International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

The *In Vitro* Antioxidant Activity of Aqueous and Alcohol Extract of Medicinal Plant Individually and In Polyherbal Combination Using DPPH Method

Ms. Dipali A. Rathod[1], Dr. Ranajit D. Tijare[2], Ms. Sayali G. Thakare[3]

[1]B. Pharma Student Ishwar Deshmukh Institute of Pharmacy, Digras Maharashtra, India
[2] Principal, Department of Pharmacognosy, Ishwar Deshmukh institute of Pharmacy, Digras.
[3]B.Pharma Student Ishwar Deshmukh Institute of Pharmacy ,Digras Maharashtra ,India
Sant gadge Baba Amaravati University, Amaravati Maharashtra India

Abstract: The present study deal with the aqueous alcohol extract of *Allium sativum* (bulb), *Phyllanthus emblica* (fruits) and *Curcuma longa* (rhizomes) were used for relative analysis of antioxidant activity. Antioxidant activity was determined by DPPH (1,1-dipharyl-2-picrylhydrazyl) method and expressed with ascorbic acid. It was found that *Phyllanthus emblica*, *Trigonella foenum graecum* and *Curcuma longa* had more antioxidant activity than the *Allium sativum.* In combination for all four herbal drug were the *in vitro* antioxidant activity was more significant with DPPH radical scavenging potential. *In vitro* screening used for the study and evaluation of herbal drug for antioxidant activity in combination or individually.

Keywords: Antioxidant activity, Ascorbic acid, ethanobotanical, radical scavenging ,culinary

Introduction:

1.1 Herbs:-

The term “HERB” is derived from the latin word “Herbe”

Herb can be defined as “any plant which can be leaves, stem, flowers, roots and seeds used for different purpose like food, medicine, flavoring and perfumes. Any other part of the plant, which is usually dried, is referred to as a spice. These include, for example, barks (cinnamon), berries (peppercorns), seeds (cumin), roots (turmeric), flowers (chamomile), buds (cloves) and stigmas of flowers (saffron).

Herbs are used for both culinary and medicinal purposes sometimes even spiritual ones.

Herbs is also described as small, seed bearing plant with fleshy parts, not only these herbs also includes shrubs , vines, annuals, trees and primitive plants. Herbs have been used to augment cosmetics, preserve foods and cure illnesses.

The definition of a medicinal herb is more expansive than that of a culinary herb. Medicinal herb may be shrubs or other woody plants, but culinary herbs are limited to the leaves only of non- woody plants. Any portion of the plant may be considered herbs in medicinal and spiritual use, including the plants “fruits and vegetables.

Fresh herbs often contain higher antioxidant levels compared to processed or dried herbs Consuming herbs may help to prevent and manage heart disease, cancer and diabetes. It may also help to reduce blood clots and provide anti-inflammatory and anti-tumor properties .
Herbs have been used to cover up unpleasant household odors, to enhance the flavor of dull foods, to disguise body odor, and to mask the unpleasant flavor of meats that were going badly.

1.2 Why do we need herbs:-

Herbs have been used since ancient times for their medicinal properties, mostly concentrated into teas and tinctures. The information carried or concerned by the olden books or the people is considered as ethanobotanical uses of the plant, which can be used to screen the same plant for the various biological screening, based on the chemical constituents.
This will be the new trend in the haling of the body organs and the body cells from the acute and chronic disease or the infections.
Every day our body cells face a threat, viruses and infection attack them. Free radicals also can damage your cells and DNA. Some cells can heal from the damage, while others cannot. Scientists believe molecules called free radicals can contribute to the aging process. They may also play a part in disease like cancer, diabetes and heart diseases.
Antioxidants are chemicals that help stop or limit damage caused by free radicals. Your body uses antioxidants to balance free radicals. This keeps them from causing damage to other cells. Antioxidants can protect and reverse some of the damage. They also boost your immunity. Herbal medicine is the use of plants to treat disease and enhance general health and wellbeing.

1.3 Herbal medicine:-

The chemical compounds in plants that have medical value have an impact on how the human body functions physiologically. In traditional medicine, plants have a special function since they include phytochemicals such alkaloids, flavonoids, terpenoids, steroids, carotenoids, and other phenolic compounds. The antioxidant properties of these phytoconstituents work as a barrier against many microorganisms and other diseases. A lot of them are utilised as dietary supplements and spices. Drugs known as antioxidants have the capacity to shield the body from oxidative stress brought on by free radicals. Numerous diseases, including cancer, diabetes, and neurodegenerative disorders, have been proven to be significantly triggered by oxidative stress. Lipid, protein, and carbohydrate oxidation by hazardous reactive species results in DNA mutation and damages cells.
Synthetic antioxidants cause Geno poisoning, whereas natural antioxidants do not. These plants' phytochemicals or secondary metabolites make them useful as a preventative measure against a variety of pathogenic illnesses. Antioxidant action is present in many phytochemicals, including anthocyanins, flavonoids, and other phenolic compounds. Due to the pathogens' medication resistance, attempts have been made to identify their replacement for the treatment of diseases. With the understanding of plant extracts' antioxidant activity, they may play a key role in the treatment of microbial diseases. Herbs are employed as natural antioxidant resources by parents. Four plants fenugreek, garlic, turmeric, and amla that are utilised as herbal supplements and medicines all over the world are the subject of the current study. The current study's goal was to look into phytochemicals. Refer table no.1.1

Table no 01: A brief summary of selected plant

Name of the plant	Family of the plant	Property of the plant
Garlic (*Allium sativum*)	Liliaceous	It has antiseptic, anticoagulant. Antioxidant, analgesic etc
Fenugreek (*Trigonella foenum-graecum*)	Leguminous	It has anti-ageing, antioxidant, antidiabetic etc
Turmeric *(Curcuma longa)*	Zingiberaceae	It has antimicrobial, antioxidant, anti-cancer, anti-inflammatory etc.
Amla (*Phyllanthus emblica)*	Euphorbiaceous	It has antioxidant activity, anti-cancer, analgesic etc

Why? Selected the combination drug of Amla, Fenugreek, Garlic, Turmeric

a few different polyherbal medication extracts using the DPPH technique. To increase or boost a few polyherbal drugs' antioxidant capacity. Check the antioxidant activity on pharmacological screening to investigate the chemical elements of herbs. To determine the impact of a medicine on The studied plant material showed significant activity compared to the individual plants, as the plant material combined the activity *in vitro* with the antioxidant activity

Objective

Study the combined in vitro antioxidant properties of bodily cells, in vitro research is used. To improve the combined formulation's efficacy above other herbal medicines.

Studying a plant's antioxidant activity is essential for choosing the right medication for a variety of diseases, including diabetes, liver damage, heart disease, and many others. This is because the antioxidant property of the plant is what prevents various damages from free radicals.

The present study includes utilization of 1:1 ratio of extracts, which may be more effective as antioxidant potential using DPPH method. The 1:1 ratio for individual and combined extract.

Material And Equipments

Chemical : Folin – Ciocalteu's phenol reagent, Na_2CO_3, $AlCl_3$, 1, 1- diphenyl-2-picrylhydrazyl(DPPH), 2,2'- azino-bis (3- ethylbenzothiazoline-6- sulfonic acid),(ABTS), Potassium persulfate ($K_2S_2O_8$), Potassium ferricyanide, Trichloroacetic acid, Ferric chloride, Ferrozine, Peroxidase from horseradish, Hydrogen Peroxide were obtained from (Sigma – Aldrich) Ethanol 99% and distilled water, crude course powder of Amla, Fnugreek , Turmeric, Garlic.40 gm Each crude course drug sample.

Methodology And Experimental Work

Collection: The species of Garlic, Fenugreek, Amla, and Turmeric taken from fresh farms field. Plant petals, room, fruits, were shade dried and crushed in course powder form. From each dried powder take 40 gm of sample

Maceration process : Maceration is an extraction method that involves keeping a plant in touch with a liquid (solution) for an extended period of time. It is carried out at room temperature. It involves submerging a liquid (such as water, oil, or alcohol). Plant material is combined with the extracting solvent

in a tight container and left to stand at room temperature for a while, stirring frequently, until the soluble matter dissolves. The mixture is filtered (by pressing moist solid particles). Filtration or decantation are used to clarify the combined liquid. 24 hours of maceration are spent at room temperature.

Extraction: Extraction techniques aim to extract the active compounds from the species with certain selectivity and sensitivity. Several well-known methods have been used to extraction of active components from spices, such as liquid phase (extraction using solvent). Solid phase extraction, and supercritical fluid extraction (extraction using CO_2 in its supercritical state). It is proven that different solvents or solvent mixture applied on the same spice sample can lead to different extraction efficiencies. A list of solvents used for Extraction of spices has been provided. The solvents include methanol, methanol/water mixture (1:1), Trichloroacetic acid, acetone, toluene, ethanol, ethyl Acetone and water. Methanol/water (1:1) and ethanol/ water (1:1) mixture are used most frequently. To address the complexity of the extraction characteristics and optimize parameters for solvent extraction of phenolic compounds and antioxidants, response surface methodology by central composite design has been employed to statistically optimize the extraction condition.

Material and preparation of extract:

Garlic (*Allium sativum)*, Fenugreek (*Trigonella foenum-graecum)*, Turmeric (*curcuma longa)*, and Amla (*Phyllanthus Emblica*) are the plants that were employed in this study. Fresh plant material, which were procured from the farm, cleaned under running water before being air dried. Following placement of all crude drugs in a suitable state, cutting into pieces, and three days of heating in a hot air oven, the medication was ground into a course powder using a grinder.

Crude Extraction: Initial screening of plant for possible antioxidant activity typically being by using crude aqueous or ethanol extraction and followed by various organic methods.

Solvent extract: 40 gm of dried plant was extracted with 100ml of 99% v/v Ethanol in round bottom conical flask. Which sealed with foil and allowed to stand for 72 hours. Letter the flask content were filtered to obtained crude Ethanolic extracts and at 4^0C when not in use

Antioxidant Activity Using DPPH Method:

The antioxidant activity of the plant extracts and the standard was assessed on the basis of the radical scavenging effect on the stable 1,1- Diphneyl-2-picrylhydrazyl (DPPH. 0.002%)method.

The diluted working solution of the test extract (10 mg/ml) was prepared using the respective solvents, In 3 ml of total reaction solution, 2 ml of extract/ standard solution of plant and 1.0 ml of DPPH were mixed and allowed to react at 37^0 C for 30 min. after ward's, absorbance value were measured at 520 nm and converted into percent antioxidant activity. The percentage antioxidant activity was calculated by following formula.

Percent (%) inhibition of DPPH radical scavenging activity = (A – B)/A.100

Where, A = Absorbance of the blank and

B = Absorbance of the sample

Result And Discussion:

Result : For the analysis of phytochemicals and *in vitro* antioxidant activity using the DPPH method, the mixed extract of herbal plants was used in the current study. The numerous plants, including *Curcuma longa*, *Phyllanthus emblica*, *Trigonella foenum-graecum*, and *Allium sativum*, were combined. Three copies of the outcome were given.

Phytochemicals: Phytochemical analysis preliminary characterization of plant extracts containing bioactive compounds. Phytochemical screening of the different extracts revealed the presence of various phytoconstituents such as phenols and terpenoids. The turmeric extract did not contain saponins, whereas other components such as tannins, glycosides, alkaloids and carbohydrates were present as shown in Table 6.1. The garlic extract showed the presence of various phytoconstituents such as phenols, flavonoids, saponins, glycosides, alkaloids, steroids and carbohydrates, while the content of tannins was absent in the garlic extract. The alcohol extract of fenugreek showed the presence of phenols, flavonoids, saponins, tannins, glycosides, terpenoids, alkaloids and carbohydrates. The steroid component was not present in the alcohol extract of fenugreek. The results are presented in Table No. 6.1. The alcohol extract of amla showed the presence of phenols, flavonoids, saponins, tannins, glycosides

Table no 02: Phyto-chemicals screening protocol details for the selected plants

Phytoconstituent	*Curcuma longa*	*Allium sativum*	*Trigonella foenum-graecum*	*Phyllanthus emblica*
Phenols	+	+	+	+
Saponins	-	+	+	+
Flavonoids	+	+	+	+
Steroids	+	+	-	+
Glycosides	-	-	+	+
Alkaloids	-	+	+	+
Terpenoids	+	+	+	+
Carbohydrates	-	+	-	+

***In vitro* antioxidant activity determination**

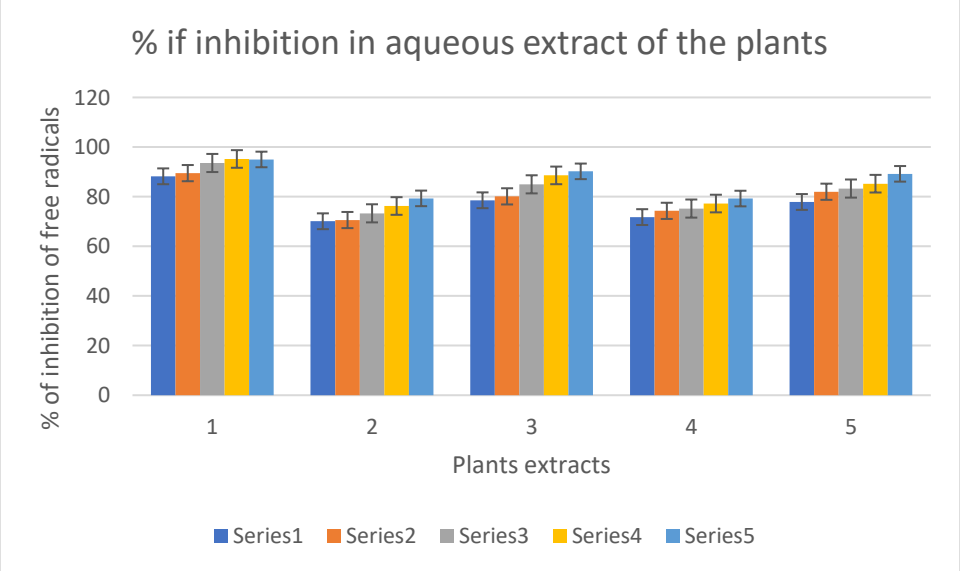
The various extracts were prepared from selected plant material and all were evaluated for their *in vitro* antioxidant activity. The present study showed that among the four herbal extracts of the plant . Amla exhibited the highest antioxidant activity and turmeric showed the least antioxidant activity compared to Amla. Turmeric showed 81.77% antioxidant activity in alcohol extract and 79.25% aqueous extract. Garlic showed 80.71% antioxidant activity in alcohol extract and 79.32 % aqueous extract, Fenugreek showed 90.50% antioxidant activity and 89.21% aqueous extract . Amla 91.37% antioxidant activity in alcohol extract and 90.21% aqueous extract . The ascorbic acid standard in alcohol extract showed 97% antioxidant activity and 90% in aqueous extract . There are numerous articles reporting the antioxidant activity of these plant extracts see Table No. 3&4 Graphical representation: Antioxidant activity of herbal plants.

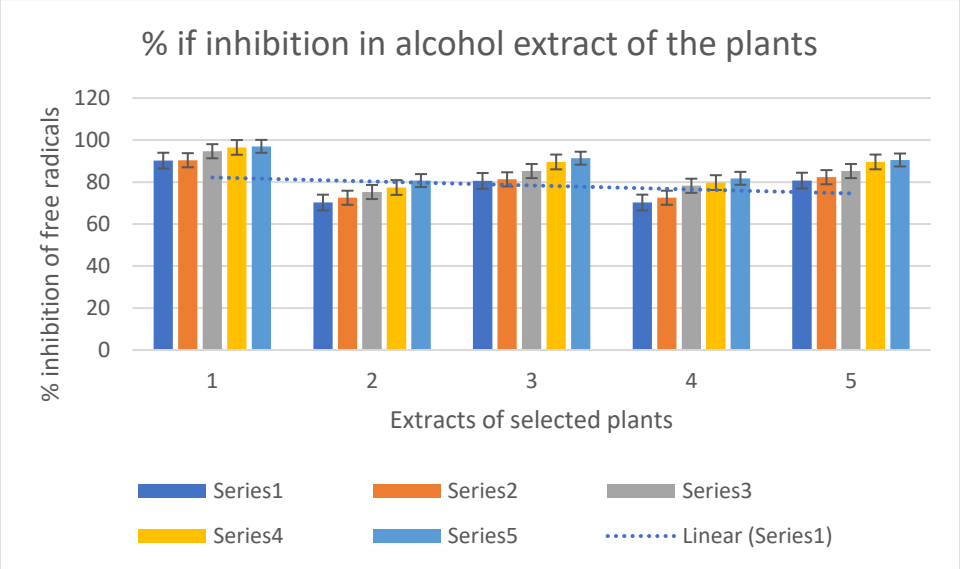
Table No.03: Showing % if inhibition in alcohol extract of the plants .

Concentrations of the Extract (µg/ml)	Alcohol extract of the plant with percentage of inhibition of free radical (% inhibition)				
	ascorbic acid standard	*Allium sativum*	*Phyllanthus emblica*	*Curcuma longa*	*Trigonella foenum graecum*
100	90.21	70.24	80.54	70.25	80.69
200	90.40	72.52	81.29	72.53	82.31
300	94.68	75.26	85.25	78.24	85.24
400	96.50	77.41	89.57	79.74	89.59
500	97	80.71	91.37	81.77	90.50

Table No.04: Showing % if inhibition in aqueous extract of the plants .

Concentrations of the Extract (µg/ml)	Aqueous extract of the plant with percentage of inhibition of free radical (% inhibition)				
	ascorbic acid standard	*Allium sativum*	*Phyllanthus emblica*	*Curcuma longa*	*Trigonella foenum graecum*
100	88.21	70.10	78.55	71.78	77.87
200	89.49	70.59	80.13	74.29	81.99
300	93.58	73.29	84.98	75.22	83.28
400	95.20	76.25	88.58	77.24	85.24
500	95	79.32	90.21	79.25	89.21





Discussion

According to the results of the phytochemical screening, alcohol and aqueous plant extracts contain phenols, flavonoids, saponins, glycosides, terpenoids, alkaloids, steroids, carotenoids, and carbohydrates. Chemical evidence supported this. An analysis of the selected plant extracts of amla, garlic, Fenugreek, and turmeric for their antioxidant activities was done. The most popular method for evaluating plant extracts' capacity to scavenge free radicals is the DPPH radical scavenging test. According to the results, amla demonstrated stronger antioxidant effects, even if all drugs have good anti-oxidant properties. Antioxidant trend was therefore turmeric < Garlic < Fenugreek < Amla.

From lowest to highest. This finding demonstrated the ability of the chosen plants to suppress free radicals and may serve as the body's main antioxidants in people. The report of present study revealed that all pathogenic microbes have different sensitivity against individual extract, added in growth medium.

Summary

After gathering the plants and properly drying each one separately, we began macerating the solution to concentrate it. Next, we filtered the extracted solution. In the current investigation, the extracts from the chosen medicinal plants, *Phyllanthus emblica*, *curcuma longa*, and *Allium sativum*, were prepared using the DPPH free radical scavenging method in order to determine the evolution o*f in vitro* antioxidant activity. The findings of this study further confirm the idea that some plants are potential sources of natural antioxidants. Between four chosen plants, there are considerable differences in total alcohol and aqueous concentration and total antioxidant capacity. After that, combination cluster analysis was helpful and enabled the selection of the finest plants based on the outcomes of various antioxidant capacity methods, offering.

Conclusion

The DPPH method was used in the current study to test the *in vitro* antioxidant activity of plant extracts such as turmeric, garlic, Fenugreek, and amla individually and collectively. The results showed that the combination of the plants had significantly higher antioxidant activity (free radical scavenging potential) than each individual extract. This effort will be helpful in developing polyherbal formulations and

screening more new, innovative crude medicines for antioxidant properties. One of the preventive parameters for testing and developing new treatments to slow the spread of other diseases is antioxidant activity. In general, the current study or observation worsens the chronic health issue, thus it is necessary to restore both mild and chronic disease health.

Bibliography

1. Baselga-Escudero, L.; Souza-Mello, V.; Pascual- Serrano, A.; Rachid, T.; Voci, A.; Demori, I.; Grasselli, E. Beneficial effects of the Mediterranean spices and aromas on non-alcoholic fatty liver disease. Trends Food Sci. Technol. 2017, 61, 141- 159.
2. Ahmed MA., Ibrahim OM., Elham AB., 2010. Effect of bio and mineral phosphorus fertilizer on the growth, productivity and nutritional value of fenugreek (Trigonellafoenumgraecum L.) in Newly Cultivated Land. Res. J. Agriculture and Biological Sci, 6 (3): 339 – 48.
3. Patra, K.; Jana, K.; Mandal, D.P.; Bhattacharjee, S. Evaluation of the antioxidant activity of extracts and active principles of commonly consumed Indian spices. J. Environ. Pathol. Toxicol. Oncol. 2016, 35, 299–315.
4. .Singhal, P.; Signal, N.; Sakhare, D.; Sharma. K.A. A comparative evaluation of in vitro antioxidant activity of some commonly used spices of northern India. Nat. Prod. J. 2017, 7, 131–136.
5. Ene-Obong, H.; Onuoha, N.; Aburime, L.; Mbah, O. Chemical composition and antioxidant activities of some indigenous spices consumed in Nigeria. Food Chem. 2017
6. Serafini, M.; Peluso, I. Functional foods for health: The interrelated antioxidant and antiinflammatory role of fruits, vegetables, herbs, spices and cocoa in humans. Curr. Pharm. Des. 2016, 22, 6701-6715.
7. Saravanan G, Prakash J. Effect of garlic (Allium sativum) on lipid peroxidation in experimental myocardial infarction in rats. J Ethnopharmacol. 2004;94(1):155–158. Doi: 10.1016/j.jep.2004.04.029. [PubMed]
8. Amagase H, Petesch BL, Matsuura H, Kasuga S, Itakura Y. Recent advances on the nutritional effects associated with the use of garlic as a supplement: intake of garlic and its bioactive components. J. Nutr. 2001;131(3):955S–962S.
9. Tan B.L., Norhaizan M.E., Liew W.-P.-P., Rahman H.S. Antioxidant and Oxidative Stress: A Mutual Interplay in Age-Related Diseases. Front. Pharmacol. 2018;9:1162. Doi: 10.3389/fphar.2018.01162.
10. Jakubczyk K., Kałduńska J., Dec K., Kawczuga D., Janda K. Antioxidant properties of small-molecule non-enzymatic compounds. Pol. Merkur. Lekarski. 2020;48:128–132.