ORIGINAL ARTICLE

Energy-Dispersive X-ray Microanalysis of a Herbal Antimicrobial: Fifatrol

K. Ramachandra Reddy[1]*, Chetan Sahni[2], N. K. Prasad[3], N. S. Anuraag[3], K. Hari Chandana[4], K. Vinay[5], Sanchit Sharma[6]

[1]Department of Rasa Shastra and Bhaishajya Kalpana, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India, [2]Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India, [3]Department of Metallurgical Engineering, IIT (BHU), Varanasi, Uttar Pradesh, India, [4]Vydehi Institute of Medical Sciences and Research Centre is in Whitefield, Bengaluru, Karnataka, India, [5]Department of Community Medicine, Bharati Vidyapeeth University Medical College and Hospital, Sangli, Maharashta, India, [6]Department of Research & Development, New Product Development, AIMIL Pharmaceuticals (I) Ltd., Delhi, India

Abstract

Ayurveda is a natural system of medicine, which is acceptable worldwide now days. Most of the medications used in this traditional system belong to natural drugs derived from the plants and other natural resources. These naturally derived antimicrobial drugs are equally effective as the other synthetically designed drugs of other medicinal systems against the different microbes. A detailed compositional analysis of any drug is very necessary to establish its role. Energy-dispersive X-ray (EDX) or energy-dispersive spectroscopy is a kind of microanalysis of any elemental structure. It is based on the detection of characteristic X-rays generated under electron microscopy. It provides both quantitative as well as qualitative information in elemental analysis. It is very important and useful technique to study a new drug to understand the drug delivery as well as nanoparticles in composition of any drug. In the present study, we have used a plant based extract antimicrobial drug named Fifatrol for detailed compositional analysis by EDX microanalysis under scanning electron microscope to see the different proinflammatory and anti-inflammatory elements such as Ca, S, O, Na, and B.

Key words: Antimicrobial, Ayurveda, energy-dispersive spectroscopy, energy-dispersive X-ray, Fifatrol, Herbal, scanning electron microscope

INTRODUCTION

In Ayurveda practices, many plants extracts have herbal and medicinal properties to cure the various diseases and their symptoms.[1] Although, the advancement and research in medicine is going synchronously and several medicines and vaccines being developed in recent decade against wide spectrum of micro-organisms. Still, the infectious diseases are continued to rise in present scenario. The development of antimicrobial resistance against these synthetically designed drugs could be a major reason. It has been also seen that plant-based extracts are far safer in comparison to the synthetically formulated drugs.[2] If we go through the ayurvedic dietary supplements and medicines, there are more than thousands herbal products available which are quite efficient against different micro-organisms.[3] The ayurvedic antimicrobials formed by combination of different plant extracts have shown better antimicrobial activity against the wide spectrum of bacteria and other micro-organisms. It has also been seen that such plant extracts have low bacterial resistance as compared to the synthetically designed drugs.[4-6] Many inorganic trace elements have proinflammatory and anti-inflammatory properties. These elements are also present in plant-based extract which is difficult to formulate in the synthetically designed drugs.[7] In the viral infections like COVID-19, proinflammatory immune reaction plays a key role to eliminate the viral load at initial phase. This proinflammatory immune response is double-edged sword. The dysregulated proinflammatory markers like cytokines

Address for correspondence:
K. Ramachandra Reddy, Deparment of Rasa Shastra and Bhaishajya Kalpana, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India. E-mail: krcreddy@bhu.ac.in

Received: 26-11-2022
Revised: 17-01-2023
Accepted: 23-01-2023

may create a cytokine storm like condition which leads to programmed cell death and tissue damage. Many researchers had proved antioxidant and anti-inflammatory effect of Boron in dietary supplementation.[8] Karnad *et al*. suggested that calcium carbonate and calcium gluconate have anti-inflammatory property comparable to that of aspirin (non-steroidal anti-inflammatory agent).[9] In the present study, Fifatrol (a multidrug formulation) formed by extracted from different plants was analyzed for detailed compositional analysis by energy-dispersive X-ray (EDX) microanalysis under scanning electron microscope (SEM). EDX microanalysis is being used in several biomedical research areas nowadays. It is a very useful tool to understand the drug delivery and therapeutic efficiency of any drug by analyzing its microanalysis at its nanoparticle size.

MATERIALS AND METHODS

EDX microanalysis or energy-dispersive spectroscopy (EDS)

The element detection for analytical study of any drug by electron microscope (EM) is not very common. Although, some diagnostic and research work is going on this domain worldwide. One of the most common approach through EM is EDS, more commonly known as electron probe X-ray microanalysis (EDX).[10] In the EDX microanalysis, electron beams hits the atoms of different elements present in the study material and, in turn, it generates characteristic specific X-rays.[11] There are two results of this electron bombardment, elastic scattering, and inelastic scattering. The elastic scattering determines the shape of the interaction volume, while the inelastic scattering determines the size of the interaction volume.[12] The X-rays emitted after electron beam collision are of different wavelengths. They are measured by photon-energy sensitive detector. The specific characteristic of these detected X-rays reflects the information of the elements present in the study material.[13] The use of EDS or EDX analysis in several herbomineral compounds had been established in the recent studies.[14,15] Field emission scanning electron microscopy with EDX (FESEM-EDS) was also used in quantitative analysis of Swarna Makshika Bhasma and Rajat Bhasma.[16,17] SEM-EDX analysis along with pharmacodynamics study of any mineral compound can provide the accurate estimation of bioavailability as well as safety dose estimation of study compound.[18,19] SEM-EDX quantitative and qualitative analysis is also useful to maintain the inherent quality of the ancient Indian system of medicine.[20]

Fifatrol is multidrug composition of plant-based extracts. To evaluate the elemental composition of the Fifatrol Tablet, it was manually crushed into powder form using a mortar and pestle. The crushed powder was coated with gold using sputtering to ensure that the sample was conductive. In secondary electron mode, the gold-sputtered powder Fifatrol sample was then observed under the EVO - SEM MA15/18. The chemical composition of the Fifatrol tablet was then probed using the EDS attachment of SEM. Further, mapping of the elements present in the sample was also done. The compounds formed from the constituent elements of the powder Fifatrol tablet were determined using X-ray diffraction. Rigaku Miniflex 600 Desktop, X-ray diffraction system, was employed with Copper K-alpha radiation as the source with wavelength 1.542 Å. Diffraction was carried out at a scan rate of 5°/min and a step size of 0.02 was maintained to obtain the peaks in the 2θ range of 20–60°.

RESULTS

The particles of Fifatrol tablet observed under SEM show random shapes [Figure 1] as they were crushed manually. As seen in the figure, the particles at different magnification show a difference in contrast. This contrast difference might be arising due to the different elemental distribution in the sample. Furthermore, different elements have different conductivities. The one's with relatively lesser conductivity appear brighter as charge up of electrons occur and the relatively conductive samples appear darker.

The EDS attachment of SEM was used to determine the various elements present in the Fifatrol sample. The spectrum of constituent elements is shown in Figure 1d and their approximate percentages are as tabulated in Table 1.

The calcium, sulfur, and boron were main trace elements found in the EDS. Figure 2 depicts the distribution of elements such as Ca, S, O, Na, and B in the sample obtained by the area mapping function of the EDS attachment. It also reveals that the elements are uniformly distributed throughout the sample. It also provides an indirect reference on the concentration of each element in the sample by the relative intensity of their mapping.

The X-ray diffraction pattern of Fifatrol powder tabl*et al*so showing the calcium and boron compounds [Figure 3].

Table 1: Elemental composition of Fifatrol tablet determined using EDS attachment in SEM

Element	Weight %
Calcium	15.0
Sulfur	20.0
Oxygen	33.0
Sodium	5.0
Boron	17.0
Iron	4.0
Zinc	2.0
Silver	4.0

EDS: Energy-dispersive spectroscopy, SEM: Scanning electron microscope

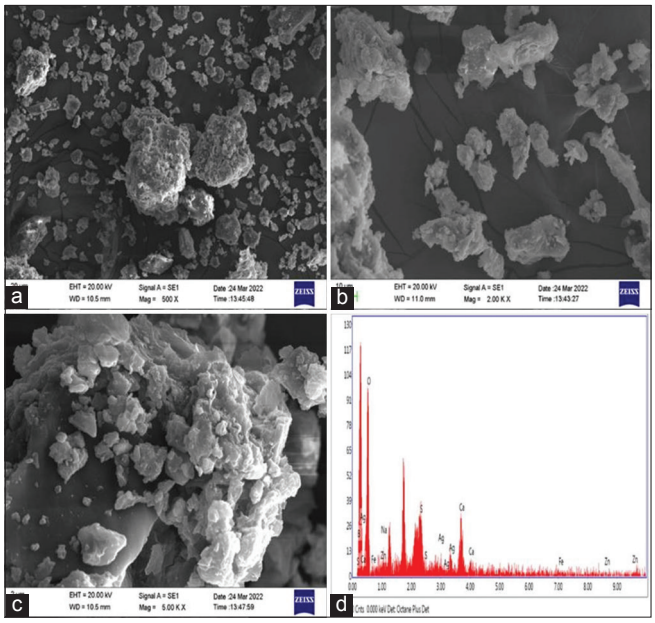

Figure 1: Scanning electron images of manually crushed Fifatrol tablet at (a) 500 X, (b) 200 kX, (c) 500 kX magnifications, and (d) energy spectrum of elements present in the Fifatrol tablet detected using scanning electron microscope-energy-dispersive spectroscopy mode

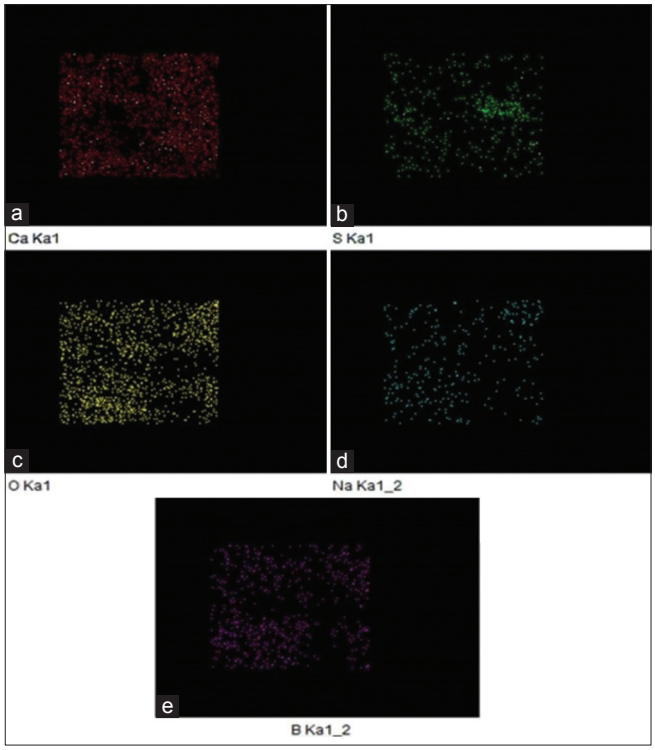

Figure 2: Energy-dispersive spectroscopy-scanning electron microscope mode element wise- (a) calcium, (b) sulfur, (c) oxygen, (d) sodium, (e) boron compositional mapping of Fifatrol tablet in powder form

DISCUSSION

Calcium compounds such as calcium chloride, calcium carbonate, and calcium gluconate are well-documented

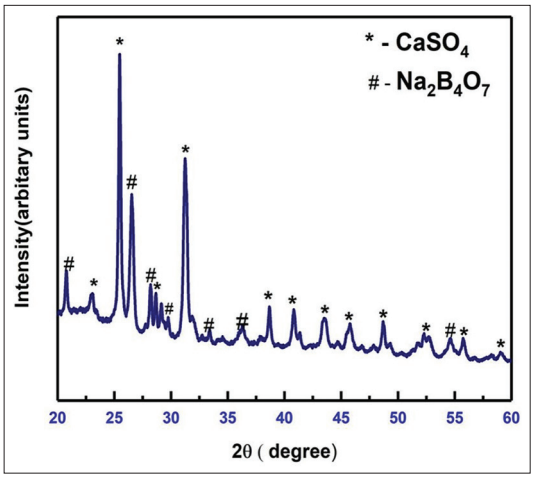

Figure 3: X-ray diffraction pattern of Fifatrol powder tablet

to suppress the inflammation in several conditions such as urticaria, pruritis, and erythema. Several theories have been proposed in favor of anti-inflammatory activity of calcium compounds. Piller proposed in his theory that calcium dobesilate suppresses the inflammation by reducing the number of circulating monocytes and disrupting the action of macrophage.[21] Calcium gluconate also shows anti-inflammatory activity by reducing the production of platelet aggregating factor in endothelium.[9] Many enzymes such as superoxide dismutase, peroxidase, glutathione peroxidase, and glutathione reductase reduce the inflammation. Calcium glubionate has a important role in enhancing the activity of these enzymes.[22,23] Supplemental calcium and vitamin D reduce indicators of cancer-promoting inflammation in normal colorectal tissue in humans.[24,25]

Boron is also an important trace element which has a major role in biological functions.[26,27] In 1995, Penland, described the important physiological role of Boron in inflammatory state of several diseases.[28] It was also found that higher intake of dietary Boron reduces the inflammation in inflammatory joint diseases.[29,30] It has also been reported that Boron has important role in to modulation of inflammatory responses in animals and humans. It also shows antioxidant activity by inhibiting production of reactive oxygen species.[31] Several antibacterial agents like borinic acid picolinate esters, which have Boron in their content have additional anti-inflammatory propert.[32,33] It is also reported that Boron along with curcumin has strong antiviral role against SARS-CoV-2.[34] Gündüz *et al*. (2021) formulated the novel boron containing compounds which had cytotoxic and antimicrobial effect against HepG2 cancer cell line.[35] Antibiotics like Boromycin which contains boron in its constituents have potent activity against certain viruses, Gram-positive bacteria, and protozoan parasites.[36] Dopaboroxazolidone, boron containing compound of boroxazolidones group, has potential as an attractive neuroactive drug against many neuromotor disorders.[37] Microelements such as sulfur and iron are integral part of certain proteins like CDGSH

iron-sulfur domain-containing protein 1 localized on the outer membrane of mitochondria and plays key roles in regulating cell death and oxidative stress.[38]

CONCLUSION

Such combination of activities is ideal for the treatment of microbial infections with inflammatory consequences. In the present analytical study of the herbal drug named Fifatrol, using EDS with SEM, it was found that Fifatrol has essential elements such as Ca, S, O, Na, and B which have proinflammatory and anti-inflammatory properties.

ACKNOWLEDGMENTS

None.

REFERENCES

1. Patwardhan B, Vaidya AD, Chorghade M. Ayurveda and natural products drug discovery. Curr Sci 2004;86:789-99.
2. Barrett B, Kiefer D, Rabago D. Assessing the risks and benefits of herbal medicine: An overview of scientific evidence. Altern Ther Health Med 1999;5:40-9.
3. World Health Organization. WHO General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. Geneva: World Health Organization; 2000.
4. Vickers A, Zollman C. What is complementary medicine? BMJ 1999;319:693-6.
5. De Smet PA. Herbal remedies. N Engl J Med 2002;347:2046-56.
6. Dawson W. Herbal medicines and the EU directive. JR Coll Physicians Edinb 2005;35:25-7.
7. Acaroz U, Ince S, Arslan-Acaroz D, Gurler Z, Kucukkurt I, Demirel HH, *et al*. The ameliorative effects of boron against acrylamide-induced oxidative stress, inflammatory response, and metabolic changes in rats. Food Chem Toxicol 2018;118:745-52.
8. Ince S, Kucukkurt I, Cigerci IH, Fatih Fidan A, Eryavuz A. The effects of dietary boric acid and borax supplementation on lipid peroxidation, antioxidant activity, and DNA damage in rats. J Trace Elem Med Biol 2010;24:161-4.
9. Karnad AS, Patil PA, Majagi SI. Calcium enhances antiinflammatory activity of aspirin in albino rats. Indian J Pharmacol 2006;38:397-402.
10. Fernandez-Segura E, Warley A. Electron probe X-ray microanalysis for the study of cell physiology. Methods Cell Biol 2008;88:19-43.
11. Samuelson DA. Energy dispersive X-ray microanalysis. In: Free Radical and Antioxidant Protocols. Germany: Springer; 1998. p. 413-24.
12. Roomans GM. Introduction to X-ray microanalysis in biology. J Electron Microsc Tech 1988;9:3-17.
13. Scimeca M, Orlandi A, Terrenato I, Bischetti S, Bonanno E. Assessment of metal contaminants in non-small cell lung cancer by EDX microanalysis. Eur J Histochem 2014;58:2403.
14. Wele A, De S, Dalvi M, Devi N, Pandit V. Nanoparticles of biotite mica as Krishna Vajra Abhraka Bhasma: Synthesis and characterization. J Ayurveda Integr Med 2021;12:269-82.
15. Belge R, Pandey R, Itankar P. Synthesis, characterization and antimicrobial study of Vanga Bhasma prepared with special reference to Rasatarangini. Int J Ayurveda Pharma Res 2021;9:1-16.
16. Bhardwaj R, Johar S, Kapila A, Sharma A. Physicochemical study and quantitative analysis Swarna Makshika Bhasma. Int J Pharm Biol Sci Arch 2021;9:7-15.
17. Kar P, Banerjee S, Chhetri A, Sen A. Synthesis, physicochemical characterization and biological activity of synthesized Silver and Rajat Bhasma nanoparticles using *Clerodendrum inerme*. J Phytol 2021;13:64-71.
18. Kamble S, Wanjari A, Rathi B, Rajput D. Pharmaceutico-analytical study of Muktashukti Pishti and Muktashukti bhasma and comparative evaluation of their relative oral bioavailability. J Pharm Res Int 2021;33:1-9.
19. Paudel R, Karn G, Aryal G, Giri J, Adhikari R, Sharma ML. Synthesis, characterization, biological study of synthesized Lauha Bhasma. J Nepal Chem Soc 2022;43:4-15.
20. Bhatnagar N, Pareek A. Comparing the physico-chemical characteristics of formulated and marketed Yashada bhasma. Res J Pharm Technol 2021;14:6392-8.
21. Piller NB. Assessment of the anti-inflammatory action of calcium dobesilate. Effect on macrophages attaching to subcutaneously implanted coverslips in guinea pigs. Arzneimittelforschung 1990;40:698-700.
22. Willhite CC, Karyakina NA, Yokel RA, Yenugadhati N, Wisniewski TM, Arnold IM, *et al*. Systematic review of potential health risks posed by pharmaceutical, occupational and consumer exposures to metallic and nanoscale aluminum, aluminum oxides, aluminum hydroxide and its soluble salts. Crit Rev Toxicol 2014;44 Suppl 4:1-80.
23. Soetan KO, Olaiya CO, Oyewole OE. The importance of mineral elements for humans, domestic animals and plants-A review. Afr J Food Sci 2010;4:200-22.
24. Gibbs DC, Fedirko V, Baron JA, Barry EL, Flanders WD, McCullough ML, *et al*. Inflammation modulation by Vitamin D and calcium in the morphologically normal colorectal mucosa of patients with colorectal adenoma in a clinical trial. Cancer Prev Res (Phila) 2021;14:65-76.
25. Zeng H, Safratowich BD, Liu Z, Bukowski MR, Ishaq SL. Adequacy of calcium and Vitamin D reduces inflammation, β-catenin signaling, and dysbiotic *Parasutterela* bacteria in the colon of C57BL/6 mice fed

a western-style diet. J Nutr Biochem 2021;92:108613.
26. Arciniega-Martínez IM, Romero-Aguilar KS, Farfán-García ED, García-Machorro J, Reséndiz-Albor AA, Soriano-Ursúa MA. Diversity of effects induced by boron-containing compounds on immune response cells and on antibodies in basal state. J Trace Elem Med Biol 2022;69:126901.
27. Routray I, Ali S. Boron induces lymphocyte proliferation and modulates the priming effects of lipopolysaccharide on macrophages. PLoS One 2016;11:e0150607.
28. Penland JG. The importance of boron nutrition for brain and psychological function. Biol Trace Elem Res 1998;66:299-317.
29. Newnham RE. Essentiality of boron for healthy bones and joints. Environ Health Perspect 1994;102 Suppl 7:83-5.
30. Korkmaz M, Sayli U, Sayli BS, Bakirdere S, Titretir S, Yavuz Ataman O, *et al*. Estimation of human daily boron exposure in a boron-rich area. Br J Nutr 2007;98:571-5.
31. Zhao C, Han Y, Wang C, Ren M, Hu Q, Gu Y, *et al*. Transcriptome profiling of duodenum reveals the importance of boron supplementation in modulating immune activities in rats. Biol Trace Elem Res 2022;200:3762-73.
32. Benkovic SJ, Baker SJ, Alley MR, Woo YH, Zhang YK, Akama T, *et al*. Identification of borinic esters as inhibitors of bacterial cell growth and bacterial methyltransferases, CcrM and MenH. J Med Chem 2005;48:7468-76.
33. Baker SJ, Akama T, Zhang YK, Sauro V, Pandit C, Singh R, *et al*. Identification of a novel boron-containing antibacterial agent (AN0128) with anti-inflammatory activity, for the potential treatment of cutaneous diseases. Bioorg Med Chem Lett 2006;16:5963-7.
34. Scorei IR, Biță A, Mogoşanu GD. Letter to the editor: Boron enhances the antiviral activity of the curcumin against SARS-CoV-2. Rom J Morphol Embryol 2020;61:967-70.
35. Gündüz MK, Bolat M, Kaymak G, Berikten D, Köse DA. Therapeutic effects of newly synthesized boron compounds (BGM and BGD) on hepatocellular carcinoma. Biol Trace Elem Res 2022;200:134-46.
36. de Carvalho LP, Groeger-Otero S, Kreidenweiss A, Kremsner PG, Mordmüller B, Held J. Boromycin has rapid-onset antibiotic activity against asexual and sexual blood stages of *Plasmodium falciparum*. Front Cell Infect Microbiol 2021;11:802294.
37. Abad-García A, Ocampo-Néstor AL, Das BC, Farfán-García ED, Bello M, Trujillo-Ferrara JG, *et al*. Interactions of a boron-containing levodopa derivative on D(2) dopamine receptor and its effects in a Parkinson disease model. J Biol Inorg Chem 2022;27:121-31.
38. Hua J, Gao Z, Zhong S, Wei B, Zhu J, Ying R. CISD1 protects against atherosclerosis by suppressing lipid accumulation and inflammation via mediating Drp1. Biochem Biophys Res Commun 2021;577:80-8.

Source of Support: Nil. **Conflicts of Interest:** None declared.