IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

COMPARSION STUDIES OF POLYHERBAL FORMULATION IN ESSSENTIAL OIL AND INFUSION BY ANTI-OXIDANT METHOD.

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Abstract: In this study investigation, *Nigella sativa* (black seed), *Trigonella foenum graecum (fenugreek,), Trachyspermum ammi (Ajwain)* polyherbal formulation are mainly focused in antioxidant activity by DPPH (2,2-dipheny1picryl-1-hydrazine) free radical scavenging assay. The main aim of the studies is to evaluate strong antioxidant activity. The Polyherbal formulation of Essential oil and infusion were compared to standard ascorbic acid. As a result, obtained the IC50 value of volatile oil and infusion filtrate (43.98mg/ml and 39.997mg/ml) for ascorbic acid(36.981mg/ml) respectively. Hence, infusion shows more antioxidant activity compared to essential oil.

Keywords - Polyherbal formulation, antioxidant, DPPH etc.

I.INTRODUCTION

Antioxidants and artificial substance which prevent generation of free radicals. Free radical is unstable substances it is also referred as Reactive oxygen species (ROS)when free radical increased represents an Oxidative stress[(1)]. Oxidative stress is defined as imbalance state of producing and accumulating of oxygen. Free radicals occur in many shapes, size and Chemical configuration. Generally, they steal the electron from the other substances[(2)]. The stolen electrons in the human body can cause damage in the cell membrane, cellular protein lipids, DNA and other structure. They play major role in cell division[(3)].

There are thousands or different substances can act as antioxidants. The important antioxidants such as vitamin A, C, E, Beta-carotene and some minerals like lycopene, lutein[(4).] When it came to public attention, many scientists have undergone studies in the free radicals which lead to damages in the DNA or cellular mechanisms. It also includes chronic diseases, loss of vision, cancer &aging. Less intake of fruits and vegetables can also lead to antioxidant[(5)].

There are some scientific confirmations can reduce antioxidant property including chronic or heart diseases. Some plant-based products like fruits and vegetables or whole grains[(6)]. Most of the anti-oxidant activity are established from the plants source with a wide range physical and chemical properties[(7)]. The actively and inexpensive method is 2,2-dipheny1picryl-1-hydrazine (DPPH) assay which are commonly used in the experiments.2,2- dipheny1picryl-1-hydrazine (DPPH) is a dark crystalline powder of a stable molecules. The electrons from the nitrogen atom are decreased by taking the electrons from the hydrogen atom corresponding to the hydrazine. DPPH shows a maximum absorption range in UV spectrophotometric against the blank at 517nm[(8)].when DPPH solution is mixed with a ethanol gives raise to violet colour complex, colored complex is due to the absorption disappears as the electron pair off. On mixing the DPPH solution with a test substance donate hydrogen atom, it decreases the violet colour, the decolorization is due to reducing ability of electron and gives raise to yellow colored complex from the picryl group still is present. Compared to the reference standard group of ascorbic acid[(9)].

This *Nigella sativa* (black seed)[(10)], *Trigonella foenum graecum (fenugreek)* [(11)]*Trachyspermum ammi (Ajwain)*[(12)]has been reported to have antioxidant properties. The main aim of the work is to evaluate the health beneficial effects in Antioxidant.

II.NEED OF THE STUDY

Generation of free radicals can cause damages to the DNA. To overcome this herbal medicine is gaining more importance in developing and developed countries. Herbal medicines and plant possess no side effects and toxicity. It represents health beneficial in the herbal medicines. Polyherbal formulation used in the studies of anti-oxidant are *Nigella sativa* (black seed), *Trigonella foenum graecum (fenugreek,), Trachyspermum ammi (Ajwain)* has been reported in ancient literature as a drug useful in treating the Antioxidant activity.

III.RESEARCH METHODOLOGY

3.1 MATERIALS AND METHOD

The raw material used in the poly herbal formulation *Nigella sativa* (kalonji), *Trigonellafoenum-graecum*(fenugreek), *Trachyspermum ammi* (Ajwain) were procured locally from Amruth kesari herbal drug vendor Hosakerehalli, Bengaluru-560085.The raw material was identified and authenticated by prof Dr Sathish and Dr Urmila department of pharmacognosy, Nargund college of pharmacy.

3.1.2Infusion

The seeds were dried in shadow and grinded into coarsely powder.100gm of crude drug is moistened, in a suitable vessel with a cover, 300 ml boiling water is added, and the vessel is covered tightly and allowed to stand for 30 min. The mixture is strained and the infusion filtrate measure 250 ml[13][14].

3.1.3Essential oil

Crude drug was subjected to vaccum distillation, 200gm of mixture is added into the solvent 2/3rd of the distillation flask and liquid is heated under reduced vaccum pressure. The liquid turns into vapour form and vice-versa. Liquid was collected in the receiver. Now these vapors are pass through water condenser and condensate is collected in receiver. The condensate polyherbal formulation is found to be 105ml of essential oil[15].

3.1.4 ANTIOXIDANT ACTIVITY BY DPPH ASSAY

0.1mg/ml stock solution of ascorbic acid was prepared. Sample extracts of essential oil and infusion aliquots0.1ml, 0.2ml, 0.3ml, 0.4ml, 0.5ml, 0.6ml were taken and this concentration is prepared by various methods. To the mixture 1ml of DPPH (500mM in ethanol) and make up the volume with ethanol[16]. Shake vigorously and kept 30mins protected from the sunlight. Read the absorbance 517nm UV spectrophotometric against the blank (ethanol serves as blank). BHA (Butylated Hydroxy Anisole) is another name of Ascorbic acid acts as a standard compound. Perform the experiments in triplicate. Read the absorbance and calculate according the below mentioned formula. DetermineIC50 value which is the concentration of the sample is 50% inhibited of the DPPH free radical was calculated using log dose inhibition curve[16].

The percentage DPPH radical scavenging activity is calculated as follows:
%DPPH scavenging effect= [(Abs Control-Abs sample)/Abs control] *100
Where Abs control-absorbance of control
Abs sample-absorbance of sample

3.2 Statistical analysis

The date was obtained from these studies is mean ± SD. All the test sample were analyzed by descriptive statistics data method by using Microsoft excel.

IV. RESULTS AND DISCUSSION

4.1 Results of Descriptive Statics of Study Variables

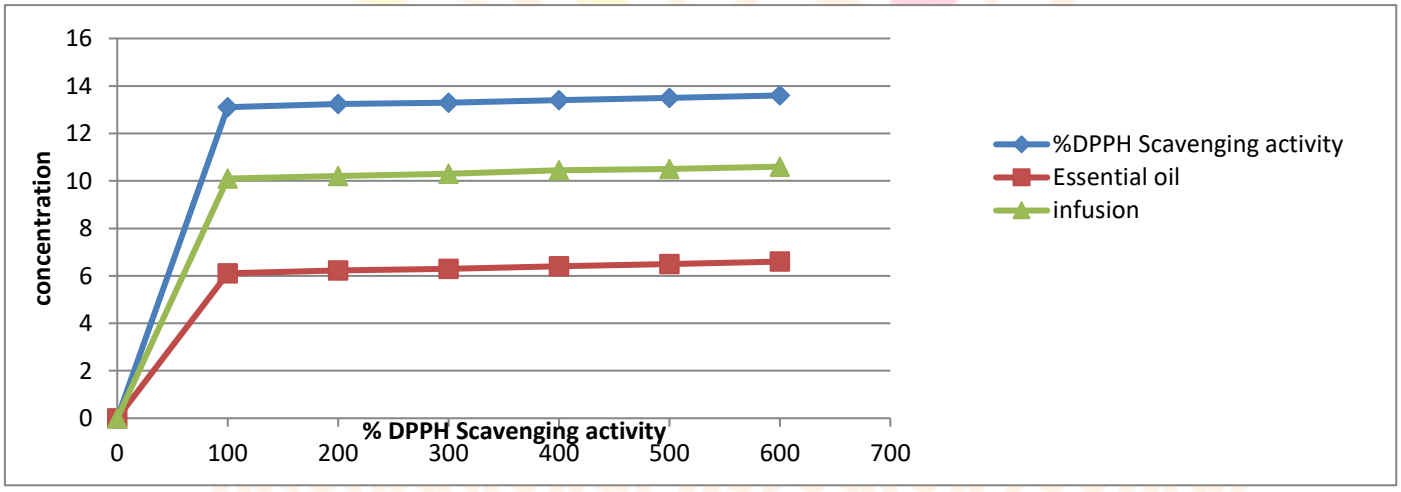
Table 4.1 Standard: Ascorbic acid

Concentration in µg/ml	%DPPH Scavenging activity	IC $_{50}$ in mg/ml
100	13.1	36.981
200	13.24	
300	13.3	
400	13.4	
500	13.5	
600	13.6	

Table:4.2Infusion And Essential oil

Concentration in µg/ml	% DPPH Scavenging activity		IC_{50} in mg/ml	
	ESSENTIAL OIL	INFUSION	Essential OIL	INFUSION
100	6.1	10.1	43.986	39.997
200	6.23	10.2		
300	6.3	10.3		
400	6.4	10.45		
500	6.5	10.5		
600	6.6	10.6		

Ascorbic acid, Infusion and Essential oil



The inhibitory concentration of polyherbal extract IC_{50} values showed table:4.2 that Essential oils **43.986mg/ml** - exhibited Antioxidant activity lower than the Standard-Ascorbic acid table:4.1 **36.981mg/ml**.

The Observed IC_{50} values showed that Essential oils at **43.986mg/ml** exhibited Antioxidant activity lower than the Infusion extract at **39.997mg/ml**.

Thereby, the observed IC50 values showed that Infusion extract exhibited highest antioxidant activity followed by Essential oils. Further, Infusion extract was used in Study.

In this study investigation of polyherbal formulation *Nigella sativa* (black seed), *Trigonella foenum graecum (fenugreek,), Trachyspermum ammi (Ajwain)* of essential oil and infusion filtrate shown a strong antioxidant property by DPPH assay as compared to ascorbic acid. The IC50 value of essential oil and infusion filtrate (43.98mg/ml and 39.997mg/ml) for ascorbic acid(36.981mg/ml) respectively. As a result, infusion filtrate shows more antioxidant activity than essential oil.

ACKNOWLEDGMENT

First and foremost, I would to like to thank first GOD, the Almighty for the blessings without them nothing can be achieved. The author is thankful to staff members and management of Nargund college of Pharmacy. We thank the VGST (GRD-178) RGUHS for supporting the present work. The management at Nargund College of Pharmacy Bengaluru for supporting and providing necessary infrastructural support. Our sincere thanks to Dr Rajeev D Hiremath for the guidance provided.

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