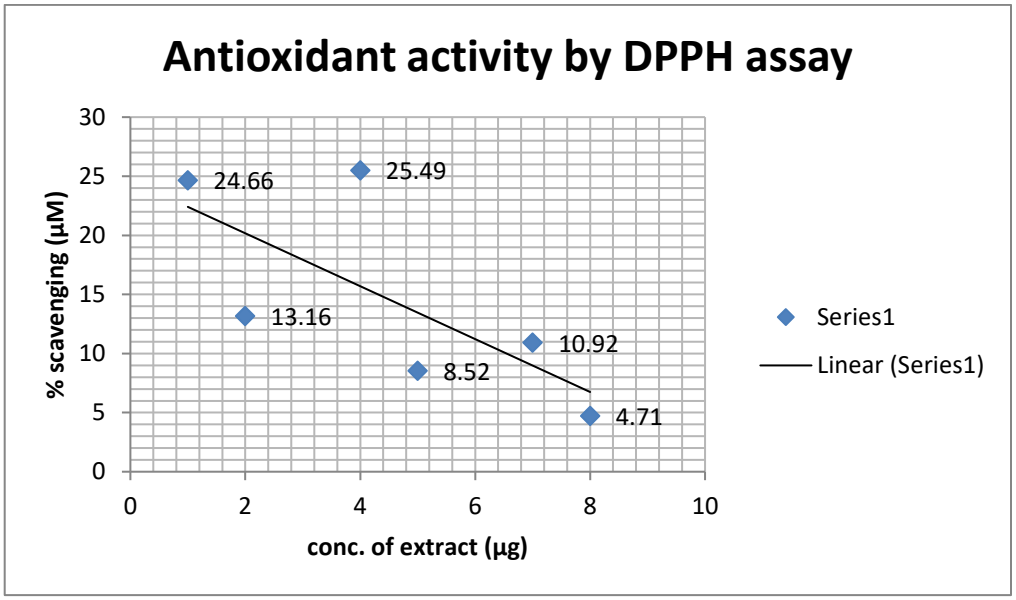

Figure 5.3: This graph shows the Antioxidant activity by DPPH assay

DISCUSSION

In this study, the Antioxidant activity of the Anti-diabetic ayurvedic medicines was determined using ABTS and DPPH assay by spectrophotometric analysis. It has been observed that the percentage scavenging increased with concentration as the OD decreased.

CHAPTER 6

CONCLUSION

Conclusion

Negative influence of the reactive oxygen species on living organisms and on the stability of food products is responsible for a significant interest in substances exhibiting antioxidant properties and in the methods of estimating them. In most such methods, the ability of antioxidants to trap free radicals is measured by the reaction kinetics between the examined antioxidant and the radical. The methods applying chromogen compounds are commonly used due to their ease, speed and sensitivity most popular being those employing the stable DPPH or ABTS.

As results from the presented data:

- ABTS and DPPH is adequate for measuring antioxidant characteristics when the spectrum of examined antioxidant or real biological system does not coincide with the wavelength used to monitor colored radical depletion;
- The Antioxidant activity of the Anti-diabetic ayurvedic medicines was determined using ABTS and DPPH assay by spectrophotometric analysis. It has been observed that the percentage scavenging increased with concentration as the OD decreased.

CHAPTER 7

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