

**ANTIOXIDANT AND XANTHINE OXIDASE INHIBITORY
POTENTIAL OF AQUEOUS EXTRACT OF *FERULA ASAFOETIDA***

Running title: Antioxidant of aqueous extract of aqueous asafoetida.

ABSTRACT :

Background: *Ferula asafoetida* is a dried latex that is exuded from rhizome or taproot species. Organosulfides are primarily responsible for flavour and odour of asafoetida. *Ferula asafoetida* is a natural medicine good for asthma and bronchitis. is also used to relieve stomach gas, digestive issues. It is usually added while cooking to harmonise the sweet, sour, salty, spicy taste of the food. Increased activity of xanthine oxidase is involved in the medical condition known as gout, which is characterized by hyperuricemia that leads to deposition of uric acid in the joints resulting in painful inflammation.

Aim: To analyse the anti-oxidant and xanthine oxidase inhibitory potential of aqueous extract of *Ferula asafoetida*.

Materials and methods: Preparation of the aqueous seed extract of *Ferula asafoetida* done by hot percolation method. Phytochemical screening, *in vitro* antioxidant activity and xanthine oxidase inhibitory potential was done by standard procedures. The data were analyzed statistically by a one - way analysis of variance (ANOVA) followed by Duncan's multiple range test was used to see the statistical significance among the groups. The results with the $p < 0.05$ level were considered to be statistically significant.

Results: The phytochemical screening revealed that the extract is rich in phytoconstituents. DPPH radical scavenging activity established the potent *in vitro* antioxidant activity ($p < 0.05$) of *Ferula asafoetida* extract. The extract was also efficient in inhibiting the activity of xanthine oxidase enzyme ($p < 0.05$) in a concentration dependent manner.

Discussion: The extract has potent antioxidant and xanthine oxidase inhibitory potential, although the activities are less compared to the standard drug.

Conclusion: The *Ferula asafoetida* extract can be used to treat gout and to combat various other disorders associated with xanthine oxidase activity.

Keyword: *Ferula asafoetida*, antioxidant, phytochemical screening, gout, xanthine oxidase, Innovative technology, Novel method

INTRODUCTION:

Plants have been a constant source of drugs and recently, much emphasis has been placed on finding novel therapeutic agents from medicinal plants (1). Today many people prefer to use medicinal plants rather than chemical drugs (2). Spices are known to possess several medicinal properties. A number of health favorable physiological effects of dietary spices have been experimentally documented in recent decades (2). The efficacy of these chemopreventive agents against cancer has been related with their antioxidant potential to reduce /or inhibit free radical mediated damage to cellular macromolecules, such as DNA, lipids and proteins (3). The protective action of the antioxidants are related with their potential to decrease oxidative stress and induce phase II detoxifying enzymes such as glutathione S-transferase and quinone reductase (4).

Ferula asafoetida is reported to be effective in lowering blood pressure and blood sugar level, having anti cancer effects, and protects brain health. It is a perennial plant belonging to the *Umbelliferae* family. The flowers are small and dirty yellow coloured produced in large compounds. The syrup prepared by decoction of asafoetida has been widely used because of its prominent activity it possesses and it is used for whooping cough and asthma and various types of respiratory disorders. Previous studies reported the promising activities of *Ferula asafoetida* as having muscle relaxant, memory enhancing, digestive enzyme, antioxidant, antispasmodic, hypotensive, hepatoprotective, antiviral, antifungal, anticancer, anxiolytics, and anthelmintic activities (5). The volatile oil in the gum resin is eliminated through the lungs, making it suitable for the treatment of asthma. The odor of asafoetida is imparted to the breath, secretions, flatus, and gastric eructations (1). From old ages, farmers in Malir, Karachi area of Sindh, are applying asafoetida for preventing the plants from root diseases particularly nematode attacks and increasing yield ((6). Chemicals called coumarins in asafoetida can thin the blood. Ethanolic extract of Asafoetida oleo gum-resin has shown antifungal activity against *Mucor dimorphosporous*, *Penicillium commune* and *Fusarium solani* (7),(8)

Xanthine oxidase serves as an important biological source of oxygen-derived free radicals that contribute to the oxidative damage of living tissues. Xanthine oxidase is associated with the formation of uric acid from xanthine and hypoxanthine which in turn is derived from purine catabolism (9),(10),(11) Therefore, defects associated with the purine degradation pathway can result in an increase in the uric acid level which is a risk factor for the development of gout. XO inhibitors which block the terminal step in uric acid biosynthesis, can reduce the plasma uric acid level, and are usually employed for the treatment of gout (12),(13),(14).Our team has extensive knowledge and research experience that has translate into high quality publications (15),(16),(17),(18),(19), (20), (21), (22), (23), (24),(25), (26), (27),(28),(29),(30) Hence the aim of the present study is to assess the xanthine oxidase inhibitory potential of *Ferula asafoetida*.

MATERIALS AND METHODS

Preparation of aqueous extract of *Ferula asafoetida*:

Ferula asafoetida was purchased from a herbal health care center. Crushed and made into powder. 80% of methanolic extract was obtained (31).

Assessment of preliminary phytochemical screening

Test for phlobatannins:

1 ml of the extract was treated with 1ml of 1% of HCL and boiled for 10 minutes. The formation of red colour precipitate indicates the presence of phlobatannin.

Test for carbohydrates:

Three to five drops of molisch agent was added with 1 ml of the extract and then 1 ml of concentrated sulphuric acid was added carefully through the side of the tube.

Test for flavonoids:

Few drops of 1% liquid ammonia were taken in a test tube and along with it 1ml of extracted indicating presence of flavonoids.

Test for alkaloids:

2ml of sample was mixed with 2 ml of HCL. Then 6 drops of HCN was added and further 2 drops of picric reamish pale yellow indicating the presence of alkaloids.

Test for terpenoids:

2 ml of sample along with 2ml of chloroform and 3ml of concentrated H₂SO₄ was added.
Red - presence of terpenoids.

Test for proteins:

1 ml of ninhydrin was dissolved in 1ml of acetone in a small amount.
Purple - presence of protein.

Detection of saponins:

Foam test : a fraction of the extract was vigorously shaken with water and observed for Persistent foam.

Test for steroids:

1ml of chloroform was mixed with 1ml of extract and 10 drops of acetic anhydride and five drops of concentrated sulphuric acid were added and mixed.
Dark red and dark pink - presence of steroids.

Assessment of *in vitro* antioxidant activity by DPPH free radical scavenging activity:

Scavenging of 2,2 - diphenyl-1- picrylhydrazyl radical was assessed by the method of DPPH solution was added to 1 ml of extract at different concentrations. The mixture was kept at room temperature for 50 minutes and the activity was measured at 517 nm. Ascorbic acid at the same concentration was used as standard.

$$\text{DPPH scavenging activity (\%)} = (\text{control OD} - \text{sample OD}) / \text{control OD} \times 100$$

***In vitro* xanthine inhibitory activity of aqueous extract of *ferula asafoetida* :**

In vitro xanthine oxidase inhibitory of the extract was assessed as per the method (32) . In brief the assay mixture consisted of 1ml of fraction, 2.9 ml of phosphate buffer and 0.1ml of xanthine oxidase enzyme solution, which was prepared immediately before use. After pre-incubation at 25° for 15 minutes, the reaction was initiated by the addition of 2ml of the substrate solution. The assay mixture was incubated at 25° for 30 minutes. The reaction was then stopped by the addition of 1ml of 1N hydrochloric acid and the absorbance was measured at 290 nm using a UV spectrophotometer. xoz activity was expressed as the % inhibition of xo in the above assay system calculated as % of inhibition as follows :

$$\text{Inhibitory activity (\%)} = (1 - \text{as}/\text{ac}) \times 100$$

where as - absorbance in presence of test substance, ac - absorbance of control.

Statistical analysis:

The data were subjected to statistical analysis using one -way analysis of variance(ANOVA) and Duncan's multiple range test to assess the significance of individual variations between the groups. Decan's test was considered at the level of $P < 0.05$.

Results

Phytochemical screening of aqueous extract of *Ferula asafoetida* : Amino acids are less in concentration of ferula asafoetida. Proteins are more in concentration of ferula asafoetida. Flavonoids are more in concentration of ferula asafoetida. Alkaloids are more in concentration of ferula asafoetida. Terpenoids are more in concentration of ferula asafoetida. Saponins are more in concentration of ferula asafoetida. Steroids are more in concentration of ferula asafoetida. Carbohydrates are less in concentration of ferula asafoetida .

S.No	Phytochemical	Presence
1	Amino acid	-
2	Proteins	+
3	Flavonoids	+
4	Alkaloids	+
5	Terpenoids	+
6	Saponins	+
78	Steroids	+
9	Carbohydrates	-

Table 1: Phytochemical screening of aqueous extract of *Ferula asafoetida*

Figure 1: *In vitro* antioxidant activity of *Ferula asafoetida*

Antioxidant potential of aqueous extract of *Ferula asafoetida*

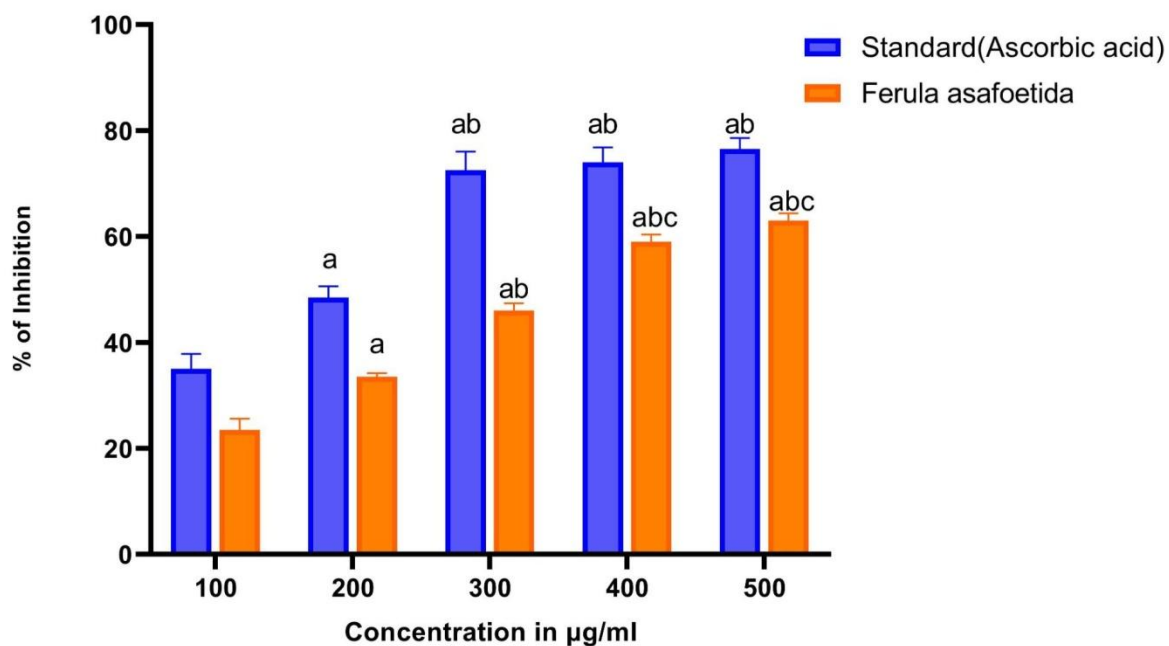


Figure 1 represents antioxidant potential of aqueous extracts of *Ferula asafoetida* with standard Ascorbic acid- DPPH Assay. X axis represents concentration in µg/ml and y axis represents the inhibitory potential of the extracts. Blue bar represents standard ascorbic acid and orange bar represents aqueous extracts of *Ferula asafoetida*. Each bar represents Mean \pm SEM of 3 independent observations. Significance at $p < 0.05$

Figure 2: In vitro xanthine oxidase inhibitory activity of *Ferula asafoetida* extract

Xanthine oxidase inhibitory potential of aqueous extract of *Ferula asafoetida*

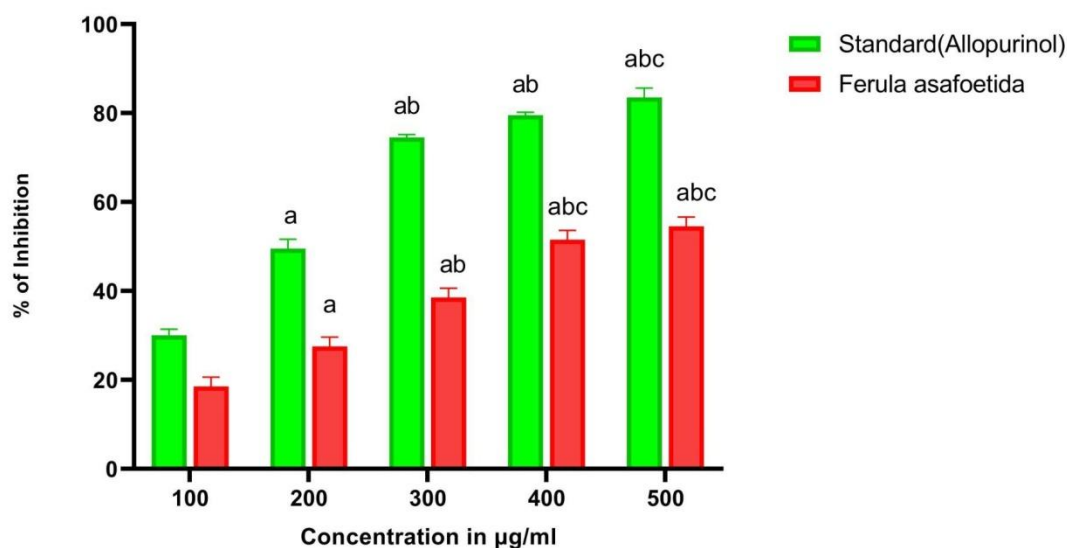


Figure 2: Represents xanthine oxidase inhibitory potential of aqueous extracts of *Ferula asafoetida* with standard Allopurinol. X axis represents concentration in µg/ml and the y axis represents the inhibitory potential of the extracts. Green bar represents standard drug Allopurinol and red bar represents aqueous extracts of *Ferula asafoetida*. Each bar represents Mean \pm SEM of 3 independent observations. Significance at $p < 0.05$

DISCUSSION

The phytochemical screening showed that the extract is rich in protein, flavonoids, alkaloids, terpenoids, saponins, steroids (Table 1). Free radicals and other reactive oxygen species by-products might be generated by living cells as a result of physiological and biochemical processes. These can cause oxidative damage to the biomolecules like lipids, proteins and DNA, which can lead to many chronic diseases, such as diabetes, cancer, aging, and other degenerative diseases (33). Medicinal plants contain biologically active compounds

known as phytochemicals which constitute a broad spectrum of chemical structures that have disease preventative properties. Thus, preliminary phytochemical screening of plants is an important aspect in determining the chemical constituents in plant materials (34). The presence of phytochemicals in the asafoetida extract might have contributed to the wide range of its therapeutic properties.

The extract also possesses significant antioxidant activity as evident from the DPPH radical scavenging activity (Figure 1), although the activity is less compared to standard vitamin-C. Natural antioxidants present in medicinal plants can help to prevent oxidative damage and reduce the risk of diseases (35). The continued search among plant secondary metabolites for natural antioxidants has gained importance in recent years because of the increasing awareness of herbal remedies as potential sources of phenolic oxidants (36). The antioxidant activity of our extract also accounts for its disease preventive activity.

The study also established the *in vitro* xanthine oxidase inhibitory potential of *Ferula asafoetida* extract (Figure 2). The activity of the extract is compared with the standard drug allopurinol. Allopurinol is an important clinically used inhibitor of xanthine oxidase that is used in the treatment of gout (37). However, the use of this drug is associated with various side effects such as hepatitis, nephropathy, and allergic reactions (38). Thus, the search for novel xanthine oxidase inhibitors with a greater therapeutic activity and lesser side effects are desired not only to treat gout but also for preventing other diseases associated with the xanthine oxidase activity (39). Since our extract is having inhibitory activity towards xanthine oxidase and is natural in origin, it can be used to treat gout if detailed studies were done on the plant. From these investigations, we can say that asafoetida possesses significant antimicrobial activity due to presence of various phytoconstituents and it could be a resource of new antibiotic compounds and antioxidant compounds.

CONCLUSION

The study can be concluded that aqueous extract of *Ferula asafoetida* possessed potent *in vitro* antioxidant and antigout activities. Further studies on the therapeutic role of *F.asafoetida* on *in vitro* cell lines and animal experiments need to be done in order to ascertain the potential of this extract towards the development of new drug.

REFERENCES

1. Mahendra P, Bisht S. *Ferula asafoetida*: Traditional uses and pharmacological activity. *Pharmacogn Rev.* 2012 Jul;6(12):141–6.
2. Amalraj A, Gopi S. Biological activities and medicinal properties of *Asafoetida*: A review. *Afr J Tradit Complement Altern Med.* 2017 Jul;7(3):347–59.
3. Saleem M, Alam A, Sultana S. *Asafoetida* inhibits early events of carcinogenesis: a chemopreventive study. *Life Sci.* 2001 Mar 9;68(16):1913–21.
4. Mala KN, Thomas J, Syam DS, Maliakel B, Krishnakumar IM. Safety and Efficacy of *Ferula asafoetida* in Functional Dyspepsia: A Randomized, Double-Blinded, Placebo-Controlled Study [Internet]. Vol. 2018, Evidence-Based Complementary and Alternative Medicine. 2018. p. 1–11. Available from: <http://dx.doi.org/10.1155/2018/4813601>
5. Iranshahy M, Iranshahi M. Traditional uses, phytochemistry and pharmacology of *asafoetida* (*Ferula assa-foetida* oleo-gum-resin)—A review [Internet]. Vol. 134, Journal of Ethnopharmacology. 2011. p. 1–10. Available from: <http://dx.doi.org/10.1016/j.jep.2010.11.067>
6. Sultana V, Ehteshamul-Haque S, Ara J, Athar M. Comparative efficacy of brown, green and red seaweeds in the control of root infecting fungi and okra [Internet]. Vol. 2, International Journal of Environmental Science & Technology. 2005. p. 129–32. Available from: <http://dx.doi.org/10.1007/bf03325866>
7. Al-Hazmi AS, TariqJaveed M. Effects of different inoculum densities of *Trichoderma harzianum* and *Trichoderma viride* against *Meloidogyne javanica* on tomato [Internet]. Vol. 23, Saudi Journal of Biological Sciences. 2016. p. 288–92. Available from: <http://dx.doi.org/10.1016/j.sjbs.2015.04.007>
8. Phytochemical Analysis [Internet]. Vol. 30. 2019. Available from: <http://dx.doi.org/10.1002/pca.v30.2>
9. Duong NT, Vinh PD, Thuong PT, Hoai NT, Thanh LN, Tran The Bach, et al. Xanthine oxidase inhibitors from *Archidendron clypearia* (Jack.) I.C. Nielsen: Results from systematic screening of Vietnamese medicinal plants [Internet]. Vol. 10, Asian Pacific Journal of Tropical Medicine. 2017. p. 549–56. Available from: <http://dx.doi.org/10.1016/j.apjtm.2017.06.002>
10. Wu F, Zhu J, Li G, Wang J, Veeraraghavan VP, Krishna Mohan S, et al. Biologically synthesized green gold nanoparticles from Siberian ginseng induce growth-inhibitory effect on melanoma cells (B16). *Artif Cells Nanomed Biotechnol.* 2019 Dec;47(1):3297–305.
11. Chen F, Tang Y, Sun Y, Veeraraghavan VP, Mohan SK, Cui C. 6-shogaol, a active constituents of ginger prevents UVB radiation mediated inflammation and oxidative stress through modulating Nrf2 signaling in human epidermal keratinocytes (HaCaT cells). *J Photochem Photobiol B.* 2019 Aug;197:111518.
12. Hudaib MM, Tawaha KA, Mohammad MK, Assaf AM, Issa AY, Alali FQ, et al. Xanthine oxidase inhibitory activity of the methanolic extracts of selected Jordanian medicinal plants. *Pharmacogn Mag.* 2011 Oct;7(28):320–4.

13. Li Z, Veeraraghavan VP, Mohan SK, Bolla SR, Lakshmanan H, Kumaran S, et al. Apoptotic induction and anti-metastatic activity of eugenol encapsulated chitosan nanopolymer on rat glioma C6 cells via alleviating the MMP signaling pathway [Internet]. Vol. 203, *Journal of Photochemistry and Photobiology B: Biology*. 2020. p. 111773. Available from: <http://dx.doi.org/10.1016/j.jphotobiol.2019.111773>
14. Babu S, Jayaraman S. An update on β -sitosterol: A potential herbal nutraceutical for diabetic management. *Biomed Pharmacother*. 2020 Nov;131:110702.
15. Malaikolundhan H, Mookkan G, Krishnamoorthi G, Matheswaran N, Alsawalha M, Veeraraghavan VP, et al. Anticarcinogenic effect of gold nanoparticles synthesized from *Albizia lebbek* on HCT-116 colon cancer cell lines. *Artif Cells Nanomed Biotechnol*. 2020 Dec;48(1):1206–13.
16. Han X, Jiang X, Guo L, Wang Y, Veeraraghavan VP, Krishna Mohan S, et al. Anticarcinogenic potential of gold nanoparticles synthesized from *Trichosanthes kirilowii* in colon cancer cells through the induction of apoptotic pathway. *Artif Cells Nanomed Biotechnol*. 2019 Dec;47(1):3577–84.
17. Gothai S, Muniandy K, Gnanaraj C, Ibrahim IAA, Shahzad N, Al-Ghamdi SS, et al. Pharmacological insights into antioxidants against colorectal cancer: A detailed review of the possible mechanisms. *Biomed Pharmacother*. 2018 Nov;107:1514–22.
18. Veeraraghavan VP, Hussain S, Balakrishna JP, Dhawale L, Kullappan M, Ambrose JM, et al. A Comprehensive and Critical Review on Ethnopharmacological Importance of Desert Truffles: *Terfezia claveryi*, *Terfezia boudieri*, and *Tirmania nivea* [Internet]. *Food Reviews International*. 2021. p. 1–20. Available from: <http://dx.doi.org/10.1080/87559129.2021.1889581>
19. Sathya S, Ragul V, Veeraraghavan VP, Singh L, Niyas Ahamed MI. An in vitro study on hexavalent chromium [Cr(VI)] remediation using iron oxide nanoparticles based beads. *Environmental Nanotechnology, Monitoring & Management*. 2020 Dec 1;14:100333.
20. Yang Z, Pu M, Dong X, Ji F, Priya Veeraraghavan V, Yang H. Piperine loaded zinc oxide nanocomposite inhibits the PI3K/AKT/mTOR signaling pathway via attenuating the development of gastric carcinoma: In vitro and in vivo studies. *Arabian Journal of Chemistry*. 2020 May 1;13(5):5501–16.
21. Rajendran P, Alzahrani AM, Rengarajan T, Veeraraghavan VP, Krishna Mohan S. Consumption of reused vegetable oil intensifies BRCA1 mutations. *Crit Rev Food Sci Nutr*. 2020 Oct 27;1–8.
22. Barma MD, Muthupandiyan I, Samuel SR, Amaechi BT. Inhibition of *Streptococcus mutans*, antioxidant property and cytotoxicity of novel nano-zinc oxide varnish. *Arch Oral Biol*. 2021 Jun;126:105132.
23. Samuel SR. Can 5-year-olds sensibly self-report the impact of developmental enamel defects on their quality of life? *Int J Paediatr Dent*. 2021 Mar;31(2):285–6.
24. Samuel SR, Kuduruthullah S, Khair AMB, Shayeb MA, Elkaseh A, Varma SR. Dental

pain, parental SARS-CoV-2 fear and distress on quality of life of 2 to 6 year-old children during COVID-19. *Int J Paediatr Dent*. 2021 May;31(3):436–41.

25. Tang Y, Rajendran P, Veeraraghavan VP, Hussain S, Balakrishna JP, Chinnathambi A, et al. Osteogenic differentiation and mineralization potential of zinc oxide nanoparticles from *Scutellaria baicalensis* on human osteoblast-like MG-63 cells [Internet]. Vol. 119, *Materials Science and Engineering: C*. 2021. p. 111656. Available from: <http://dx.doi.org/10.1016/j.msec.2020.111656>
26. Yin Z, Yang Y, Guo T, Veeraraghavan VP, Wang X. Potential chemotherapeutic effect of betalain against human non-small cell lung cancer through PI3K/Akt/mTOR signaling pathway. *Environ Toxicol*. 2021 Jun;36(6):1011–20.
27. Veeraraghavan VP, Periadurai ND, Karunakaran T, Hussain S, Surapaneni KM, Jiao X. Green synthesis of silver nanoparticles from aqueous extract of *Scutellaria barbata* and coating on the cotton fabric for antimicrobial applications and wound healing activity in fibroblast cells (L929). *Saudi J Biol Sci*. 2021 Jul;28(7):3633–40.
28. Mickymaray S, Alfaiz FA, Paramasivam A, Veeraraghavan VP, Periadurai ND, Surapaneni KM, et al. Rhaponticin suppresses osteosarcoma through the inhibition of PI3K-Akt-mTOR pathway. *Saudi J Biol Sci*. 2021 Jul;28(7):3641–9.
29. Teja KV, Ramesh S. Is a filled lateral canal – A sign of superiority? [Internet]. Vol. 15, *Journal of Dental Sciences*. 2020. p. 562–3. Available from: <http://dx.doi.org/10.1016/j.jds.2020.02.009>
30. Kadanakuppe S, Hiremath S. Social and Behavioural Factors Associated with Dental Caries Experience among Adolescent School Children in Bengaluru City, India [Internet]. Vol. 14, *British Journal of Medicine and Medical Research*. 2016. p. 1–10. Available from: <http://dx.doi.org/10.9734/bjmmr/2016/24021>
31. Kahraman C, Topcu G, Bedir E, Irem Tatli I, Ekizoglu M, Akdemir ZS. Phytochemical screening and evaluation of the antimicrobial and antioxidant activities of *Ferula caspica* M. Bieb. extracts [Internet]. Vol. 27, *Saudi Pharmaceutical Journal*. 2019. p. 525–31. Available from: <http://dx.doi.org/10.1016/j.jsps.2019.01.016>
32. Deeb HKE, El Deeb HK, Al Khadrawy FM, Abd El-Hameid AK. Inhibitory effect of *Ferula asafoetida* L. (Umbelliferae) on *Blastocystis* sp. subtype 3 growth in vitro [Internet]. Vol. 111, *Parasitology Research*. 2012. p. 1213–21. Available from: <http://dx.doi.org/10.1007/s00436-012-2955-1>
33. Y ACAR, Arunakumarn C And Ranjith. Preliminary Phytochemical Screening of Medicinal Plants used in Traditional Medicine [Internet]. Vol. 6, *International Journal for Modern Trends in Science and Technology*. 2020. p. 109–12. Available from: <http://dx.doi.org/10.46501/ijmtst061019>
34. Madike LN, Takaidza S, Pillay M. Preliminary Phytochemical Screening of Crude Extracts from the Leaves, Stems, and Roots of *Tulbaghia violacea* [Internet]. Vol. 9, *International Journal of Pharmacognosy and Phytochemical Research*. 2017. Available from: <http://dx.doi.org/10.25258/phyto.v9i10.10453>

35. Sahoo S, Ghosh G, Das D, Nayak S. Phytochemical investigation and In vitro antioxidant activity of an indigenous medicinal plant *Alpinia nigra* B.L. Burt [Internet]. Vol. 3, Asian Pacific Journal of Tropical Biomedicine. 2013. p. 871–6. Available from: [http://dx.doi.org/10.1016/s2221-1691\(13\)60171-9](http://dx.doi.org/10.1016/s2221-1691(13)60171-9)
36. Ark R, Reddy ARK, Grace JR. In vitro Evaluation of Antioxidant Activity of *Brugeiera Gymnorhiza* and *Aegialitis Rotundifolia* [Internet]. Vol. 05, Medicinal & Aromatic Plants. 2016. Available from: <http://dx.doi.org/10.4172/2167-0412.1000231>
37. Hoey BM, Butler J, Halliwell B. On the specificity of allopurinol and oxypurinol as inhibitors of xanthine oxidase. A pulse radiolysis determination of rate constants for reaction of allopurinol and oxypurinol with hydroxyl radicals. *Free Radic Res Commun.* 1988;4(4):259–63.
38. Fagugli RM, Gentile G, Ferrara G, Brugnano R. Acute renal and hepatic failure associated with allopurinol treatment. *Clin Nephrol.* 2008 Dec;70(6):523–6.
39. El-Desoukey RMA. Comparative Antimicrobial Effect of *Boswellia*, Lichen, Coffea peel, *Asafoetida*, *Saussurea Costus*, *Rhatany*, Anise and Cumin Extracts on Some Animal Pathogens [Internet]. Vol. 5, Open Access Journal of Veterinary Science & Research. 2020. p. 1–9. Available from: <http://dx.doi.org/10.23880/oajvsr-16000193>