JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

ANTIOXIDANT POTENTIAL OF SPICES AND VITAMINS SYNERGISTICALLY

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Abstract

Antioxidant is very potent for every human body. It reduces the effect of free radical generation by donating the electron. Curcumin, piperine, and eugenol are some well-known antioxidants, even some vitamins viz. vitamin C and E, also have that type of property. This research paper contains the evaluation of antioxidant activity between mixtures of spices (curcumin, piperine, and eugenol) and vitamins (vitamin A, B complex, C, D, E and vitamin B3) by DPPH* and Nitric oxide method. Synergistically it shows the maximum antioxidant activity in the mixture of eugenol and vitamin B complex (1:1) with 96.82% inhibition by DPPH* and same with 76.81% inhibition by NO method, whereas the mixture of piperine and vitamin B3 (1:1) showed minimum antioxidant potential with 11.56% and 11.30% inhibition respectively.

Keywords: Curcumin, Piperine, Eugenol, Vitamins, Antioxidant Activity, DPPH*, and NO method.

1. Introduction

Nowadays, when people in developed as well as developing countries are very much concerned about health and immunity due to corona, they are looking for more efficient natural dietary products which can combat such lethal microbial attacks by boosting the immune system. For the same they are taking many edible natural products viz., spices (curcumin, piperine, and eugenol, etc.), which have antioxidant, anti-inflammatory, antitumorigenic, anticarcinogenic, glucose, and cholesterol-lowering activities (Milda et al., 2015). Vitamins {vitamin A, B complex (B1, B2, B3, B5, B6, B9, and B12), C, vitamin E, etc.} are the supplements that control the natural cellular growth, critical functions, and body development, also become the part of a daily diet. Vitamin A is fat soluble which includes retinol, retinal, retinoic acid, and several provitamins A carotenoids, act as lipoperoxyl radical scavenger and reduced lipid oxidation. Vitamin C is also called as ascorbic acid, it has a switch-over role from being an antioxidant in physiologic conditions to a pro-oxidant under pathologic conditions. (Chakraborthy et al., 2014). It also acts as a co-oxidant with vitamin E (tocopherol) and reduces oxidative stress. Vitamin D not only metabolizes calcium but also induces some non-classical actions (Mokhtari et al., 2017). Whereas, amongst vitamin B complex, B3 being a micronutrient shows less stability and bioavailability, but degrades quickly, so is suitable for entrapment by the electrospinning process. A scientific investigation revealed that natural vitamins are more absorbable by the body rather than synthetic vitamins (Sílvia et al., 2022).

The origin of the harmful process called oxidative stress lies in the extreme production of free radicals such as reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive sulfur species (RSS) with half-lives of only a few nanoseconds or a reduced ability to deactivate them, whose effects can seriously alter cell structures (e.g., membranes) and damage biomolecules such as lipids, lipoproteins, proteins, and nucleic acids (Nimse et al., 2015; Lobo et al., 2010; Dizdaroglu, 1992) , triggering a variety of patho-physiological discorders, such as arthritis, arteriosclerosis, diabetes, inflammation, cancer, cardiovascular diseases, neuronal disorders,

skin ulcers and genotoxicity (Kourounakis et al. 1999; Gulcin et al. 2002). Reactive oxygen species (ROS), such as superoxide anion radical O2-, hydroxyl radical (OH-), and peroxyl radical (ROO-), are constantly generated in vivo both by aerobic metabolism and exogenous sources such as UV radiation, environmental pollution, thermal stress, and the diet. In clinical settings, ROS are also important to signal transduction molecules and mediators of damage in cellular processes, such as apoptosis and cell necrosis for organisms (Michael, 2006).

Here, antioxidants play a vital role in the growth and repair of damaged cells (Santos-Sánchez et al., 2019). It's antiaging properties can reduce oxidative stress like lipid oxidation, DNA and proteins rupture, etc. Antioxidants that fit in this definition include free radical scavengers, singlet oxygen quenchers, inactivators of peroxides and other reactive oxygen species (ROS), metal ion chelators, quenchers of secondary oxidation products, and inhibitors of pro-oxidative enzymes. Thus, one of the main factors involved in oxidative stress reduction is increased antioxidant potential. Among various types of antioxidants, natural vitamins (eg. A, B, C, and E, and carotenoids) represent a wide group of chemically distinct, water-soluble, and biologically active compounds which serve to limit or delay the oxidative damage of important macromolecules of cells in humans by scavenging those free radicals and lower the risk of certain chronic diseases (Mohajeri et al., 2009; Brewer, 2011; Skorna et al., 2016; Bendich et al., 1986; McCall et al., 1999). It is broadly recognized that the most vital structural characteristic which facilitates effective antioxidant activity is the presence of one or more conjugated OH groups or COOH groups, which boosts the ability of such a molecule to quench the free radicals (Mohajeri et al., 2009; Brewer, 2011). Therefore, the chemical structure of an antioxidant determines its intrinsic reactivity towards free radicals and other ROS and hence, the antioxidant activity. Food manufacturers use antioxidants to stabilize food lipids and thus prevent the quality deterioration of the products.

The effectiveness of antioxidants is generally influenced by several factors, including their structural features, concentration, temperature, type of oxidation substrate, and physical state of the system as well as the presence of pro-oxidants and synergists. The efficiency of antioxidants also depends on their location in the system (e.g. distribution at the interface. However, little is known about the antioxidant capacity and the mechanism involved by each biochemical component either through reducing power or radical scavenging activity of vitamins and spices. It might also be attributed to the combined activity of these minor components through synergistic effects (Dizdaroglu1992; Mohajeri et al., 2006). They are broadly classified by their mechanism of action as primary and secondary antioxidants. Primary antioxidants such as tocopherols and some phenolic compounds inhibit the chain reaction of oxidation by acting as hydrogen donors or free radical acceptors and generation of more stable radicals. Whereas, secondary antioxidants prevent or retard oxidation by suppressing the oxidation promoters, including metal ions, singlet oxygen, pro-oxidative enzymes, and other oxidants.

Many epidemiological studies suggest that the consumption of fruits and vegetable-rich diets having antioxidants in abundance inversely correlates with the risk of cardiovascular diseases and certain forms of cancer. Numerous enzymatic and non-enzymatic antioxidant defence mechanisms that the organism itself created to ensure its survival combat the ongoing threat of oxygen radical-induced harm to cells, tissues, and organisms. (Brewer, 2011).

For this purpose, the present study focuses on the evaluation of the antioxidant potential of mixtures of various vitamins (viz., vitamin A, vitamin B complex, vitamin C, vitamin D, vitamin E, and vitamin B3) and spices (viz., curcumin, piperine, and eugenol), and their synergistic combination has been tested by DPPH* and NO method.

The main curcumenoid found in the well-known Indian spice turmeric (Curcuma longa) belongs to the family *Zingiberaceae*, is curcumin, has a variety of therapeutic benefits, and is found throughout the tropics of Asia, Africa, and Australia. It also contains phenolics, terpenoids, and flavonoids, in its rhizomes. Demethoxycurcumin and bisdemethoxycurcumin are the other two forms of curcumenoids (Pandey et al., 2014). In oriental nations, Black pepper (Piper nigrum), is a widely used spice. Ascorbic acid, beta-carotene, camphene, lauric acid, linalylautate, myristicin, palmitic acid, terpinen-4-ol, piperine, and feruperine are just a few of the numerous antioxidants found in black pepper (Suhag, 2006). It has been found that all the phenolic amides isolated from black pepper have strong antioxidant properties. These properties are comparable to those of the synthetic antioxidants butylated hydroxyanisole and butylated hydroxytoluene and are greater than those of the naturally occurring antioxidants α-tocopherol and feruperine (Asimi et al., 2013). Black pepper's major bioactive component, piperine, has been shown to have hydroxyl radical scavenger properties at low doses. Recently, nano-encapsulated black pepper was created using hydroxypropyl beta-cyclodextrin, and it showed improved antioxidant activity (Teixeira et al., 2013). Additionally, piperine has been found to increase the antioxidant power of curcumin (Mehta et al., 2012). Clove (Syzygium aromaticum) belongs to the Myrataceae family. These are flower buds, which have uneven shapes akin to nails. The essential oil in high-quality clove buds, which

makes up 15-20% of the total oil, is dominated by eugenol (70–85%), eugenyl acetate (15%), and β-caryophyllene (5–12%) (Zachariah et al., 2005). Methyl amyl ketone, methylsalicylate, humulene, benzaldehyde, ylangene, and chavicol are the other constituents. Compared to other essential oils, clove essential oil has the highest antioxidant capacity; it may even be among the highest known for a meal or supplement. Compared to the synthetic antioxidants BHA and pyrogallol, clove and eugenol have better antioxidant activity (Dorman et al., 2000). It also shows a significant inhibitory effect against hydroxyl radicals.

2. Materials and Methods

Curcumin, Piperine, Eugenol and Gallic acid were purchased from CDH Pvt. Ltd. New Delhi, India. 1, 1-Diphenyl-2-picrylhydrazyl (DPPH*) was purchased from HiMedia Pvt. Ltd. Mumbai, India. Methanol AR was purchased from Loba Chemie Pvt. Ltd. Mumbai, India. Sulfanilic acid GR was purchased from E.MERCK Ltd. Mumbai. Sodium Nitroprusside was purchased from CDH Pvt. Ltd. New Delhi, India. Sodium Dihydrogen Orthophosphate Dihydrate (Extra Pure) was purchased from Loba Chemie Pvt. Ltd. Mumbai, di-Sodium Hydrogen Orthophosphate Anhydrous was purchased from QUALIGENS FINE CHEMICALS, Mumbai, India. 1-Naphthylamine AR was purchased from Loba Chemie Pvt. Ltd. Mumbai, India. Vitamins such as Vitamin A, B complex, C, D, E, and B3 were purchased from USV Pvt. Ltd. Mumbai, Pfizer Ltd. Mumbai, SPECTROCHEM Pvt. Ltd. Mumbai, MANKIND PHARMA Ltd. New Delhi, HiMedia Pvt. Ltd. Mumbai, and Sisco Research Laboratories Pvt. Ltd. Mumbai, India respectively.

UV- Visible spectrophotometer (Systronic, Model No. 104) was used to record the samples' absorbance. The experiments were performed and accomplished in the Department of Chemistry, CMP Degree College (A Constituent PG College of University of Allahabad) and Department of Biochemistry, SHUATS, Prayagraj.

2.1. 1, 1-Diphenyl-2-picrylhydrazyl (DPPH* Assay) (Pandey et al., 2014)

DPPH* was used as a radical scavenger and gallic acid as the standard. DPPH* has an odd electron due to which it gives maximum absorption at 517 nm and the procedure follows the decrease in absorption and showed the antioxidant activity of that compound.

The formula used for the calculation is:-

% Inhibition of DPPH* activity = $\frac{(A-B)}{A}$ x 100

Where A= absorbance of control, B= absorbance of sample

2.2. Nitric Oxide (NO Assay)

As the method was described by Mishra et al., 2015, an aqueous solution of Sodium Nitroprusside at physiological pH generates nitric oxide and it interacts with oxygen to produce nitrite ions (NO_2^-), which get estimated using Griess reagent. After this, Scavengers of nitric oxide compete with oxygen to reduce the production of nitrite ions. The absorbance of all the samples was recorded at 546 nm. The same formulae were used for the calculation of absorbance as mentioned above in DPPH* assay.

2.3. Experiment

2.3.1. DPPH* scavenging assay

Stock solutions (1mg/10 mL) of curcumin, piperine, eugenol, vitamin A, vitamin, B complex, vitamin C, vitamin D, vitamin E, and vitamin B_3 were prepared in methanol. The stock solution of DPPH* was also prepared at the concentration of 2mg/100 mL in methanol.

Different concentrations such as 2 mL, 1.5 mL, 1 mL, 0.5 mL, 0.25 mL, 0.125 mL of curcumin, and all the vitamins, were taken out in the test tube separately and made up the volume up to 2 mL by adding methanol respectively. Now add 2 mL DPPH* solution in each test tube and make the volume up to 4 mL and kept the solutions for 30 minutes in dark. After the incubation, the absorbance of all the samples was recorded by UV-spectrophotometer. Similarly, performed further experiments for piperine and eugenol with the same vitamins.

2.3.2. Nitric Oxide scavenging assay

In this assay, stock solutions of Sodium nitroprusside were prepared at the concentration of 10 mM, phosphate buffer (7.4 pH) at the concentration of 10 mM, and Naphthylamine at the concentration of 5% in distilled water. The stock solution of Sulfanilc acid at the concentration of 0.33% was prepared in 20% glacial acetic acid and Stock solutions of curcumin, piperine, eugenol, vitamin A, vitamin, B complex, vitamin C, vitamin D, vitamin E, and vitamin B_3 were prepared in methanol at the concentration of 1mg/10 mL.

In a test tube, took 2 mL of Sodium nitroprusside with 0.5 mL of phosphate buffer and 0.5 mL of curcumin and incubate the solution for 150 min. After incubation took out 0.5 mL of this solution in a separate test tube and 1 mL of Sulfanilic acid was added. Stand it for 5 min for completing the diazotization process. After this, 1 mL of Naphthylamine was added, mixed, and allowed to stand for 30 minutes. At the end of this assay, pink color chromophores were observed. Now the absorbance of all samples was recorded at 546 nm. The same procedure was followed for the other samples.

3. Results and Discussion

3.1. The antioxidant potential value of Curcumin, Eugenol, and Piperine with Vitamins were evaluated by DPPH*assay (Table 1.)

Table 1: Antioxidant Activity of Curcumin, Eugenol, and Piperine with Vitamins

S.No.	Curcumin, Piperine, Eugenol with different Vitamins	% Antioxidant activity (by DPPH* assay)
1	Curcumin	72.32
2	Eugenol	64.12
3	Piperine	15.05
4	Curcumin : Vitamin A (1:1)	94.21
5	Eugenol : Vitamin A (1:1)	97.10
6	Piperine : Vitamin A (1:1)	25.14
7	Curcumin : Vitamin B (1:1)	96.24
8	Eugenol : Vitamin B (1:1)	98.26
9	Piperine : Vitamin B (1:1)	97.68
10	Curcumin : Vitamin C (1:1)	95.95
11	Eugenol : Vitamin C (1:1)	96.82
12	Piperine : Vitamin C (1:1)	97.39
13	Curcumin : Vitamin D (1:1)	93.35
14	Eugenol : Vitamin D (1:1)	96.24
15	Piperine : Vitamin D (1:1)	19.36
16	Curcumin : Vitamin E (1:1)	94.50
17	Eugenol : Vitamin E (1:1)	93.06
18	Piperine : Vitamin E (1:1)	88.43
19	Curcumin : Vitamin B3 (1:1)	89.30
20	Eugenol : Vitamin B3 (1:1)	97.97
21	Piperine : Vitamin B3 (1:1)	11.56
22	Gallic Acid (Standard)	95.66

The curcumin, eugenol, and piperine showed antioxidant potential with 72.32, 64.12, and 15.05% DPPH* inhibition respectively as compared to gallic acid (standard), which shows comparable values referring paper Pandey et al. 2014.

The mixture of curcumin and vitamin A (1:1) showed antioxidant activity with 94.21% while eugenol and vitamin A (1:1), piperine and vitamin A (1:1) exhibited antioxidant potential with 97.10 and 25.14% respectively. The mixture of curcumin and vitamin B (1:1) showed antioxidant activity with 96.24% while eugenol and vitamin B (1:1), piperine and vitamin B (1:1) exhibited antioxidant potential with 98.26 and 97.68% respectively. The mixture of curcumin and vitamin C (1:1) showed antioxidant activity with 95.95% while eugenol and vitamin C (1:1), piperine and vitamin C (1:1) exhibited antioxidant potential with 96.82 and 97.39% respectively. The mixture of curcumin and vitamin D (1:1) showed antioxidant activity with 93.35% while eugenol and vitamin D (1:1), piperine and vitamin D (1:1) exhibited antioxidant potential with 96.24 and 19.36% respectively. The mixture of curcumin and vitamin E (1:1) showed antioxidant activity with 94.50% while eugenol and vitamin E (1:1), piperine and vitamin E (1:1) exhibited antioxidant potential with 93.06 and 88.43% respectively. The mixture of curcumin and vitamin B3 (1:1) showed antioxidant activity with 89.30% while eugenol and vitamin B3 (1:1), piperine and vitamin B3 (1:1) exhibited antioxidant potential with 97.97 and 11.56% respectively. (from Table 1).

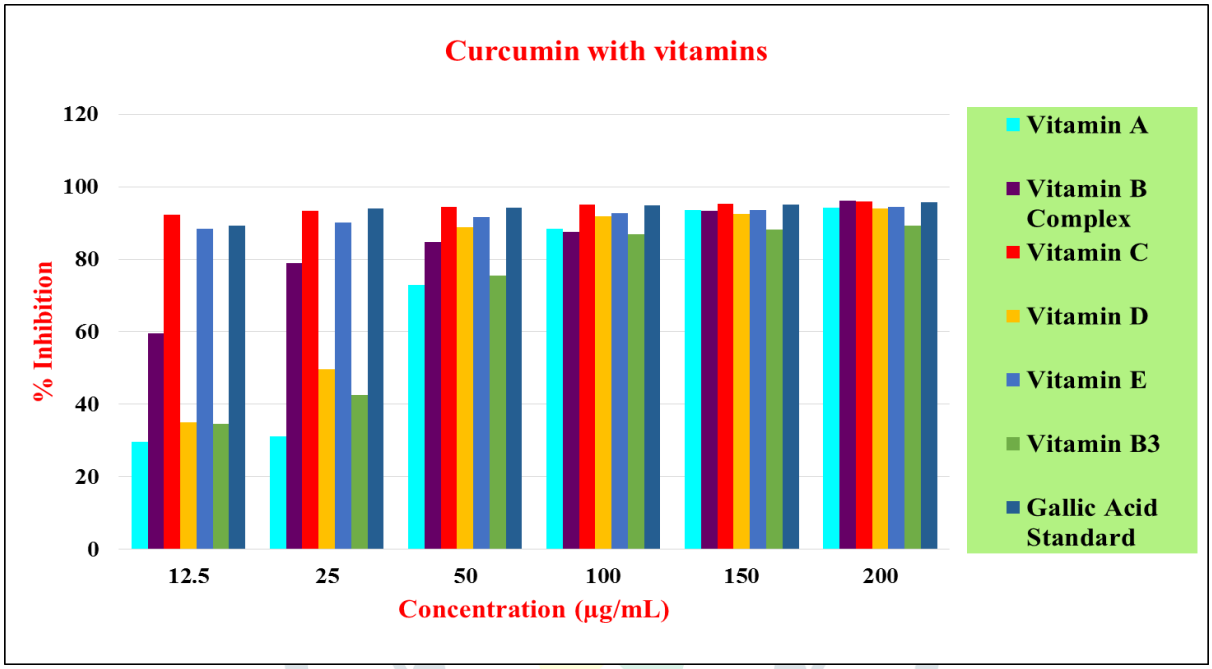

Fig. 1. Mixture of Curcumin with Vitamins

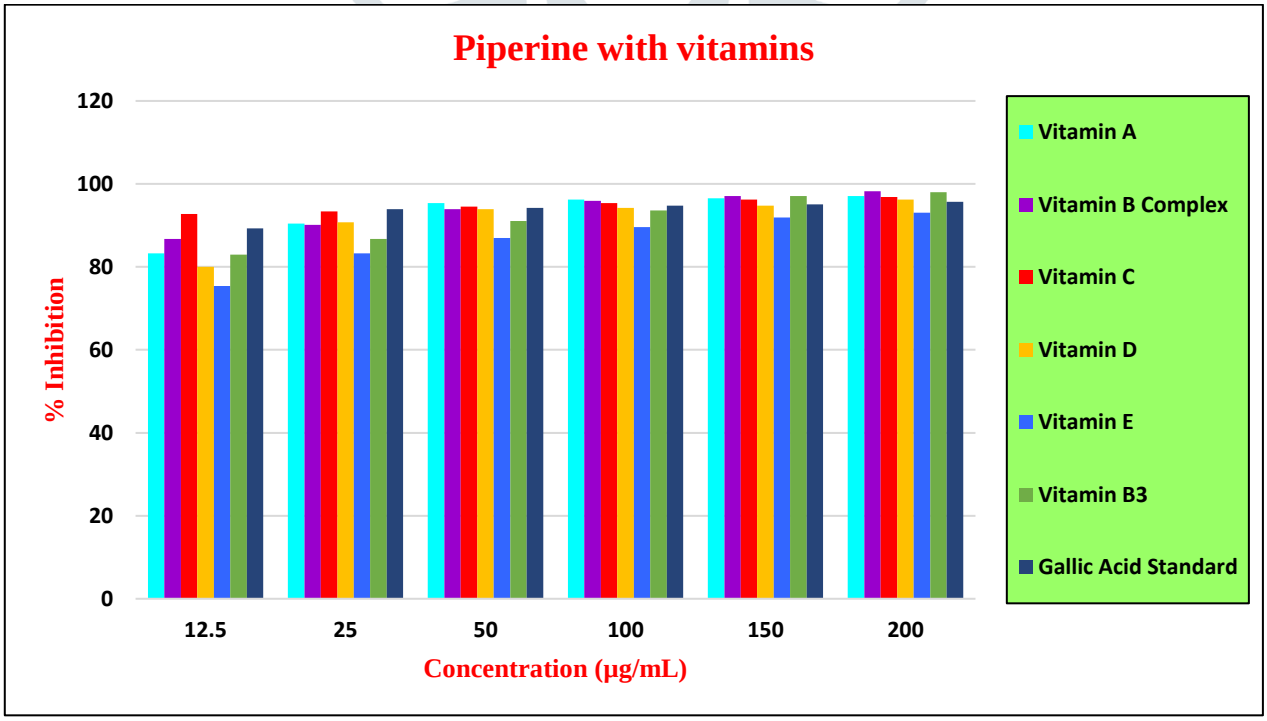

Fig. 2. Mixture of Piperine with Vitamins

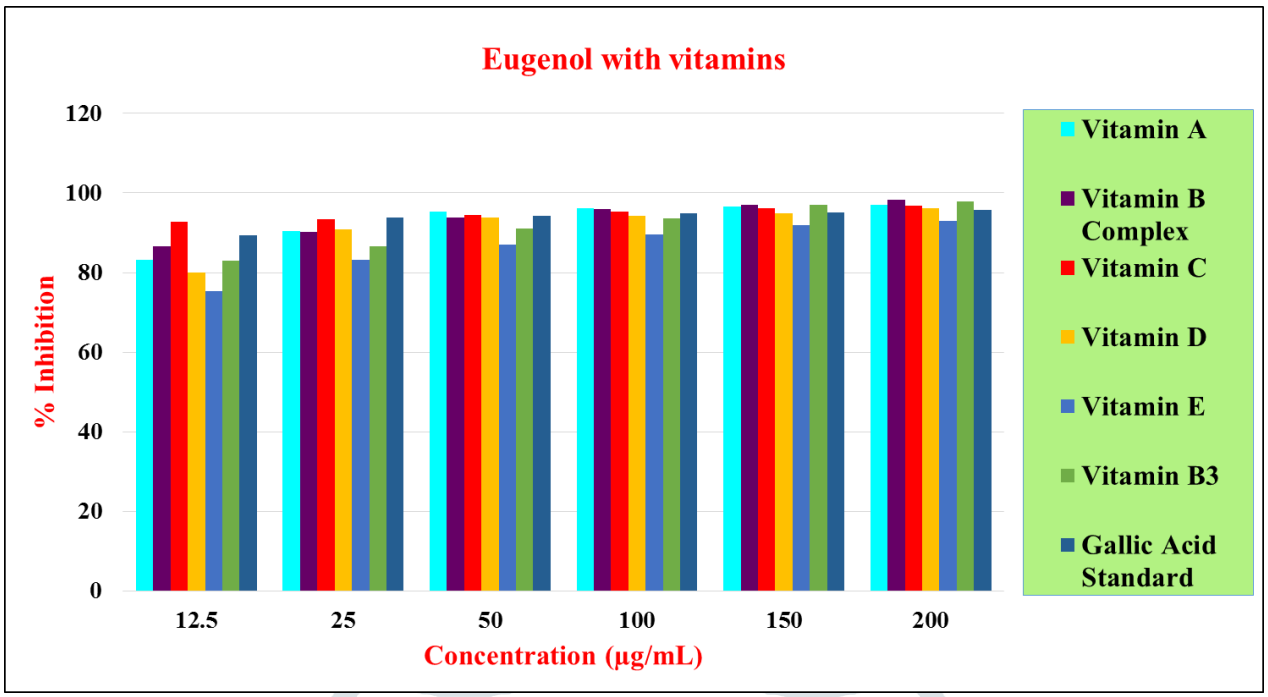

Fig. 3. Mixture of Eugenol with Vitamins

The mixture of curcumin and vitamin B complex (1:1) showed higher antioxidant activity with 96.24% inhibition in comparison to curcumin and vitamin A (1:1), curcumin and vitamin C (1:1), curcumin and vitamin D (1:1) and curcumin and vitamin E (1:1), curcumin and vitamin B3 (1:1) with DPPH* inhibition of 94.21, 95.95, 93.95, 94.50 and 89.30% respectively. In the same way, the mixture of eugenol and vitamin B complex (1:1) showed higher antioxidant activity with 98.26% inhibition in comparison to eugenol and vitamin A (1:1), eugenol and vitamin C (1:1), eugenol and vitamin D (1:1) and eugenol and vitamin E (1:1), eugenol and vitamin B3 (1:1) with DPPH* inhibition of 97.10, 96.82, 96.24, 93.06 and 97.97% respectively. In order that the mixture of piperine and vitamin B (1:1) showed higher antioxidant activity with 97.68% inhibition in comparison to piperine and vitamin A (1:1), piperine and vitamin C (1:1), piperine and vitamin D (1:1), and piperine and vitamin E (1:1), piperine and vitamin B3 (1:1) with DPPH* inhibition of 25.14, 97.39, 19.36, 88.43 and 11.56% respectively. It is very fascinating to notice that among all these mixtures, the mixture of eugenol and vitamin B complex showed a higher antioxidant potential value than gallic acid. (from Fig. 1, 2, 3, and Table 1. respectively).

3.2. The antioxidant potential value of Curcumin, Eugenol, and Piperine with different Vitamins were evaluated by NO assay (Table 2.)

Table 2. Antioxidant Potential of Curcumin, Piperine, Eugenol with Vitamins (by NO assay)

S.No.	Curcumin, Piperine, Eugenol with different Vitamins	% Antioxidant activity (by NO assay)
1	Curcumin	50.63
2	Eugenol	42.12
3	Piperine	09.06
4	Curcumin : Vitamin A (1:1)	72.21
5	Eugenol : Vitamin A (1:1)	75.36
6	Piperine : Vitamin A (1:1)	13.75
7	Curcumin : Vitamin B (1:1)	74.24
8	Eugenol : Vitamin B (1:1)	76.81
9	Piperine : Vitamin B (1:1)	75.03
10	Curcumin : Vitamin C (1:1)	73.45
11	Eugenol : Vitamin C (1:1)	74.87

12	Piperine : Vitamin C (1:1)	75.27
13	Curcumin : Vitamin D (1:1)	71.96
14	Eugenol : Vitamin D (1:1)	74.84
15	Piperine : Vitamin D (1:1)	14.81
16	Curcumin : Vitamin E (1:1)	72.12
17	Eugenol : Vitamin E (1:1)	71 .15
18	Piperine : Vitamin E (1:1)	66.48
19	Curcumin : Vitamin B3 (1:1)	67.69
20	Eugenol : Vitamin B3 (1:1)	75.58
21	Piperine : Vitamin B3 (1:1)	11.30
22	Gallic Acid (Standard)	73.64

The curcumin, eugenol and piperine showed antioxidant potential with 50.63, 42.12 and 09.06% NO inhibition, respectively, compared to gallic acid (standard). The mixture of curcumin and vitamin A (1:1) showed antioxidant activity with 72.21% while eugenol and vitamin A (1:1), piperine and vitamin A (1:1) exhibited antioxidant potential with 75.36 and 13.75% respectively. The mixture of curcumin and vitamin B (1:1) showed antioxidant activity with 74.24% while eugenol and vitamin B (1:1), piperine and vitamin B (1:1) exhibited antioxidant potential with 76.81 and 75.03% respectively. The mixture of curcumin and vitamin C (1:1) showed antioxidant activity with 73.45% while eugenol and vitamin C (1:1), piperine and vitamin C (1:1) exhibited antioxidant potential with 74.87 and 75.27% respectively. The mixture of curcumin and vitamin D (1:1) showed antioxidant activity with 71.96% while eugenol and vitamin D (1:1), piperine and vitamin D (1:1) exhibited antioxidant potential with 74.84 and 14.81% respectively. The mixture of curcumin and vitamin E (1:1) showed antioxidant activity with 72.12% while eugenol and vitamin E (1:1), piperine and vitamin E (1:1) exhibited antioxidant potential with 71.15 and 66.48% respectively. The mixture of curcumin and vitamin B3 (1:1) showed antioxidant activity with 67.69% while eugenol and vitamin B3 (1:1), piperine and vitamin B3 (1:1) exhibited antioxidant potential with 75.58 and 11.30% respectively. (from Table 2).

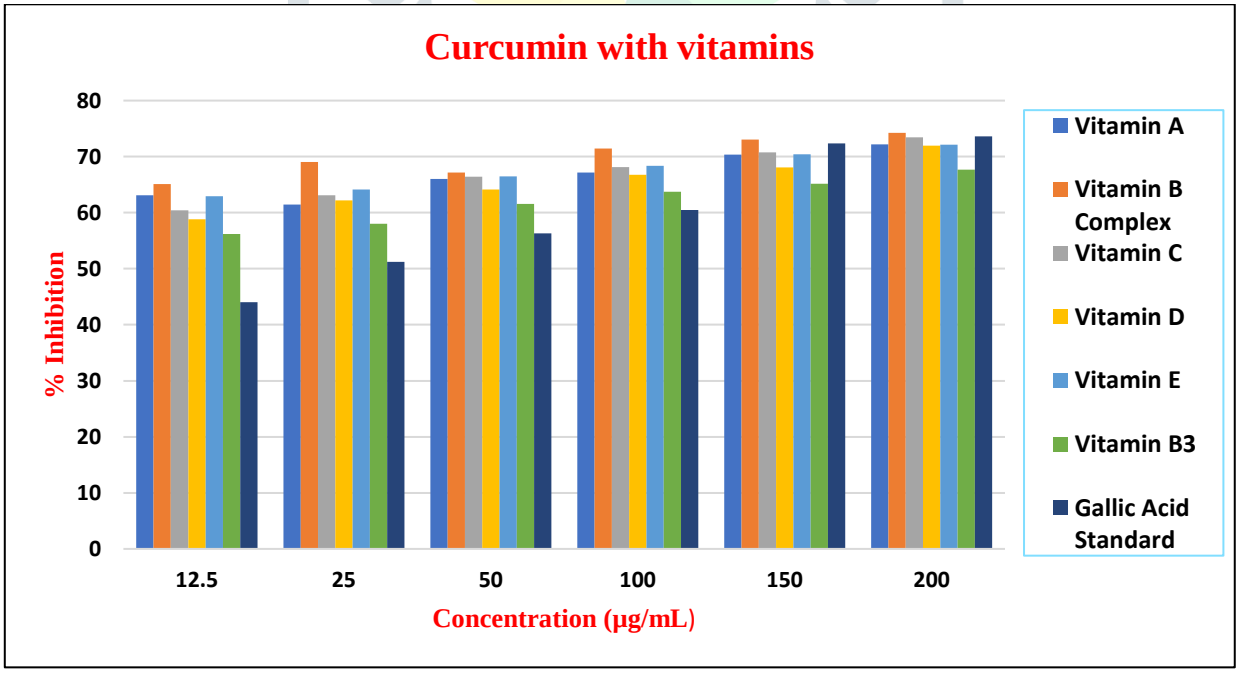

Fig.4. Mixture of Curcumin with Vitamins

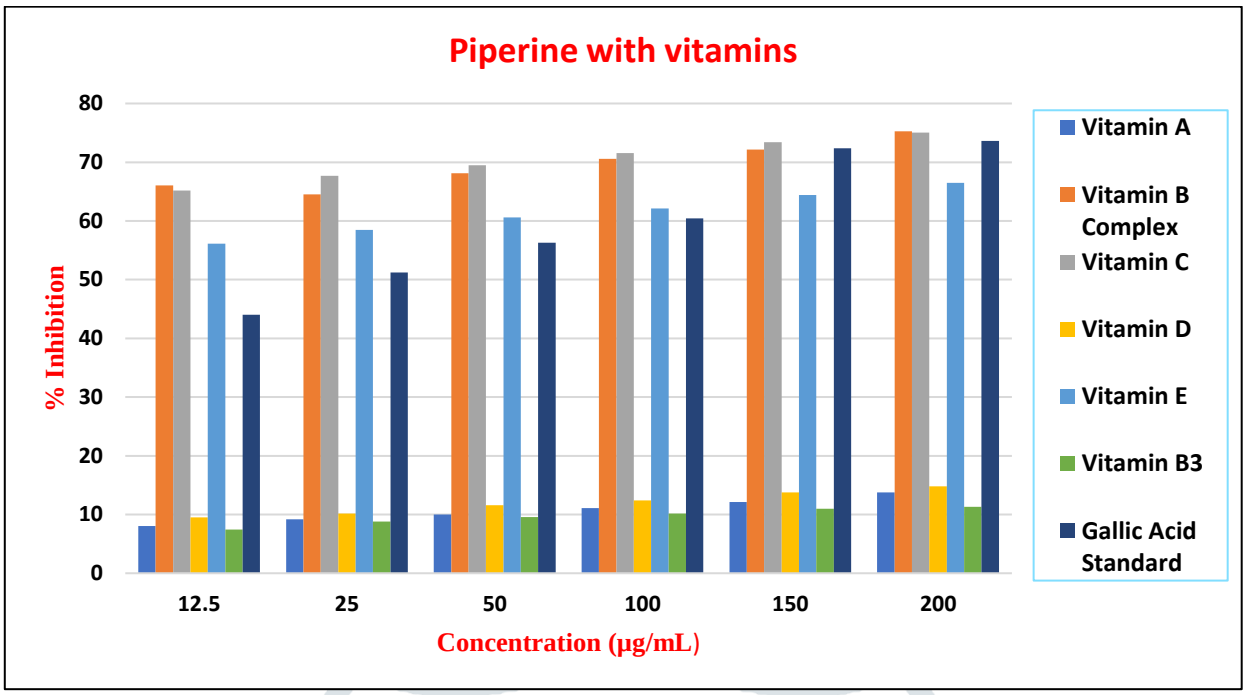

Fig.5. Mixture of Piperine with Vitamins

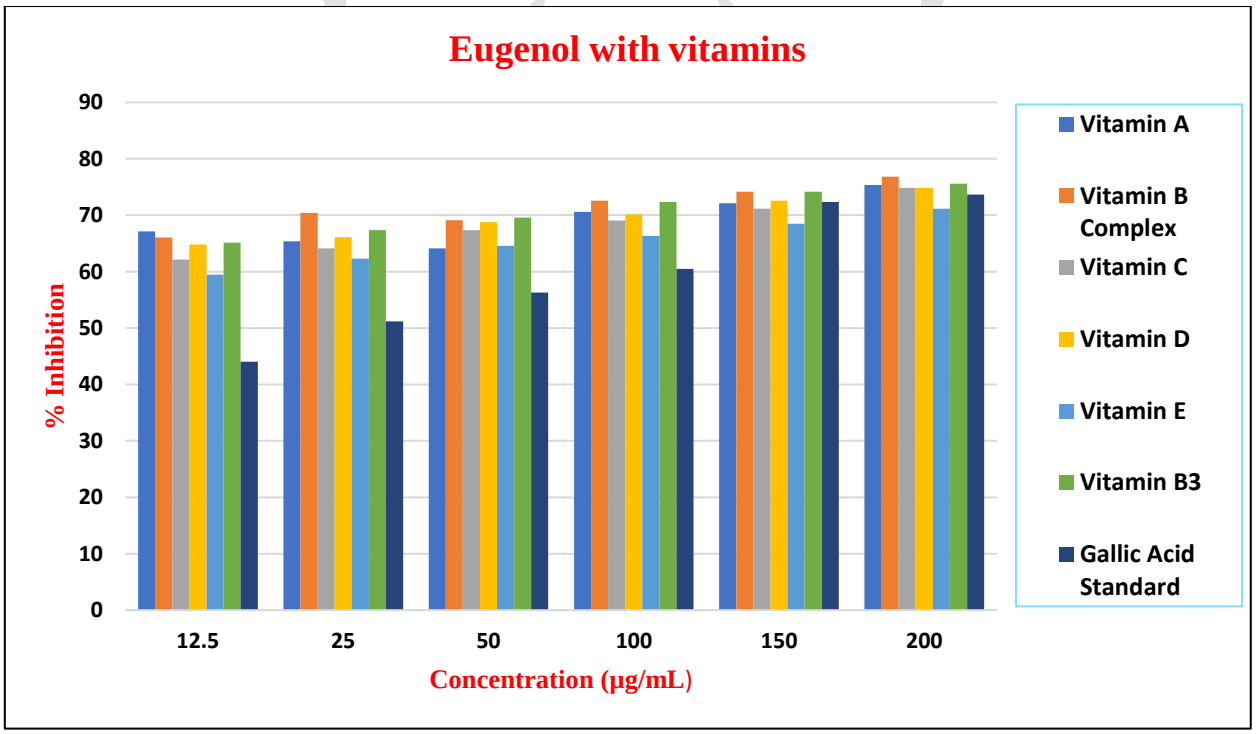

Fig.6. Mixture of Eugenol with Vitamins

The mixture of curcumin and vitamin B complex (1:1) showed higher antioxidant activity with 74.24% inhibition in comparison to curcumin and vitamin A (1:1), curcumin and vitamin C (1:1), curcumin and vitamin D (1:1) and curcumin and vitamin E (1:1), curcumin and vitamin B3 (1:1) with NO inhibition of 72.21, 73.45, 71.96, 72.12 and 67.69% respectively. Also, the mixture of eugenol and vitamin B complex (1:1) showed higher antioxidant activity with 76.81% inhibition in comparison to eugenol and vitamin A (1:1), eugenol and vitamin C (1:1), eugenol and vitamin D (1:1) and eugenol and vitamin E (1:1), eugenol and vitamin B3 (1:1) with NO inhibition of 75.36, 74.87, 74.84, 71.15 and 75.58% respectively. Similarly, the mixture of piperine and vitamin B complex (1:1) showed higher antioxidant activity with 75.27% inhibition in comparison to piperine and vitamin A (1:1), piperine and vitamin C (1:1), piperine and vitamin D (1:1) and piperine and vitamin E (1:1), piperine and vitamin B3 (1:1) with NO inhibition of 13.75, 75.03, 14.81, 66.48, 11.30% respectively. (from Fig. 4, 5, 6 and, Table 2. respectively).

When compared the antioxidant potential of mixtures of curcumin, piperine, and eugenol with different vitamins by DPPH* and NO assay, we found the same order of result, that maximum antioxidant potential was shown by the mixture of eugenol and vitamin B complex (1:1) with 96.82% inhibition by DPPH* method and same with 76.81% inhibition by NO method, which justifies our results, whereas the mixture of piperine and vitamin B3 (1:1) showed minimum antioxidant potential with 11.56% inhibition and 11.30% inhibition by DPPH* and NO method respectively. Nitric oxide assay justifies our outcomes by showing a similar kind of order of antioxidant potential of mixtures of curcumin, piperine, and eugenol with different vitamins like DPPH*.

The high antioxidant activity of the mixture of eugenol and vitamin B complex is due to the presence of one free phenolic group in eugenol and different vitamins of B viz., B1, B2, B3, B5, B6, B9, and B12 in the vitamin B complex. These phenolic groups are good hydrogen donors making the compound highly antioxidant. It also proved the synergistic effect of the mixtures.

4. Conclusion

Results from the above studies, concluded that the mixtures of the bioactive constituents of spices with vitamins showed synergistic effects and good antioxidant activity except for vitamin B3. This research would be very helpful in treating various kinds of oxidative stress and diseases.

5. Acknowledgment

All the authors are grateful to the Department of Chemistry, CMP Degree College (A Constituent PG College of University of Allahabad) and Department of Biochemistry, SHUATS, Prayagraj for providing the necessary facilities.

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