International Journal of Secondary Metabolite
2019, Vol. 6, No. 1, 1-9

https://dx.doi.org/10.21448/ijsm.454836

Published at http://www.ijate.net http://dergipark.gov.tr/ijsm **Research Article**

In vitro anticoagulant and antiinflammatory activities of *Geastrum fimbriatum* Fr., namely as Earthstar fungus

Nurdan Sarac [*,1], **Hakan Alli** [1], **Tuba Baygar** [2], **Aysel Ugur** [3]

[1] Department of Biology, Faculty of Science, Mugla Sitki Kocman University, 48000 Mugla, Turkey,
[2] Material Research Laboratory, Research Laboratories Center, Mugla Sitki Kocman University, 48000 Mugla, Turkey
[3] Section of Medical Microbiology, Department of Basic Sciences, Faculty of Dentistry, Gazi University, 06500 Ankara, Turkey

Abstract: Mushrooms have great potential to be used as food and pharmaceutical sources. Most of the non-edible mushrooms contain biologically active metabolites that are functional for modern medicinal applications. Within the present study, anticoagulant and antiinflammatory activities of *Geastrum fimbriatum* Fr. (Syn. *Geastrum sessile* (Sowerby) Pouzar), a mushroom naturally grown in Turkey, were investigated. The *in vitro* anticoagulant activity of the ethanolic extract obtained with a soxhlet apparatus determined by activated partial thromboplastin time (APTT) and prothrombin time (PT) assays using commercial reagents. The antiinflammatory activity of the extract was determined by lipoxygenase inhibition assay. When compared with the negative control DMSO, *G. fimbriatum* extract exhibited significant anticoagulant effects in the APTT test that evaluates the intrinsic coagulation pathway. The ethanolic extract found to prolong the coagulation time. However, no inhibition was observed in the PT test which evaluates the extrinsic coagulation pathway, The extract showed 12.92% inhibition on the lipoxygenase enzyme activity. Overall, *G. fimbriatum* ethanolic extract exhibited potent antiinflammatory activity besides being a potential source of anticoagulant. Further analysis is required to evaluate the medical use of *Geastrum* mushrooms from a pharmaceutical point of view.

ARTICLE HISTORY

Received: August 22, 2018
Revised: October 25, 2018
Accepted: December 17, 2018

KEYWORDS

Medicinal mushroom,
Lipoxygenase inhibition,
Coagulation

1. INTRODUCTION

Health problems in heart and blood vessels and also thrombosis are the major causes of death in the World [1]. It is reported by World Health Organization [2] that an estimated 17.5 million people died from cardiovascular diseases (CVDs) in 2012, representing 31% of all global deaths. It is supposed that 7.4 million of those people died due to coronary heart disease and 6.7 million died because of stroke [2]. CVDs are a group of disorders of the heart and blood

*CONTACT: Nurdan Saraç ✉ sarac_63@hotmail.com Department of Biology, Faculty of Science, Mugla Sitki Kocman University, 48000 Mugla, Turkey

ISSN-e: 2148-6905 /© IJSM 2019

vessels such as coronary, cerebrovascular and rheumatic heart diseases, deep ven thrombosis and pulmonary embolism [2]. Most require anticoagulant therapy is generally required for thromboembolic processes. Recently researchers are focusing on developing specific and potent anticoagulant and antithrombotic agents [1]. Anti-coagulants that mentioned above present some restrictions to their use, and some are used under a critical control due to hemorrhagic risk and limitation in the administration, however, they have effective use [3]. That's why investigating new, safer, and more effective anti-coagulant drugs, with less hemorrhagic risk and fewer side effects or interaction with drugs and food has been gaining much importance [4].

Inflammation is a complex biological process, induced by microbial infection or tissue damage [5]. There are different types of rheumatic disorders such as rheumatic fever, rheumatoid arthritis, ankylosing spondylitis, polyarthritis nodosa, systemic lupus erythematosus and osteoarthritis, which are called as inflammatory diseases [6]. Inflammation is a body defense reaction which tries to eliminate or limit the spread of injurious agent. Inflammatory reaction involves various components that can contribute to the associated symptoms and tissue injury. These components may be listed as oedema, leukocyte infiltration, and granuloma formation [7].

Arachidonic acid is released from the cell membranes due to the cell damage associated with inflammation [8]. There are two metabolic pathways that arachidonic acid undergoes: the cyclooxygenase (COX) pathway involving cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) to produce the prostaglandins and thromboxanes [9]; and the lipoxygenase (LOX) pathway, involving 5-lipoxygenase (5-LOX), 12-lipoxygenase (12-LOX) and 15-lipoxygenase (15-LOX), to produce the leukotrienes and hydroperoxy fatty acids [10,11].

Lipoxygenases are involved in the biosynthesis of leukotrienes which have a key role in several inflammatory diseases such as cancer, arthritis, asthma and allergic diseases. For this reason, lipoxygenase inhibitors may be potentially used with their medicinal benefits in prevention of these inflammatory cases [12].

Lipoxygenases (LOXs) (LOX; EC 1.13.11.12) are the group of non-heme iron-containing dioxygenases which catalyze the biosynthesis of leukotrienes. Leukotrienes take part as initiators of inflammation so their inhibition is directly related to anti-inflammatory activity [13].

Mushrooms have been used since long times for the treatments of various diseases, but still it has not been approved in main stream science as drugs or medical treatments [14-16]. Both fruiting body and mycelia of different mushroom known to contain different compounds such as flavonoids, alkaloids, polysaccharides, polyglucans, polyphenol, steroids, terpenoids, polyketides and dietary fibers which exert several pharmacological activities [17-18]. *Geastrum* species are mushrooms of the class Gasteromycetes in which the hymenium is enclosed until spores are matured. This genus has cosmopolitan distribution especially in the sandy soil forests of Asia, Africa, Australia, Europe, Mexico, North America and South America [19-21]. Medicinal uses of *Geastrum* species have not been well documented but some members of this class have been reported to have bioactive potentials [22-23]. In the study reported the rare appearance of *Geastrum* species and its ethnomedicinal uses as wound healing by ethnic tribes of Northern Odisha, India [24]. Further, some species of *Geastrum* have also been reported to have antimicrobial activity from southern India [25].

In this study the anticoagulant and antiinflammatory activities of the ethanolic extract of *G. fimbriatum* were investigated. To our knowledge, this is the first study related to the anticoagulant and antiinflammatory activities of this mushroom.

2. MATERIALS AND METHODS

2.1. Mushroom Collection and Extraction Procedure

The mushroom samples were collected from Golcuk village (Gediz-Kutahya), Turkey and identified by Dr. Alli (Figure 1). The samples were brought to the laboratory, dried in an incubator at 40°C. The dried and powdered mushroom samples (20 g) were extracted with ethanol (Merck) (400 ml) using the Soxhlet apparatus at 4 h. The extract was evaporated and then kept in small sterile opac bottles under refrigerated conditions until used [26,27].

Figure 1. *Geastrum fimbriatum* Fr. (Syn. *Geastrum sessile* (Sowerby) Pouzar)

2.2. Anticoagulant Activity

Activated partial thromboplastin time (APTT) and prothrombin time (PT) were performed using commercial human plasma (purchased from Sigma Aldrich). 10 mg/ml concentration of the *G. fimbriatum* extract was prepared with pure dimethyl sulphoxide (DMSO). The APTT and PT were measured via Pacific Hemostasis™ APTT and PT reagents (Thermo Fisher Scientific) according to the manufactures guidelines.

In the APTT test, the calcium chloride (0.02 M) prewarmed at 37°C. 100 µl of commercial plasma (Sigma, P9523) and 10 µl of the *G. fimbriatum* extract (10 mg/ml) transferred to the tube and also prewarmed at 37°C. After then, 100 µl of APTT-XL added to the tube and mixed. The plasma-reagent mixture was incubated at 37°C for 3-5 minutes (activation time). Forcibly added 100 µl prewarmed calcium chloride and coagulation times in the tubes were recorded [28,29].

In the PT test, 100 µl of commercial plasma (Sigma, P9523) and 10 µl of the *G. fimbriatum* extract (10 mg/ml) were transferred to the test tube. The mixture was incubated at 37°C for 1 min and then 200 µl of heated Thromboplastin-DS (at 37°C) added to the tube. Immediately the stopwatch was operated and coagulation times in the tubes were recorded [28,29].

Acetylsalicylic acid (0.5 mM final concentration) and pure DMSO were used as positive control and negative control, respectively.

2.3. Lipoxygenase Inhibition Assay

15-Lipoxygenase (15-LOX) inhibition activity was assayed using the Lipoxygenase Inhibitor Screening Assay Kit (Cayman Chemical Company, Ann Arbor, MI, USA) according to the manufacturer's guidelines. 15-LOX from *soybean* was purchased from Cayman Chemical Company (Ann Arbor, MI, USA). Linolenic acid was used as a substrate [30].

Residual 15-LOX activity was determined after pre-incubation for 5 min at room temperature with 100 µg/ml *G. fimbriatum* extract. Firstly, 10 µl of *G. fimbriatum* extract (100 mg/ml), 90 µl of 15-LOX, 10 µl of 0.1 M Tris-HCl buffer (pH 7.4) and 10 µl of 1 mM linolenic acid were added to a plate and incubated for 5 min. Then, the reaction was stopped by adding 100 µl chromogen and the plate was incubated in a shaker for 5 minutes and the absorbance was measured at 550 nm. The 100% initial activity was obtained from the well which incubated with DMSO. Nordihydroguaiaretic acid (NDGA) at 100 µM final concentration was used as a positive control. Data analysis was performed according to the instructions supplied with the assay kit.

3. RESULTS

In this study, the anticoagulant and antiinflammatory activities of the ethanolic extract of *G. fimbriatum* were investigated. To carry out coagulation tests, two standard *in vitro* tests, including PT and APTT tests were applied. These two tests cover both intrinsic and extrinsic blood coagulation pathways. This assay was made with commercial human plasma, using acetylsalicylic acid as a positive control. The anticoagulant activity of the *G. fimbriatum* extract was shown in Table 1.

Table 1. The anticoagulant activity of *G. fimbriatum* extract at 10 mg/ml concentration

Sample	PT (sec)	APTT (sec)
G. fimbriatum extract	22	67
Negative control (DMSO)	24	50
Acetylsalicylic acid	23	71

The *G. fimbriatum* extract exhibited notable anticoagulant effects in the APTT test that was applied to evaluate the intrinsic coagulation pathway, compared with the negative control DMSO. The extract prolonged the time of coagulation. On the other hand, in the PT test that was applied to evaluate the extrinsic coagulation pathway, no inhibition was observed with the extract.

The antiinflammatory activity of the *G. fimbriatum* extract was determined with the lipoxygenase inhibition capacity. The extract showed 12.92% inhibition on the LOX enzyme activity (Table 2).

Table 2. The LOX inhibition activity of *G. fimbriatum* extract

Sample	Concentration	Inhibition (%)
G. fimbriatum extract	100 µg/ml	12.92
NDGA	100 µM	40.00

4. DISCUSSION

Enzyme promoting processes are involved in the blood coagulation [31]. At the end of these processes; fibrin is formed from fibrinogen and blood is transformed to gel state from collosol state. An enzyme, the thrombase, effects the hemostasis and blood coagulation [32]. In order to explain the possible hemostatic mechanism, the compounds were tested for PT and APTT assays. PT is mainly applied to evaluate the extrinsic coagulation pathway while APTT is related to the intrinsic coagulation pathway [33]. When APPT prolongs, it suggests the inhibition of the intrinsic and/or the common pathway. On the other hand, PT prolongation gives information about the inhibition of the extrinsic and/or the common pathway [34].

Anticoagulants play a role in the prevention and treatment of thromboembolic disorders [35-36]. Anticoagulant drugs consisting of heparin and its derivatives, and vitamin K antagonists, have been the main anticoagulants in clinical practice. Despite their efficacy, major and life-threatening side effects of these agents have also been reported [37,38].

Anticoagulant drugs become crucial when there is a failure throughout the hemostasis; which leads to blood coagulation that could cause a vessel occlusion; unfractionated heparin (UFH), coumarin, low molecular weight heparin (LMWH), and the most recent drug introduced in the market - the synthetic pentasaccharide fondaparinux are the current medical options [3].

The use of anticoagulants is under a critical control as there are some restrictions for their use and also it needs to pay attention due to hemorrhagic risk and limitation in the administration [3]. Nowadays researchers focus on developing new, safer, and more effective anti-coagulant drugs, with less hemorrhagic risk and fewer side effects or interaction with drugs and food [4].

Most pharmaceutical drugs have side effects that may be experienced alongside their therapeutic actions [39]; for example, warfarin, a commonly used anticoagulant drug, demonstrates side effects that include bruising, bleeding gums, red or dark brown urine, red or black bowel motions, nosebleeds, haemoptysis, dyspnoea and dysphagia, heavier than usual menstrual periods, excessive wound bleeding, dark or blood stained vomit, severe headache and dizziness, unexplained pain, swelling and discomfort [40].

However, traditional remedies have long been known to have fewer adverse effects [41]. Therefore, the authors claim that the use of herbs with therapeutic effects and minimal side effects, could be the best option for the prevention and treatment of a wide range of diseases including cardiovascular disorders [42].

LOXs are the group of enzymes involve in the biosynthesis of leukotrienes that play an important role in the pathophysiology of various inflammatory diseases. The LOXs are classified with respect to their positional specificity of arachidonic acid oxygenation (5-LOX, 9-LOX, 12-LOX, 15-LOX)[43].

LOX enzymes are directly related to inflammatory and allergic reactions due to the formation of the leukotrienes (LTs). LTs values may increase in the case of allergic rhinitis, rheumatoid arthritis, psoriasis, asthma and colitis ulcerosa. Inhibition of the lipoxygenase pathway prevents the production of LTs. As a useful tool for cancer treatment, therapeutic strategies for inhibition of L\isoforms and/or their biologically active metabolites may be biologically and pharmacologically targeted by using lipoxygenase inhibitors [44].

Medicinal mushrooms are recognized with their extracts that profound health benefits and functionally used for traditional therapies. There are extensive studies that demonstrating the *in vitro* or *in vivo* biological activities of extracted and/or purified mushroom species such as antitumor and immunomodulating activity [45,46], anticancer [47,48], antioxidant [49], antiviral [50], antihypercholesterolemia [51], antidiabetic [52] effects.

There are drugs in the market that are used to treat antiinflammatory disorders but most of them are not toxically free. The use of antiinflammatory drugs may lead to gastrointestinal problems which is a problem for medical industry [53]. Thus, investigation of a natural antiinflammatory drug with functional properties is a necessity for pharmaceutical technology. Similar to our study, Guerradore et al. [54] reported that *Geastrum saccatum* extract inhibited the enzyme cyclooxygenase and had a promising antiinflammatory activity. *Geastrum* species have also antioxidant [54,55], antibacterial [56], and cytotoxic [54] activities. Although there are not any studies about *G. fimbriatum*, a nature muschroom growing in Mugla. The present study presents an important view about the anticoagulant and antiinflammatory activities of *G. fimbriatum* that potentially active.

5. CONCLUSION

Studies with mushrooms have been developing recently and it is figured out that potent properties of secondary metabolites from different mushroom species show great biological activites. Besides being an important dietary source, mushrooms are strong pharmaceuticals that reveals multiple therapies to diseases. There is a growing interest in active metabolites that are obtained from natural sources as an alternative to synthetic drugs. Anticoagulant and antiinflammatory characteristics of an agent enhance its functionality. In the present research, the potential use of mushroom extract as an anticoagulant and/or antiinflammatory agent has been evaluated. From the results of the study, it can be concluded that *G.* ***fimbriatum*** extract is endowed with effective anticoagulant and antiinflammatory activities. Comprehensive studies may be required to investigate the health-related activites of this mushroom.

Acknowledgments

This study was supported by the Scientific Research Project Unit of Mugla Sitki Kocman University, through the Grant number 15/205.

Orcid

Nurdan Sarac https://orcid.org/0000-0001-7676-542X
Hakan Alli https://orcid.org/0000-0001-8781-7029
Tuba Baygar https://orcid.org/0000-0002-1238-3227
Aysel Ugur https://orcid.org/0000-0002-5188-1106

6. REFERENCES

[1] Fares, A. (2013). Winter cardiovascular diseases phenomenon. *N. Am. J. Med. Sci., 5*, p.266-279.

[2] WHO – World Health Organization. Cardiovascular diseases. http://www.who.int/mediacentre/factsheets/fs317/en/ 2015. (accessed 17.03.2016).

[3] Aguirre, J., Borgeat, A. (2013). *Drugs for Thromboprophylaxis: Unfractionated Heparin, Low Molecular Weight Heparin, Warfarin, and Fondaparinux*. In: Llau JV, editor. Thromboembolism in Orthopedic Surgery. Springer, London, England, p. 53-65.

[4] Poulsen, B.K., Grove, E.L., Husted, S.E. (2012). New oral anticoagulants. *Drugs, 72*, p.1739-1753.

[5] García-Lafuente, A., Guillamón, E., Villares, A., Rostagno, M.A., Martínez, J.A. (2009). Flavonoids as anti-inflammatory agents: implications in cancer and cardiovascular disease. *Inflamm. Res., 58*, p.537-552.

[6] Anilkumar, M. (2010). Ethnomedicinal plants as anti-inflammatory and analgesic agents. Ethnomedicine: A Source of Comp. *Therap*., p.267-293.

[7] Amdekar, S., Roy, P., Singh, V., Kumar, A., Singh, R., Sharma, P. (2012). Anti-inflammatory activity of *Lactobacillus* on carrageenan-induced paw edema in male wistar rats. *Int. J. Inflam.*, 2012, p. 752015.

[8] Levick, S.P., Loch, D.C., Taylor, S.M., Janicki, J.S. (2007). Arachidonic acid metabolism as a potential mediator of cardiac fibrosis associated with inflammation. J. Immunol., 178, p.641-646.

[9] Rainsford, K.D. (2007). Anti-inflammatory drugs in the 21st century. *Subcell Biochem., 42*, p.3-27.

[10] Samuelsson, B., Dahlén, S.E., Lindgren, J.Å., Rouzer, C.A., Serhan, C.N. (1987). Leukotrienes and lipoxins: Structures, biosynthesis, and biological effects. *Science, 237*, 1171-1176.

[11] Yedgar, S., Krimsky, M., Cohen, Y., Flower, R.J. (2007). Treatment of inflammatory diseases by selective eicosanoid inhibition: A doubleedged sword? *Trends Pharmacol. Sci., 28*, p.459-464.

[12] Czapski, G.A., Czubowicz, K., Strosznajder, R.P. (2012). Evaluation of the antioxidative properties of lipoxygenase inhibitors. *Pharmacol. Rep., 64*(5), p.1179-1188.

[13] Ammon, H.P.T., Annazodo, M.I., Safayhi, H., Dhawan, B.N., Scrimal, R. (1992). Curcumin: a potent inhibitor of leukotriene B4 formation in rat peritoneal polymorphonuclear neutrophils (PMNL). *Planta Med., 58*, p.226.

[14] Bensky, D., Gamble, A. (1986). *Chinese Herbal Medicine: Materia Medica*, 2nd edn. Seattle: Eastland Press.

[15] Sullivan, R., Smith, J.E., Rowan, J., 2006. Medicinal mushrooms and cancer therapy: translating a traditional practice into Western medicine. *Perspect Bio. Med., 49*, 159-170.

[16] Rogers, R. (2011). *The Fungal Pharmacy. The Complete Guide to Medicinal Mushrooms and Lichens of North America*. North Atlantic Books, Berkeley, California.

[17] Thatoi, H.N., Singdevsachan, S.K. (2014). Diversity, nutritional composition and medicinal potential of Indian mushrooms: a review. Afr. J. Biotech., 13, p.523-545.

[18] Sevindik, M., Akgul, H., Akata, I., Allı, H., Selamoglu, Z. (2017). *Fomitopsis pinicola* in Healthful Dietary Approach and their Therapeutic Potentials. *Acta Alim., 46*(4), p.464-469.

[19] Nouhra, E.R., DeToledo, D.L. (1998). The first record of *Astraeus hygrometricus* from Argentina. *Mycol., 12*, p.112-113.

[20] Phosri, C., Watling, R., Martín, M.P., Whalley, A.J.S. (2004). The genus *Astraeus* in Thailand. *Mycotaxon, 89*, p.453-463.

[21] Fangfuk, W., Petchang, R., To-anun, C., Fukuda, M., Yamada, A. (2010). Identification of Japanese *Astraeus*, based on morphological and phylogenetic analyses. *Mycoscience, 51*, p.291-299.

[22] Wasser, S.P., Weis, A. (1999). Medicinal properties of substances occurring in higher basidimycetes mushrooms: current perspectives. *Int. J. Med. Mushrooms, 1*, p.31-62.

[23] Guerra, D.C.M.P., Azevedo, T.C.G., DeSouza, M.C.R. (2007). Antiinflammatory, antioxidant and cytotoxic actions of β- glucan-rich extract from *Geastrum saccatum* mushroom. *Int. Immunopharmacol., 7*, p.1160-1169.

[24] Panda, M.K., Tayung, K. (2015). Documentation and Ethnomedical Knowledge on Wild Edible Mushrooms among Ethnic Tribes of Northern Odisha, India. *Asian J. Pharma Clin. Res., 8*(4), p.139-143.

[25] Chittaragi, A., Naika, R., Banakar, S., Vijay, K. (2013). Phytochemical and Antifungal study of different solvent extracts of *Scleroderma bermudense* Corker. (Sclerodermataceae). *Am. J. Pharm. Tech. Res*., *3*, p.427-438.

[26] Basgedik, B., Ugur, A., Sarac, N. (2014). Antimicrobial, antioxidant, antimutagenic activities, and phenolic compounds of *Iris germanica*. *Ind. Crops Prod., 61*, 526-530.

[27] Saraç, N., Şen, B. (2014). Antioxidant, mutagenic, antimutagenic activities, and phenolic compounds of Liquidambar orientalis Mill. var. orientalis. *Ind. Crops Prod., 53*, 60-64.

[28] Schved, J.F., Biron-Andréani, C. (2005). Hématologie: Exploration de l'hémostase. *Montpellier*. pp: 19.

[29] Dandjesso, C., Klotoa, J. R., Dougnon, T. V., Sègbo, J., Atègbo, J. M., Gbaguidi, F., Dramane, K. (2012). Phytochemistry and hemostatic properties of some medicinal plants sold as anti-hemorrhagic in Cotonou markets (Benin). *Indian J. Sci. Technol*., *5*(8), 3105-3109.

[30] Ratnasary, N., Walters, M., Tsopmo, A. (2017). Antioxidant and lipoxygenase activities of polyphenol extracts from oat brans treated with polysaccharide degrading enzymes. *Heliyon*, *3*, e00351.

[31] Zhu, Z.Y. (1997). New concept of blood clotting. *Shanghai Med. J.*, *2*, p.115-117.

[32] Li, M., Jia, Z., Hu, Z., Zhang, R., Shen, T. (2008). Experimental Study on the Hemostatic Activity of the Tibetan Medicinal Herb *Lamiophlomis rotata. Phytoth. Res*., *22*, p.759-765.

[33] Liu, Z., Li, N., Gao, W., Man, S., Yin, S., Liu, C. (2012). Comparative study on hemostatic, cytotoxic and hemolytic activities of different species of Paris L. *J. Ethnopharmacol., 142*, p.789-794.

[34] Lee, W., Ku, S.K., Bae, J.S. (2015). Antiplatelet, anticoagulant, and profibrinolytic activities of baicalin. *Arch. Pharm. Res., 38*, p.893-903.

[35] Hull, R.D., Merali, T., Mills, A., Stevenson, A.L., Liang, J. (2013). Venous Thromboembolism in Elderly High-Risk Medical Patients Time Course of Events and Influence of Risk Factors. *Clin. Appl. Thromb. Hemost., 19*(4), p.357-362.

[36] John Camm, A. (2013). Managing anticoagulation for atrial fibrillation: current issues and future strategies. *J. Int. Med., 273*(1), p.31-41.

[37] Piazza, G., Nguyen, T.N., Cios, D., Labreche, M., Hohlfelder, B., Fanikos, J., Fiumara, K., Goldhaber, S.Z. (2011). Anticoagulation-associated Adverse Drug Events. *Am. J. Med., 124*(12), p.1136-1142.

[38] Alquwaizani, M., Buckley, L., Adams, C., Fanikos, J. (2013). Anticoagulants: A Review of the Pharmacology, Dosing, and Complications. *Cur. Emerg. Hos. Med. Rep., 1*(2), p.83-97.

[39] Kuhn, M., Campillos, M., Letunic, I., Jensen, L.J., Bork, P. (2010). A side effect resource to capture phenotypic effects of drugs. *Mol. Syst. Biol., 6*, p. 343-348.

[40] Pharma, A. Coumadin consumer medicine information. https://wwwebstgagovau/ebs/picmi/picmirepositorynsf/pdf?OpenAgent&id=CP-2010-CMI-02587-3. 2011 (accessed 18 November 2011).

[41] Winslow, L.C., Kroll, D.J. (1998). Herbs as medicines. *Arch. Intern. Med., 158*, p.2192-2199.

[42] Ernst, E. (2007). Herbal medicines: balancing benefits and risks. *Novartis Found Symp., 67-72*, p.212-218.

[43] Yamamoto, S. (1992). Mammalian lipoxygenases: molecular structures and functions. Biochim. *Biophys. Acta, 1128*, p.117-131.

[44] Yahaya, Y.A., Don, M.M. (2012). Evaluation of Trametes Lactinea extracts on the inhibition of Hyaluronidase, Lipoxygenase and Xanthine Oxidase activities in vitro. *J. Phy. Sci., 23*, p.1-15.

[45] Wasser, S.P. (2002). Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Appl. Microbiol. Biotech., 60*(3), p.258-274.

[46] Harada, T., Ohno, N. Dectin-1 and GM-CSF on immunomodulating activities of fungal 6-branched 1, 3-ß-glucans. Int. J. Med. Mushrooms, 10, p.101-114.

[47] Cai, Y., Luo, Q., Sun, M., Corke, H. (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sci., 74*(17), p.2157-2184.

[48] Deng, G., Lim, H., Seidman, A., Fornier, M., D'Andrea, G., Wesa, K., Yeung, S., Cunningham-Rundles, S., Vickers, A.J., Cassileth, B. (2009). A phase I/II trial of a polysaccharide extract from *Grifola frondosa* (Maitake mushroom) in breast cancer patients: immunological effects. *J. Cancer Res. Clin. Oncol., 135*, p.1215-1221.

[49] Mau, J.L., Lin, H.C., Chen, C.C. (2002). Antioxidant properties of several medicinal mushrooms. *J. Agric. Food Chem., 50*(21), p.6072-6077.

[50] Gao, Y., Zhou, S.H., Huang, M., Xu, A. (2003). Antibacterial and antiviral value of the genus *Ganoderma* P. Karst. species (Aphyllophoromy-cetideae): a review. *Int. J. Med. Mushrooms, 5*, p.235-246.

[51] Verticka, V., Vetrickova, J. (2009). Effects of yeast-derived beta-glucan on blood cholesterol and mactophage functionality. *J. Immunotoxicol., 6*, p.30-35.

[52] Gao, Y., Lan, J., Dai, X., Ye, J., Zhou, S.H. (2004). A phase I/II study of Ling Zhi mushroom *Ganoderma lucidum* (W.Curt.:Fr.) Lloyd (Aphyllophoro-mycetideae) extract in patients with type II diabetes mellitus. *Int. J. Med. Mushrooms, 6*, p.96-107.

[53] Bisht, R., Bhattacharya, S., Jaliwala, Y.A. (2014). COX and LOX inhibitory potential of *Abroma augusta* and *Desmodium gangeticum*. *J. Phytopharmacol., 3*(3), p.168-175.

[54] Guerradore, C., Azevedo, T., Desouza, M., Rego, L., Dedantas, J., Silva, F., Rocha, H., Baseia, I. (2007). Antiinflammatory, antioxidant and cytotoxic actions of ß-glucan-rich extract from *Geastrum saccatum* mushroom. Int. Immunopharmacol., 7(9), p.1160-1169.

[55] Sevindik, M., Akgul, H., Akata, I., Selamoglu, Z. (2017). *Geastrum pectinatum* as an Alternative Antioxidant Source with some Biochemical Analysis. *Medical Mycology: Open Access, 3*(2), 1-4.

[56] Chittaragi, A., Naika, R., Ashwini, H.S., Nagaraj, K. 2013. Antibacterial Potential of Geastrum triplex Jungh. Against Plant and Human Pathogens. *Int. J. PharmTech Res., 5*(4), 1456-1464.