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Thymol, an Active Constituent of Nigella Sativa, could Reduce Toxicity of Some Trace Metals (Fe(III),Cr(VI),Cu(II),V(IV) and Co(II))

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Abstract:

The objective of the present research is to develop a herbal method to reduce the toxicities caused by high levels of some essential trace elements in the human body. Nigella sativa (NS) seeds are well known for their numerous biological and pharmacological properties, which are mainly due to its active constituents. According to several research studies thymol is one of the principal active component of NS. In present research work complexation of thymol with some essential trace metals i.e; Fe (III), Cr (VI), Cu (II), V (IV) and Co (II) has been studied using pH-metry. It was observed from pH plots of ligand and complexes that thymol formed complexes with all metals successfully. Hence it could be used as chelating agent in case of toxicity caused by above mentioned metals. Thymol could perform this function either by forming complexes with the metals or by converting them into their reduced form due to its antioxidant ability. In either case toxic forms of metals will be converted into non toxic forms. Species distribution curves showed the maximum formation of ML (1:1) complex species of thymol with Fe (III), Cr (VI), Cu (II), V (IV) and Co (II) within pH 3.00-5.00. Whereas maximum ML_2 (1:2) complex specie formation seems to occur within pH 6.50-11.00. pK_a of the thymol was found to be 9.00±1.

Key words: *Nigella sativa*, Thymol, Chelating agent, Antioxidant, Essential metal toxicity, pH-metry.

Introduction

Thymol (2-isopropyl-5-methylphenol) ($C_{10}H_{14}O$) (Fig. 1) is a natural monoterpene phenol which can be isolated from the essential oils of various plants [1]. It is main constituents of *Nigella sativa* seed also [2, 3]. In several research studies different bioactivities and pharmacological properties of *N. sativa* seeds were found to be due to thymol which include anti-oxidant [4], anti-microbial [5-8] and anti-inflammatory [9] activities. Thymol has also been reported to possess anti-mutagenic [10], anti-tumor [11] and fungicide [12] effects.
Thymol has been determined in *Nigella sativa* oil through differential pulse voltammetric method. The voltammograms showed reproducible peaks at E_p +0.49 V for thymol [13]. The level of thymol in *N. sativa* seeds through high performance liquid chromatography technique has been reported to be 169.35 ± 100.12 [2].

Iron has a key role in our life, as it is an essential component of several proteins and enzymes. It can serve different functions because of the fact that it can exist in two different ionic states, ferrous and ferric iron, e.g.; it can serve as a cofactor to enzymes involved in oxidation-reduction reactions [14]. Iron (III) forms complexes with numerous ligands such as chloride, cyanide, amines etc., but oxygen containing ligands have high affinity for it [15].

Chromium is widely distributed in our entire environment including air, water, soil, plants and in animals. Cr^{3+} and Cr^{6+} are the most common form of chromium [14, 16]. Trivalent state is effective and is used as an essential mineral for human beings. It plays important role in metabolism of carbohydrate and lipid [17, 18]. In acidic solution Cr (VI) is a powerful oxidant [15].

Copper compounds commonly occurs as copper (II) salts. Since copper can easily accept and donate electron, it plays a vital role in oxidation reduction reactions of the body as well as in scavenging of free radicals [19]. It also serves as a constituent of several proteins and enzymes [20]. Several Cu (II) complexes can be obtained by treating aqueous solutions with different ligands [21].

Vanadium is another essential trace element which is necessary for growth, bones development, and healthy teeth and also for reproduction [14, 16]. The ability of vanadium to exist as V(IV) and V(V) makes it very important in several enzymatic reactions [22]. Vanadium (IV) forms complexes with several ligands, but the chemistry of vanadium (IV) is dominated by oxygen compounds or oxo-vanadium (IV) ion (VO^{2+}).

Cobalt is also an essential element for human as it is the key mineral of vitamin B_{12} [14] and therefore, it helps in red blood cells formation and nerve tissue maintenance. It helps in metabolism of carbohydrates, protein, fats and folic acid [23]. Co (II) is its most stable oxidation state and it forms numerous complexes.

Above discussed trace elements are very important as they perform a variety of essential structural and regulatory roles. But it is a fact that if the deficiency of any of them may be fatal, their excess can be equally deadly. Hence upper level (UL) of their recommended intakes (RDA) should not exceed (Table 1) [24]. If metal's intake exceeds the UL, it results in metal toxicity which is very dangerous. For example, excess iron promotes the formation of free radicals and causes cell death due to excess oxidation of cellular components. Iron overload can damage iron storing organs, especially heart and liver. Iron toxicity may result in heart diseases, diabetes, arthritis and cancer [25, 26]. High levels of chromium may cause renal failure. Specially Cr (VI) is a strong irritant and can cause allergic reactions, such as skin rashes. At high levels its inhalation may irritate nose lungs, stomach and intestines, whereas its ingestion can cause stomach upsets and ulcers, convulsions, kidney and liver damage and even death [27]. Excessive copper intake may cause headache, nausea, abdominal pain and diarrhea. Acute copper toxicity may result in heart problems, liver damage, jaundice, kidney failure, coma and death [24]. Vanadium toxicity may result in stunted growth. High doses of vanadium even may lead to death [28]. Exposure to vanadium dust may result in rhinitis, nose bleeds, conjunctivitis, and pain in chest, sore throat and cough. Large doses of cobalt might stimulate thyroid and bone marrow functions [29].

Material and Methods

Reagent and glassware: All the reagents used were of analytical grade, purchased from Merck, Bio Basic Inc., and MP Biochemicals LLC. All glassware used was of standard quality. They were properly cleaned and rinsed with distilled- deionized water. For pH-metric studies ferric chloride (hexa hydrate), potassium dichromate, copper sulphate (penta hydrate), vanadyl sulphate (penta hydrate), cobalt acetate (tetra hydrate), thymol and sodium hydroxide were used.

Instrumentation:
Electrical balance: Shimadzu, Model AX 200 was used for weighing.
pH meter: Jenway, Model 3510 pH meter.

Sample Preparation: Caliberation buffers of pH 4.00 and 7.00 were prepared using buffer tablets. NaOH (0.1 M), $FeCl_3.6H_2O$ (0.005 M), $K_2Cr_2O_7$ (0.005 M), $CuSO_4.5H_2O$ (0.005 M), $VOSO_4.5H_2O$ (0.005 M), $Co(CH_3COO)_2.4H_2O$ (0.005 M) and thymol (0.005 M) were also prepared. NaOH was standardized using oxalic acid as standard solution, every time, before use.

Experimental:

pH-metric studies: All pH metric titrations were performed at 25±5˚C. All pH metric titrations were carried out in a double walled glass cell. The temperature was controlled by circulating water through a thermostat (HAAKE, KT 33 Germany). The capacity of the cell was 100 mL. The rubber stopper of the cell contained four holes, one for micro-burette for addition of standard base, another one for purging inert gas (Nitrogen) and the third hole for the removal of oxygen and the forth for glass electrode. The solution completely deareated by passing N_2 gas for 30 minutes in a sealed flask and was protected with atmosphere.

pH-metric titration of ligand (Thymol): For this purpose, 40 mL of thymol solution (0.005M) and 10 mL of distilled deionized water were taken in the pH metric cell containing magnetic bead. Purified nitrogen gas was perged through the solution for half an hour. Then the thymol solution was titrated against 0.1M standard sodium hydroxide solution. NaOH solution was standardized using 0.05M Oxalic acid solution prior to the pH metric titration of thymol.

During titration regular stirring was maintained by means of magnetic stirrer. Standard NaOH was added in sufficiently small increments of 0.05 mL with the help of micro-burette and after each increment pH of the reaction mixture was recorded till pH was not affected by further addition of standard NaOH. pH values were plotted against the added volume of standard NaOH.

pH-metric titrations of metal{Fe(III), Cr(VI), Cu(II), V(IV) and Co(II)}- Thymol complexes: For pH metric titration of Fe (III)-thymol complex, 40mL of thymol solution (0.005M) and 10mL of Fe (III) solution (0.005M) were mixed to give 1:4 metal-ligand solution. The resulting reaction mixture was titrated against standard sodium hydroxide solution (0.1M) under the same conditions and in the same manner as previous two titrations, in order to compare pH metric titration of thymol and Fe (III)-thymol complex.

Similarly, pH metric titrations of other complexes of thymol i.e; complexes with Cr(VI), Cu(II), V(IV) and Co(II) were performed in the same manner and under the same conditions.
For each titration pH values were plotted against volume of standard NaOH added, which provide valuable information. With the help of pH-metric data species distribution curves were also plotted to find out best pH values for M (free metal), ML (1:1 complex) and ML_2 (1:2 complex) species.

Results and Discussion

pH-metric Titrations of Thymol and its Complexes: As a reference, titration was initially performed with ligand (thymol) solution. Here only one curve near pH 10.80 was observed. Initial pH of thymol was 6.72. It increased rapidly with the addition of NaOH and then became constant. pK_a of the thymol was calculated using the relation pK_a= -log K_a. It was found to be 9.00±1, which is close to the reported value 10.50 [30].

To check complexation similar titrations were repeated with complexes of thymol with some metals i.e. iron(III), chromium(VI), copper(II), vanadium (IV) and cobalt(II). Significant changes in the titration curves of complexes and ligand were observed in all the cases (Fig. 2), which shows release of H^+ ions due to complexation.

In case of Fe(III)-thymol complex, two prominent curves were observed near pH 3.00 and 10.00. First curve shows rapid reaction between metal and ligand, which may be due to formation of 1:1 metal-ligand complex (ML) and 1:2 metal-ligand complex (ML_2) species. This curve reveals prominent complexation at low pH (pH-3.00), but as the volume of added NaOH increased a drastic change in pH was observed and the pH plot goes straight in upward direction showing unavailability of H^+ ion for the neutralization. Here second curve was examined which indicates titration of excess ligand. Similarly, In Cr(VI)-thymol titration prominent difference in the titration curves of complex and ligand confirms complexation. In this case also two curves were observed, first curve starts near pH 5.50 and covers a pH range up to 8.00, and here probably maximum complex formation took place. Second curve crossed the ligand's curve and showed pH higher than the pH of ligand's curve. This indicates that complex may have some water molecules in its co-ordination sphere, which were releasing H^+ ions after complex formation and those H^+ ions were neutralizing by NaOH. In titration curve of Cu(II)-thymol complex a twist was observed at pH 5.80 and two curves near pH 6.50 and 9.50 were observed. First two changes may be due to formation of ML and ML_2 complex species respectively, whereas the third one may be due to titration of excess ligand. In case of V(IV)-thymol complex drastic change in the titration curves of complex and ligand was observed. Here first curve starts near pH 4.00 and reveals that the complex between V(IV) and thymol is stable at low pH. ML and ML_2 complex species of V(IV)-thymol complex seems to form within pH range 4.00-5.00, after which rapid increase in pH was observed with the addition of the base. Second curve was seen near pH 9.50. This curve proceed in the same manner as that of the ligand, hence it seems that this curve is due to the titration of excess ligand. In case of Co (II) -thymol complex also clear difference in titration curves of complex and ligand was observed revealing complexation. In titration curve of complex two distinct curves were observed, one near pH 8.00 and second near pH 9.00. These curves may be due to formation of ML and ML_2 species respectively (Fig. 2).

It was observed that out of all metals, Fe(III) formed complex at the lowest pH. V(IV) formed complex at moderate pH, whereas rest of the metals formed complexes at relatively higher pH. As far as stability of these complexes is concerned Fe(III) complex seems most stable, V(IV) complex seems to have moderate stability; whereas rest of the complexes were relatively of lower stabilities.

Species Distribution Curves of Thymol Complexes: With the help of pH-metric data, species distribution curves were plotted against pH and percent distribution of complex species. In each case two curves were obtained for complex, one for ML whereas second for ML_2 complex species. In case of Fe (III)-thymol complex maximum ML species was probably formed near pH 3.00, whereas maximum ML_2 formation seems to occur near pH 6.50 (Fig.3a). The complex seems stable at low pH. Species distribution curve shows that out of all studied complexes of thymol and Fe(III) formed most stable complex.

For Cr (VI)-thymol complex, it seems from the specie distribution curve that maximum complexation of ML and ML_2 took place near pH 5.00 and 8.50 respectively, whereas between pH range 6.00-6.50 each specie was 50% (Fig.3b). For Cu (II)-thymol complex specie distribution plot showed continuous decrease in the concentration of free metal ion with the addition of NaOH. Maximum percent distribution ML and ML_2 species seems to found around pH 4.00 and 7.00, whereas between pH 5.00 to 6.00 each species was 50% (Fig.3c). For V(IV)-thymol complex ML and ML_2 were seems maximum at pH 4.00 and 6.50 respectively (Fig.3d). The complex of Thymol with Co(II) showed 50% formation of each of the ML and ML_2 near pH 8.00, and their maximum formation at pH higher than 5.00 and at pH 11.00 respectively (Fig.3e).

Conclusion:

Thymol is an active constituent of many herbs [1] including *Nigella sativa* seeds [2, 31]. This component contributes very much for most of the pharmacological properties of *N. sativa* seeds such as antioxidant, anti-tumor, anti-inflammatory, antidiabatic, antitussive and antimicrobial activities. Several researches have been carried to examine its biological properties but not much work has been carried out on its complex forming abilities with metals. Keeping this point in view present research has been carried out.

Complexation of thymol with different metals i.e. Fe(III), Cr(VI), Cu(II), V(IV) and Co(II) was investigated by pH-metry. The study revealed that thymol successfully forms complexes with all metals. It was observed that out of above mentioned metals Fe(III) and V(IV) forms relatively stable complexes with thymol. Moreover, these metals formed complexes at lower pH as compared to rest of the metals. pK_a of thymol was also determined.

As we know that all metals studied in present research are required in trace quantities for the human body and their excess results in heavy metal toxicity which can cause a wide range of problems. From present research it is concluded that in case of toxicity caused by any of above mentioned metals thymol could be helpful in removing them from the body. Thymol could eliminate these metals from the body either by forming complexes with them or by converting the metals into their reduced form. In first case metals after complex formation become unable to absorb in the body whereas in the later case being anti-oxidant thymol converts toxic forms of metals in harmless and useful forms, for example Fe (III) to Fe (II), Cr

(VI) to Cr (III) etc. These reduced forms are needed by the body to perform different vital roles [32]. Furthermore, this way of treatment could be much safer than others, such as chelation therapy which may produce dangerous side effects [33, 34]. It was concluded that in addition to thymoquinone, which is another active constituent of *Nigella sativa* and has been reported to possess the same ability [35], thymol could also be used as a chelator in chelation therapy instead of drugs which are used in this technique and are potentially toxic [36].

Table 1. Recommended dietary allowances (RDA), adequate intakes (AI) and tolerable upper intakes levels (UL) of some trace elements for adults.

Parameters	***Fe* *mg/day***	***Cr* *(µg/day)***	***Cu* *(µg/day)***	***V* *(µg/day)***	***Co* *(µg/day)***
RDA	8-18	-	700-900	20-30	5-8
AI	-	25-35	-	-	-
UL	45	-	10,000	18,000	-

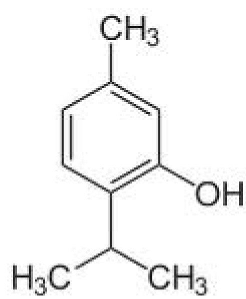


Fig. 1. Structure of thymol

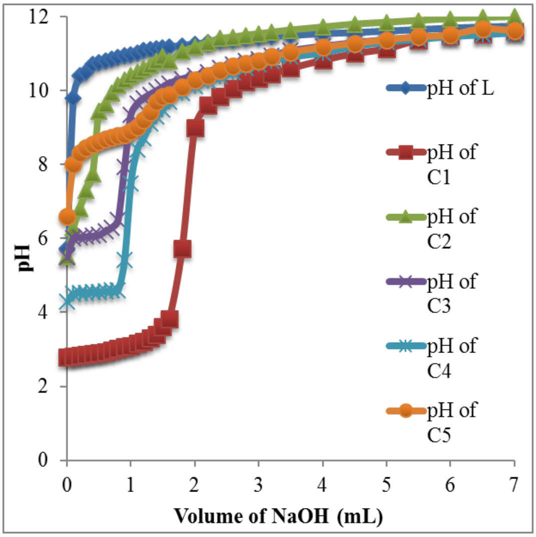
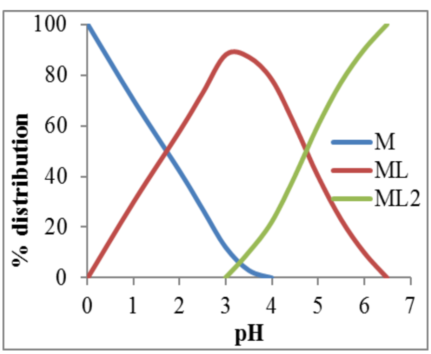


Fig. 2. pH curves of thymol and its complexes

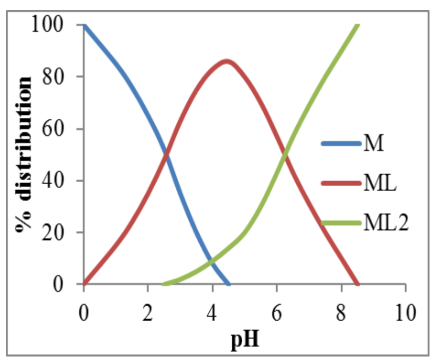
L = Thymol, C_1= Fe(III)-thymol complex, C_2= Cr(VI)-thymol complex, C_3= Cu(II)-thymol complex,

C_4= V(IV)-thymol complex, C_5= Co(II)-thymol complex.

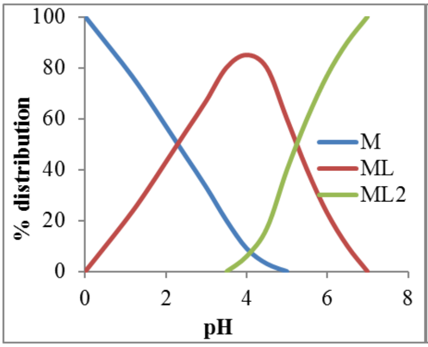
Fig. 3. Species distribution curves



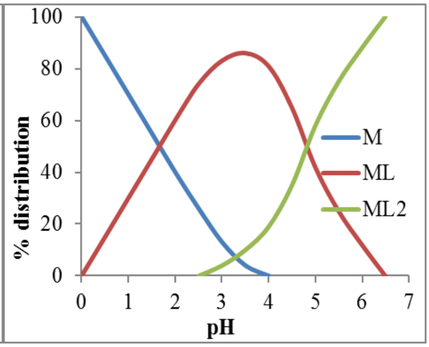
3a: Fe(III)-thymol complex



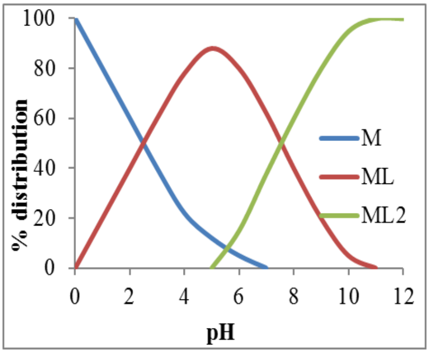
3b: Cr(VI)-thymol complex



3c: Cu(II)-thymol complex



3d: V(IV)-thymol complex



3e: Co(II)-thymol complex

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