International Journal of Engineering Research & Management Technology
ISSN: 2348-4039
May-2019 Volume 6, Issue-3
Email: editor@ijermt.org
www.ijermt.org

Physicochemical Characterization Seed oil of Seabuckthorn [SBT] (*Hippophae rhamnoides [HR]*

S. Rajesh
Research Scholar
Dept. of Pharmacy
Sunrise University, Alwar, Rajasthan

Chatter Singh
Professor
Dept. of Pharmacy
Sunrise University, Alwar, Rajasthan

ABSTRACT

This study assessed and compared Physicochemical activity of *Hippophae rhamnoides* (HR) The details about the procurement of plant material, SBT seed oil extraction, characterization of SBT seed oil, physicochemical characteristics, and preparation of the sample have been reported in our previous study.Seeds (HR and HS) were washed, air dried, crushed and extracted in petroleum ether (40-60 oC) for 7 days using a soxhlet apparatus. Petroleum ether was evaporated from the extract and the oil was filtered to clarity. The oil was stored at room temperature in amber coloured airtight bottle. To avoid oxidation, the oil was purged with nitrogen and was filled to the brim of the bottle so that there was no headspace**.**The HR seed oil were found to have non-significant changes in acid, iodine, saponification, and peroxide value through a period of 18 months The values of the aforementioned parameters were obtained at the baseline (immediately after extraction of oil; Day 1) and were compared with the standard values (AOCS) for each parameter. The baseline values were compared further with the values obtained at subsequent time-periods**.** Further, both the samples were kept at room temperature for 1 month and physicochemical properties were evaluated weekly. There was no significant difference between physicochemical values on day 1 and Day 28. These findings further support the physicochemical stability of the oil samples at room temperature for 1 month. Data was analysed using one way ANOVA.

Key words: Physicochemical, antihistaminic, rachidonic acid,polyunsaturated fatty acid, prostaglandin

1. INTRODUCTION

The seven species and eleven subspecies of the genus Hippophae, family Elaeagnaceae, are called seabuckthorn. *Hippophae rhamnoides* is geographically the most widely distributed species. Seabuckthorn, herein being referred as SBT, *Hippophae rhamnoides* L and *Hippophae salicifolia* D. Don, is one of the most promising species among the therapeutic plants with a history of varied and multidimensional uses. SBT has been used in Eastern traditional medicine for centuries. In the Chinese Pharmacopeia,sea buckthorn berries are endorsed for helping in relieving cough and for promoting digestion and blood circulation .

In India, SBT grows in the high altitudes of Himalayan region, where temperature touches as low as -30ºC during peak winters with annual precipitation up to only 9 cm. Indian Himalayan region is the second largest habitat of SBT. Ladakh is one of the natural habitats of SBT in India. It has very less vegetation and low oxygen. People living in this remote area have peculiar problems. They have limited resources to enhance the revenue from their uneconomical land holdings and industrial production. At the same time, this region also acts as a treasure house of number of medicinal, aromatic and fruit plants like SBT that can sustain a number of industries . SBT has been exploited as fuel, fodder, medicine, and fruits by the people living in Himalayan region and has huge therapeutic and nutritional potential. Many of its products have been commercialized largely in some parts of the world. Inspite of all such things, China is the only country in the world that is utilizing the full potential of SBT in food, medicinal and cosmetic products. In China, SBT industry is estimated to be worth more than INR 3,000 Crores. There are more than 200 products in the global market based on this single plant. However, in India it was not known commercially untill the year 2001 i.e. the emergence of Leh Berry on the national scene. Arachidonic acid (AA) (20:4 n‐6) is one of the most influential poly unsaturated fatty acids (PUFAs) in positively modulating the inflammatoryprocess.[1] Dietary long‐chain n‐3 PUFAs can decrease tissue AA levels and eicosanoid production, both *in vitro* and *in vivo.* This decrease results in alteration of plasma phospholipid fatty acid composition. In addition, n‐3 PUFAs increase competition for cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, thereby decreasing pro‐inflammatory prostaglandins (PGs) and leukotrienes (LTs), which subsequently can result in anti‐inflammatory activity.[2] Treatment of inflammation includes extensive use of nonsteroidal anti‐inflammatory drugs (NSAIDs).However, their use makes the patients more vulnerable to gastrointestinal and liver toxicities.[3,4] Therefore, herbal anti‐inflammatory treatments having alternative mechanisms are preferred as substitutes to NSAIDs. Seabuckthorn (SBT) is a parasol term used for most of the plant species of genus *Hippophae*, family Elaeagnaceae. Two of the most prominent Indian species of this plant are *Hippophae rhamnoides* (HR) and *Hippophae salicifolia* (HS), henceforth collectively referred to as SBT.[5] Previous studies have revealed the presence of considerable amount of n‐3 and n‐6 fatty acids, namely, alpha linolenic acid (ALA),linoleic acid (LA), and oleic acid (OA) in HR and HS seed oil which are precursors of other PUFAs such as AA and eicosapentaenoic acid (EPA) in SBT seed oil. SBT prevents soil erosion and reduces the surface run-off. SBT has a very strong and deep tap root system. SBT is used for flood protection on riverbanks or checking the streams, wasteland development and control of soil erosion through wind and water. SBT roots contain nodules that are produced by symbiotic mycorrhiza fungus –*Frankia*, which help in nitrogen fixation and improvement in soil fertility.

2. MATERIALS AND METHODS

The details about the procurement of plant material, SBT seed oil extraction, characterization of SBT seed oil, physicochemical characteristics, and preparation of the sample have been reported in our previous study.[8] National Botanical Research Institute, Lucknow, via letter number NBRI/CIF/694/2021 and NBRI/CIF/928/2021, authenticated the HR and HS seeds, respectively, and seed oil was extracted using Soxhlet apparatus by slightly modifying the method of Cenkowski *et al*.[9] To characterize the HR seed oil, physicochemical and fatty acid profiling was performed. Wistar strain albino rats (142–150 g) were procured from Central Animal House facility of SHIATS, Allahabad. Animal Ethical Committee (IAEC/SHIATS/812) endorsed the experimental protocol.

Hippophae rhamnoides from
Leh, Ladakh, India

Specific Gravity Determination Using Pycnometer

(1) Weigh the empty clean and dry pycnometer (PM), W .

(2) Place 1g of a sample in the PM.

(3) Weigh the PM containing the sample, W .

(4) Fill the PM with distilled water and clean the exterior surface of the PM with a clean, dry cloth. Determine the weight of the PM and contents, W .

(5) Empty the PM and clean it.

Specific gravity = W +W /W +W

Iodine Value

Oils are generally regarded as mixture of triglycerides (TGs). TGs comprise of three fatty acids linked to glycerol by fatty acyl esters. Fatty acids can be grouped into saturated or unsaturated based on the number of chemical bonds. Saturated fatty acids contain only single bond between the carbon atoms. The double bonds existing in the naturally occurring unsaturated fats are in the *Cis* form. *Trans* fatty acids are associated with numerous pathological processes. Double bonds can be converted into a single bond by hydrogenation. Based on the degree of unsaturation, the fatty acids can react with oxygen or halogens to

form saturated fatty acids. Hence, it is essential to know the extent to which a fatty acid is unsaturated. Iodine value is the number of grams of iodine consumed by 100g of fat. Greater the iodine value, greater is the degree of unsaturation.

Volume of Sodium thiosulphate used = [Blank- Test] ml

$$Iodine\ No.of\ fat = \frac{Equivalent\ Wt.of\ Iodine \times Volume\ of\ Na_2S_2O_3\ used \times Normality\ of\ Na_2S_2O_3 \times 100 \times 10^{-3}}{Weight\ of\ fat\ sample\ used\ for\ analysis(g)}$$

Equivalent Weight of Iodine = 127

Normality of sodium thiosulphate ($Na_2S_2O_3$) = 0.1

Acid Value

Acid value is defined as the "number of mg of KOH required to neutralize the free acid in 1g of fat, fatty oil or other related substances is determined to evaluate the rancidity of the samples". It is indicative of inadequate processing, storage, and hydrolysis of TG. It is calculated using titration against KOH (0.56 g/100 mL) and phenolphthalein as an indicator.

Concentration of titrant (NaOH) could be calculated using below formula:

$$C_{NaOH} = \frac{W_{C_8H_5KO_4} \times P_{C_8H_5KO_4} \times 1000}{V_{NaOH} \times Mw_{C_8H_5KO_4}}$$

where C_{NaOH} = Molarity of sodium hydroxide titrant (mol/L)

V_{NaOH} = Volume of sodium hydroxide titrant used (mL)

$Mw_{C_8H_5KO_4}$ = Molecular weight of potassium hydrogen phthalate (204.22 g)

$W_{C_8H_5KO_4}$ = Weight of potassium hydrogen phthalate used (g)

$P_{C_8H_5KO_4}$ = Purity of potassium hydrogen phthalate (%)

$$\text{Acid value} = \frac{V_{NaOH} \times 5.61}{W}$$

where V_{NaOH} = Volume of sodium hydroxide titrant used (mL)

W = Weight of the fatty oil being examined (g)

Saponification Value

Fats are largely known to be a reduced compound. These constitute -COOH with hydrocarbon chains and could be saturated or unsaturated. The simplest lipid formed fromfatty acids are TGs. TGs have 3 fatty

acids each in ester linkage with a single glycerol. TGs are non-polar, aqua phobic compounds. Saponification is the hydrolysis of fats or oils.

The saponification number is the "number of milligrams of KOH required to neutralize the fatty acids resulting from the complete hydrolysis of 1g of fat". It is a measure of average molecular weight (or chain length) of all the fatty acids present. It is done by simple titration with 0.5 N HCl using phenolphthalein as an indicator. It tells about the mean molecular mass.

Saponification value or number of fat = mg of KOH consumed by 1g of fat.

Weight of KOH = Normality of KOH * Equivalent weight* volume of KOH in liters

Volume of KOH consumed by 1g fat = [Blank – test] ml

Peroxide Value

Peroxide value is an important measure of the extent of oxidation of lipids, fats, and oils. Oxidation of lipids in food is not required due to non-desirable flavors, toxins, and loss of fat-soluble vitamins. It is performed by titration with sodium thiosulphate using starch as an indicator and is indicative of rancidity.

Peroxide value (mEq peroxide/kg sample) = S*N*1000/weight of the sample in g

Where S = ml Na S O (Test-Blank) and N = normality of Na S O

Physicochemical stability studies of the samples at room temperature were also performed.

3. RESULTS & DISCUSSION

This study delineated the effect of HR seed oil on overall Physicochemical cascade with the objective of providing an insight into the plausible mechanism of action. The results of density, specific

Seed Oil Extraction

Seeds (HR and HS) were washed, air dried, crushed and extracted in petroleum ether (40-60 oC) for 7 days using a soxhlet apparatus. Petroleum ether was evaporated from the extract and the oil was filtered to clarity. The oil was stored at room temperature in amber coloured airtight bottle. To avoid oxidation, the oil was purged with nitrogen and was filled to the brim of the bottle so that there was no headspace**.**

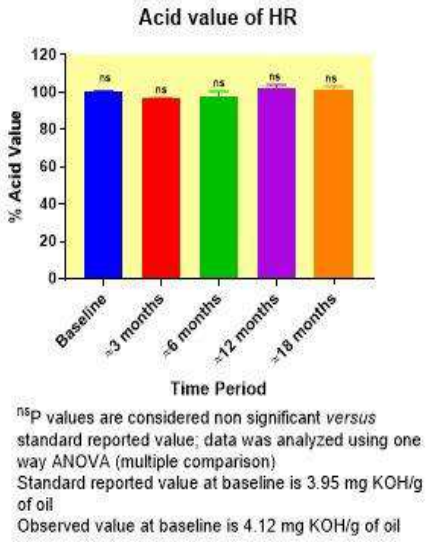
Stability Studies of the Seabuckthorn Seed Oil across Time-period in Controlled Environment and at Room Temperature through 28 Days

The HR and HS seed oil were found to have non-significant changes in acid, iodine, saponification, and peroxide value through a period of 18 months. The values of the aforementioned parameters were obtained at the baseline (immediately after extraction of oil; Day 1) and were compared with the standard values (AOCS) for each parameter.

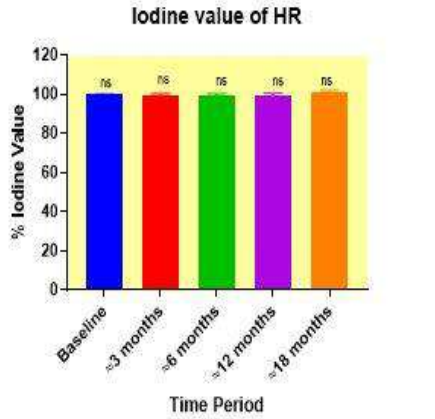
The baseline values were compared further with the values obtained at subsequent time-periods (3, 6, 12, and 18 months) **(Figure 5.3.1** . Further, both the samples were kept at room temperature for 1 month and

physicochemical properties were evaluated weekly. There was no significant difference between physicochemical values on day 1 and Day 28. These findings further support the physicochemical stability of the oil samples at room temperature for 1 month **(Figure 5.3.2 .** Data was analysed using one way ANOVA.

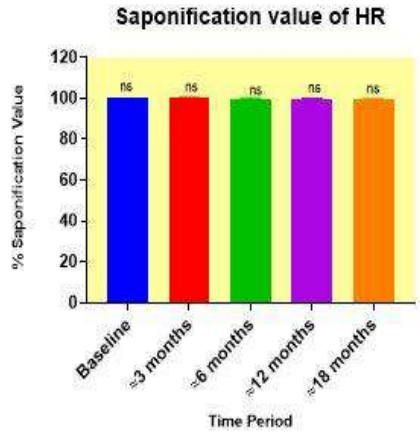
Figure 5.3.1 Acid, Iodine, Saponification, and Peroxide Value of *Hippophae rhamnoides* under Controlled Conditions through 18 Months



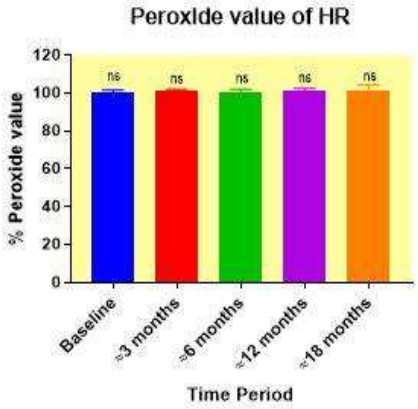
nsP values are considered non significant *versus* standard reported value; data was analyzed using one way ANOVA (multiple comparison)
Standard reported value at baseline is 3.95 mg KOH/g of oil
Observed value at baseline is 4.12 mg KOH/g of oil



nsP values are considered non significant *versus* standard reported value; data was analyzed using one way ANOVA (multiple comparison)
Standard reported value at baseline is 150.35 mg I_2/g of oil
Observed value at baseline is 152.56 mg I_2/g of oil



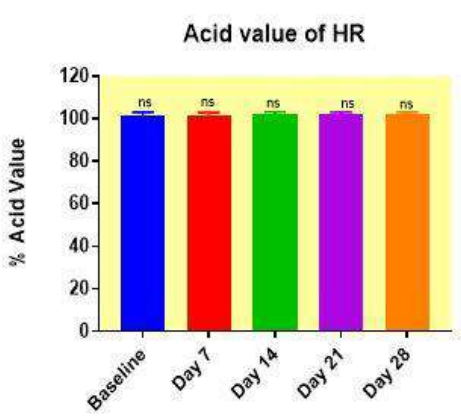
nsP values are considered non significant *versus* standard reported value; data was analyzed using one way ANOVA (multiple comparison)
Standard reported value at baseline is 190.1 mg/g of oil
Observed value at baseline is 190.34 mg/g of oil



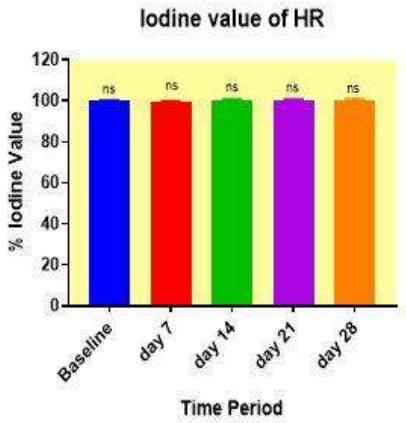
nsP values are considered non significant *versus* standard reported value; data was analyzed using one way ANOVA (multiple comparison)
Standard reported value at baseline is 17.50 mEq/Kg of oil
Observed value at baseline is 18.4 mEq/Kg of oil

Standard values are taken from Association of Official Agricultural Chemists

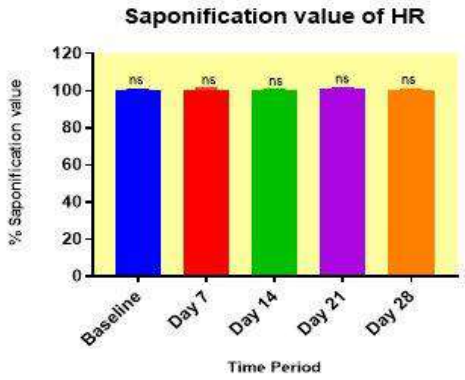
Figure 5.3.2 Acid, Iodine, Saponification, and Peroxide Value of *Hippophae rhamnoides* when stored in Room Temperature for 28 Days



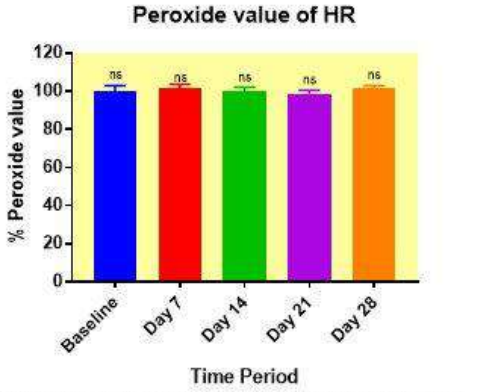
nsP values are considered non significant *versus* standard reported value; data was analyzed using one way ANOVA (multiple comparison)
Standard reported value at baseline is 3.95 mg KOH/g of oil
Observed value at baseline is 4.56 mg KOH/g of oil



nsP values are considered non significant *versus* standard reported value;data was analyzed using one way ANOVA (multiple comparison)
Standard reported value at baseline is 150.35 mg I_2/g of oil
Observed value at baseline is 153.61 mg I_2/g of oil



nsP values are considered non significant *versus* standard reported value; data was analyzed using one way ANOVA (multiple comparison)
Standard reported value at baseline is 190.1 mg/g of oil
Observed value at baseline is 190.50 mg/g of oil



nsP values are considered non significant *versus* standard reported value; data was analyzed using one way ANOVA (multiple comparison)
Standard reported value at baseline is 17.50 mEq/Kg of oil
Observed value at baseline is 18.57 mEq/Kg of oil

4. CONCLUSION

The findings of the present study may be concluded as under.Findings of Physicochemical study are helpful in identification of SBT leaves and determination of its quality & purity, stellate trichomes being the characteristic feature. Phytochemical investigation suggests 75% ethanolic extract of SBT leaves as the most promising to be developed as a nutraceutical and/ or dietary supplement agent. Animal toxicity studies indicate that 75% ethanolic extract of SBT leaves is practically non toxic in rats after oral administration and support the use of SBT leaves in traditional systems of medicine.

5. ACKNOLEGMENT

The authors are grateful to Chatter Singh, Prof., Dept. of Pharmacy, Sunrise University, Alwar, Rajasthan for providing required facilities for successful completion of this work. We would like to thank Dr. Hariom Sharma, Assistant Professor, BBAU, for his technical expertise and the infrastructural support.

6. REFERENCES

1. Abi-Hachem, R. N., Zine, A., & Van De Water, T. R. (2010). The injured cochlea as a target for inflammatory processes, initiation of cell death pathways and application of related otoprotectives strategies. Recent Pat CNS Drug Discov, 5(2), 147-163.
2. ADB Official Report. Retrieved 15th May, 2019, from https://www.adb.org/sites/default/files/project-document/67965/39229-mon- rrp.pdf
3. Antunes, L. C. M., Arena, E. T., Menendez, A., Han, J., Ferreira, R. B. R., Buckner,M. M. C., Finlay, B. B. (2011). Impact of salmonella infection on host hormone metabolism revealed by metabolomics. Infection and immunity, 79(4), 1759-1769. doi: 10.1128/IAI.01373-10
4. Auquit-Auckbur, I., Caillot, F., Arnoult, C., Menard, J. F., Drouot, L., Courville, P., Musette, P. (2011). Role of toll-like receptor 4 in the inflammation reaction surrounding silicone prosthesis. Acta Biomater, 7(5), 2047-2052. doi: 10.1016/j.actbio.2011.01.030
5. Barnig, C., & Levy, B. D. (2015). Innate immunity is a key factor for the resolution of inflammation in asthma. European Respiratory Review, 24(135), 141-153. doi: 10.1183/09059180.00012514
6. Barrera, G., Pizzimenti, S., Daga, M., Dianzani, C., Arcaro, A., Cetrangolo, G. P., Gentile, F. (2018). Lipid Peroxidation-Derived Aldehydes, 4-Hydroxynonenal and Malondialdehyde in Aging-Related Disorders. Antioxidants (Basel, Switzerland), 7(8), 102. doi: 10.3390/antiox7080102
7. Berger, A. (1999). What are leukotrienes and how do they work in asthma? BMJ, 319(7202), 90. doi: 10.1136/bmj.319.7202.90.
8. Chakraborty, M., Karmakar, I., Haldar, S., Das, A., Bala, A., & Haldar, P. K. (2016). Amelioration of oxidative DNA damage in mouse peritoneal macrophages by Hippophae salicifolia due to its proton (H(+)) donation capability: Ex vivo and in vivo studies. J Pharm Bioallied Sci, 8(3), 210-216. doi: 10.4103/0975- 7406.172663
9. Chan, G. K., Witkowski, A., Gantz, D. L., Zhang, T. O., Zanni, M. T., Jayaraman, S., & Cavigiolio, G. (2015). Myeloperoxidase-mediated Methionine Oxidation Promotes an Amyloidogenic Outcome for Apolipoprotein A-I. J Biol Chem, 290 (17), 10958-10971. doi: 10.1074/jbc.M114.630442
10. Chance, B., Sies, H., & Boveris, A. (1979). Hydroperoxide metabolism in mammalian organs. Physiol Rev, 59(3), 527-605. doi: 10.1152/physrev.1979.59.3.527
11. Charest-Morin, X., Hébert, J., Rivard, G.-É., Bonnefoy, A., Wagner, E., & Marceau, F. (2018). Comparing Pathways of Bradykinin Formation in Whole Blood From Healthy Volunteers and Patients With Hereditary Angioedema Due to C1 Inhibitor Deficiency. Frontiers in immunology, 9, 2183-2183. doi: 10.3389/fimmu.2018.02183

12. Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., Zhao, L. (2017). Inflammatory responses and inflammation-associated diseases in organs. Oncotarget, 9(6), 7204-7218. doi: 10.18632/oncotarget.23208
13. Chovatiya, R., & Medzhitov, R. (2014). Stress, Inflammation, and Defense of Homeostasis. Molecular cell, 54(2), 281-288. doi: 10.1016/j.molcel.2014.03.030
14. Choy, E. H., & Panayi, G. S. (2001). Cytokine pathways and joint inflammation in rheumatoid arthritis. N Engl J Med, 344(12), 907-916. doi: 10.1056/nejm200103223441207
15. Christman, B. W., Christman, J. W., Dworski, R., Blair, I. A., & Prakash, C. (1993). Prostaglandin E2 limits arachidonic acid availability and inhibits leukotriene B4 synthesis in rat alveolar macrophages by a nonphospholipase A2 mechanism. The Journal of Immunology, 151(4), 2096-2104.
16. Fatima, T., Snyder, C. L., Schroeder, W. R., Cram, D., Datla, R., Wishart, D., Krishna, P. (2012). Fatty acid composition of developing sea buckthorn (Hippophae rhamnoides L.) berry and the transcriptome of the mature seed. PloS one, 7(4), e34099-e34099. doi: 10.1371/journal.pone.0034099
17. Fattahi, M. J., & Mirshafiey, A. (2012). Prostaglandins and Rheumatoid Arthritis. Arthritis, 2012, 7. doi: 10.1155/2012/239310
18. Ferrante, A., Goh, D., Harvey, D. P., Robinson, B. S., Hii, C. S., Bates, E. J., Poulos, A. (1994). Neutrophil migration inhibitory properties of polyunsaturated fatty acids. The role of fatty acid structure, metabolism, and possible second messenger systems. The Journal of clinical investigation, 93(3), 1063-1070. doi: 10.1172/JCI117056
19. Ferrero-Miliani, L., Nielsen, O. H., Andersen, P. S., & Girardin, S. E. (2007). Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1beta generation. Clin Exp Immunol, 147(2), 227-235. doi: 10.1111/j.1365- 2249.2006.03261.x Funk, C. D. (2001).
20. Geetha, S., Sai Ram, M., Singh, V., Ilavazhagan, G., & Sawhney, R. C. (2002). Anti-oxidant and immunomodulatory properties of seabuckthorn (Hippophae rhamnoides)--an in vitro study. J Ethnopharmacol, 79(3), 373-378.
21. Global OTC Herbal And Traditional Medicines Market Research Report 2019. (2019). Retrieved on 15th April 2019, from URL, https://www.marketinsightsreports.com/reports/02131093784/global-otc-herbal- and-traditional-medicines-market-research-report
22. Gomes, R. N., Felipe da Costa, S., & Colquhoun, A. (2018). Eicosanoids and cancer. Clinics (Sao Paulo, Brazil), 73(suppl 1), e530s-e530s. doi: 10.6061/clinics/2018/e530s
23. Granstrom, E. (1984). The arachidonic acid cascade. The prostaglandins, thromboxanes and leukotrienes. Inflammation, 8 Suppl, S15-25.

24. Pavao-de-Souza, G. F., Zarpelon, A. C., Tedeschi, G. C., Mizokami, S. S., Sanson, J. S., Cunha, T. M., Verri, W. A., Jr. (2012). Acetic acid- and phenyl-p- benzoquinone-induced overt pain-like behavior depends on spinal activation of MAP kinases, PI(3)K and microglia in mice. Pharmacol Biochem Behav, 101(3), 320-328. doi: 10.1016/j.pbb.2012.01.018
25. Pearce, F. L. (1991). Biological effects of histamine: An overview. [journal article]. Agents and Actions, 33(1), 4-7. doi: 10.1007/bf01993112
26. Perianayagam, M. C., Balakrishnan, V. S., King, A. J., Pereira, B. J., & Jaber, B. L. (2002). C5a delays apoptosis of human neutrophils by a phosphatidylinositol 3- kinase-signaling pathway. Kidney Int, 61(2), 456-463. doi: 10.1046/j.1523- 1755.2002.00139.x
27. Singh, S., & Majumdar, D. K. (1995). Anti-inflammatory and Antipyretic Activities of Ocimum Sanctum Fixed Oil. International Journal of Pharmacognosy, 33(4), 288-292. doi: 10.3109/13880209509065380
28. Smith, W. L., DeWitt, D. L., & Garavito, R. M. (2000). Cyclooxygenases: structural, cellular, and molecular biology. Annu Rev Biochem, 69, 145-182. doi: 10.1146/annurev.biochem.69.1.145
29. Smith, W. L., Urade, Y., & Jakobsson, P. J. (2011). Enzymes of the cyclooxygenase pathways of prostanoid biosynthesis. Chem Rev, 111(10), 5821-5865. doi: 10.1021/cr2002992
30. Strzepa, A., Pritchard, K. A., & Dittel, B. N. (2017). Myeloperoxidase: A new player in autoimmunity. Cellular immunology, 317, 1-8. doi: 10.1016/j.cellimm.2017.05.002
31. Yehuda, S. (2003). Omega-6/omega-3 ratio and brain-related functions. World Rev Nutr Diet, 92, 37-56.
32. Yuan, H., Ma, Q., Ye, L., & Piao, G. (2016). The Traditional Medicine and Modern Medicine from Natural Products. Molecules, 21(5), 559.