

IN VITRO ANTI-INFLAMMATORY PROPERTIES OF VARIOUS EXTRACTS OF ANDROGRAPHIS ECHIOIDES

ABSTRACT:

BACKGROUND: *Andrographis echioides* (Acanthaceae) which is commonly known as false water willow is a herb commonly found throughout India. Some of them are medicinally important. The plants from genus *Andrographis* are used in goiter, liver diseases, fever, fertility problems, bacterial, malarial, helminthic, helminthic fungal, diarrhea and larvicidal disorders.

MATERIALS AND METHODS: The in-vitro anti-inflammatory properties of aqueous, ethanolic, chloroform extracts were evaluated by Protein denaturation assay. Aspirin was a positive control. The experiment was done in triplicates and percent inhibition of protein denaturation was calculated. The data were analysed statistically using one way analysis of variance (ONE-WAY ANOVA). Duncan's multiple tests was used to analyse the statistical significance between groups. The level of significance was considered at the levels of $p < 0.005$.

RESULTS: There was a dose-dependent increase in protease inhibition of protein denaturation in three extracts from the concentration ranging from 100 to 500 ug/ml. All the three extracts of *Andrographis echioides* showed better in-vitro anti-inflammatory effect compounds.

CONCLUSION: The study concluded an innovative finding that aqueous, ethanolic, chloroform of *Andrographis echioides* possessed potent in vitro anti-inflammatory effect attributed to its flavonoid, fenylyl glycosides compounds

Keywords: Anti-inflammatory, *Andrographis echioides*, Aspirin, Protein denaturation, innovative.

Running title: Evaluation of anti-inflammatory activity of *Andrographis Echioides*.

INTRODUCTION:

Plants are important sources of medicines and presently about 25% of pharmaceutical prescriptions within the US contain a minimum of one plant-derived ingredient. In the last five decades, roughly 121 pharmaceutical products were formulated to support the traditional knowledge obtained from various sources(1). Phytomedicine or phytotherapy or botanical medicine was collectively called herbal medicine. It refers to herbal materials or medicinal preparations that contain plant parts as active ingredients(2). The lack of scientific evidence

in herbal medicines, when compared to that of modern medicine, gradually made herbal medicine to become unpopular among people(3). Since ancient times people were dependent upon plants for their food, shelter and medicine. They used the plants for their daily life although the chemical constituents of those plants were unknown until that time(4). Before the age of Jesus Christ herbal plants were listed and described in Ayurveda(5). After 500 A.D. Many plants were listed as herbals in the renowned book *Materia Medica*. Since ancient days people were habituated to use the plants for their health(6). Knowingly or unknowingly(7). Basically, the agricultural people mainly used these plants for his or her traditional life. The tribal people have much more knowledge than others(8). Nowadays tribal medicines have developed to great extent (9)(10). India is the richest place for the source of medicinal plants and therefore the people of India use these plants in three major systems: Ayurveda, Unani and siddha(11).

Andrographis echinoides (Acanthaceae) which is usually referred to as false water willow is a very rare herb commonly found throughout India(12). *Andrographis echinoides* L. is an annual herb present throughout South Indian. However, information on the chemical composition and bioactivity of this species is extremely rare (13). The genus *Andrographis* is a native of India containing 28 species of small annual shrubs essentially distributed in tropical Asia. Some of them are medicinally important(14). The plant from genus *Andrographis* is usually utilized in goiter, liver diseases(15) , fertility problems, bacterial (16), malarial and fungal disorders(17) . Leaf juice boiled with coconut oil has controlled the falling and graying of hair (18) Rheumatoid arthritis is a chronic, systemic inflammatory disorder that may be inactive to many tissues and organs and the synovial joints (19). Statistically analyzed exhibit 1% of the population in the world affected by rheumatoid arthritis and females are affected three times more than the male. Allopathic treatment of Rheumatoid arthritis is prone to many complications. So, the present study planned to find a novel herbal antibiotic for the treatment of inflammatory conditions.

MATERIALS AND METHODS:

Chemicals

All chemicals and reagents used in this study were purchased from Sigma Chemical Company St. Louis, MO, USA; Invitrogen, USA; Eurofins Genomics India Pvt Ltd, Bangalore, India; New England Biolabs (NEB), USA; Promega, USA.

Assessment of in vitro anti-inflammatory activity

Inhibition of albumin denaturation:

The anti-inflammatory activity of the plant extract was studied by the inhibition of albumin denaturation technique which was studied according to the methods of Mizushima and Kobayashi, 1968 and Sakat et al (2010) followed with minor modifications. The reaction mixture consisted of test extracts and 1% aqueous solution of bovine serum albumin, pH of the reaction mixture was adjusted using a small amount of 1N HCl to dissolve the mixture. The plant extract with increase in concentration (100 to 500 µg/ml) were incubated at 37 °C for 20 min and later heated to 51 °C for 20 min, after the samples were cooled, the turbidity was measured at 660nm. (UV Visible Spectrophotometer Model 371, Elico India Ltd) The experiment was performed in triplicate. In this study, Aspirin was used as a standard anti-inflammatory drug.

Calculation:

$$\% \text{ Inhibition} = 100 - ((A1 - A2) / A0) * 100$$

Statistical analysis:

The data were analysed statistically using one way analysis of variance (ONE-WAY ANOVA). Duncan Multiple range test was used to analyze the statistical significance between groups. The levels of significance were considered at the levels of $p < 0.05$.

RESULTS

The present study revealed that there was a dose dependent increase in inhibition of protein denaturation in aqueous, ethanolic and chloroform extract at a concentration ranging from 100 to 500 µg/ml. ($p < 0.05$). The results showed that 400 µg/ml and 500 µg/ml showed maximum inhibition. ($p < 0.05$) (Fig 1-3)

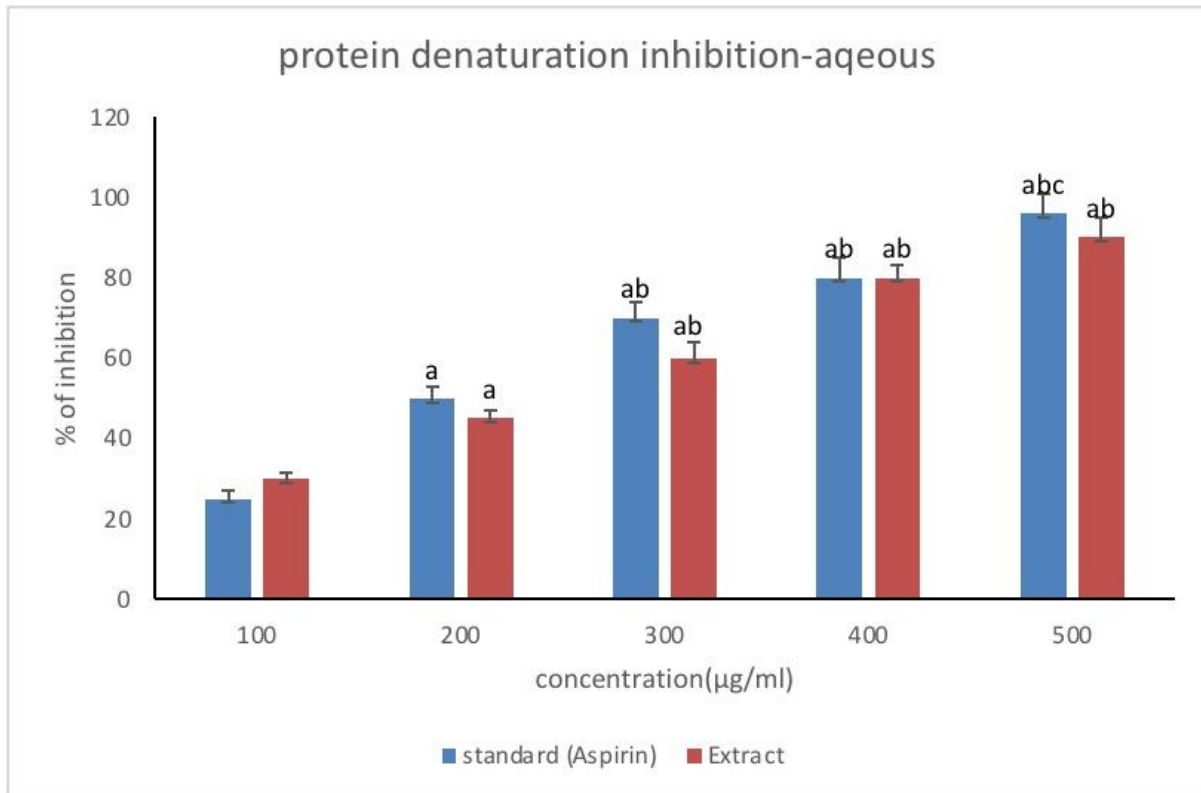


Figure 1: The figure shows the in vitro anti-inflammatory properties of aqueous extract of *Andrographis echinoides*. Each bar represents the mean \pm SD of 6 observations. Significance at the levels of $P < 0.05$. a-compared with 100 μg ; b-compared with 200 μg ; c-compared with 300 μg .

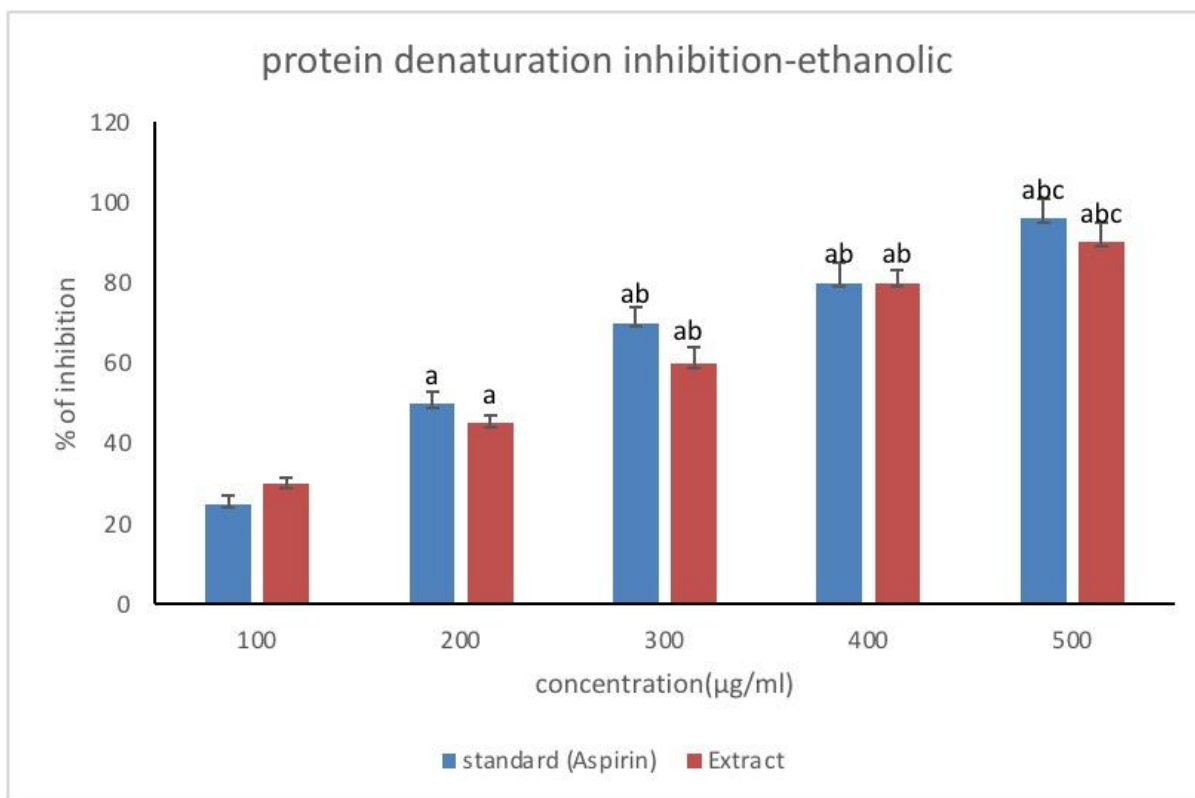


Figure 2: It Shows the in vitro anti-inflammatory properties of ethanolic extract of *Andrographis echioides*. Each bar represents the mean \pm SD of 6 observations. Significance at the levels of $P < 0.05$. a-compared with 100 μg ; b-compared with 200 μg ; c-compared with 300 μg .

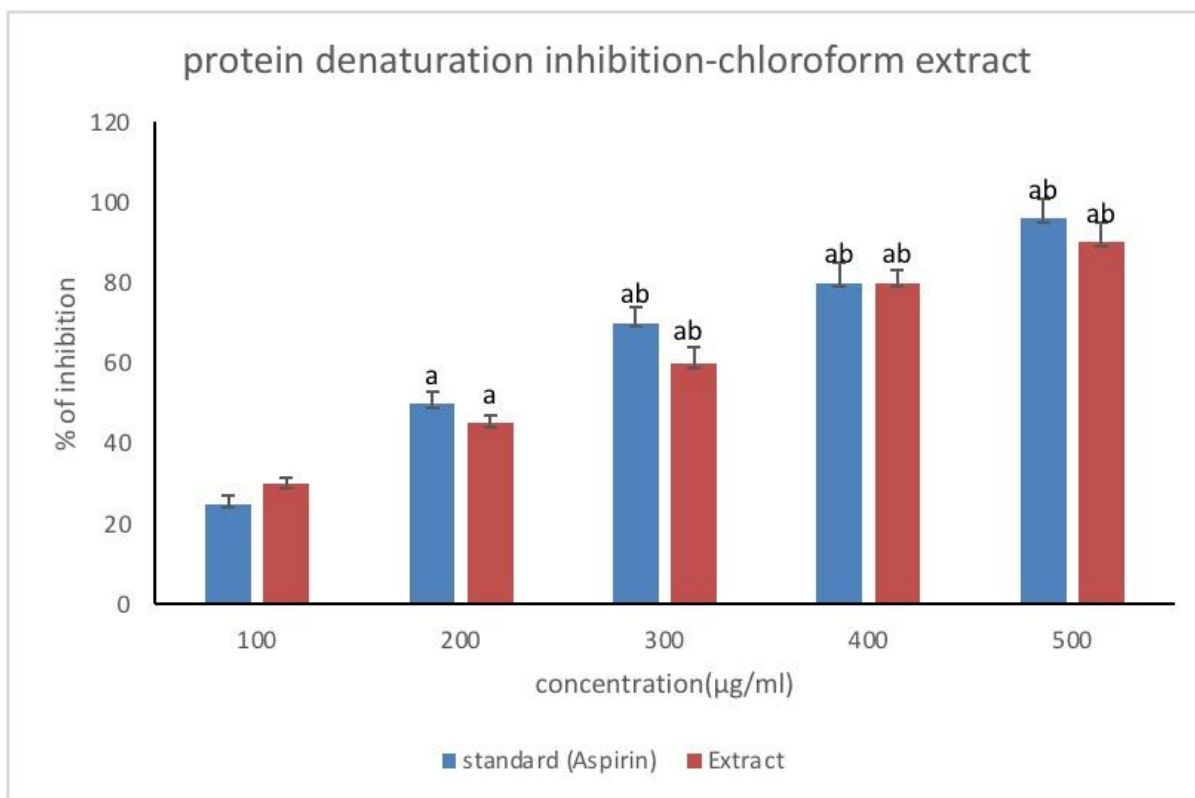


Figure 3: The figure shows in vitro anti-inflammatory properties of chloroform extract of *Andrographis echioides*. Each bar represents the mean \pm SD of 6 observations. Significance at the levels of $P < 0.05$. a-compared with 100 μg ; b-compared with 200 μg .

DISCUSSION:

The phytochemical analysis of the plant indicated various classes of molecules in different extracts of the whole plant extracts. The methanolic extract showed the significant presence of a diverse class of molecules including terpenoids, flavonoids and tannins, phenols and glycosides. On the other hand, the chloroform extract possessed a very good amount of flavonoids and steroids(20). Flavonoids have antioxidant activities and they have the property of preventing oxidative cell damage and carcinogenesis(21). Steroids, abundant in many plants, have been shown to have hypercholesterolemia effects, diuretics and also exhibit anti-leukemic, antipyretic, and derivatives of steroids are active as anticancer and antiviral agents(22). Steroids have been reported to stimulate menstrual discharge and diminish the secretion of milk(23). Phytochemicals have long been recognized to possess many properties including antioxidant, anti-allergic, anti-inflammatory, anti-viral, anti-proliferative and anti-carcinogenic (24) (21). The presence of free radicals and oxidative stress are implicated in

many diseases like stroke, asthma, cancer, atherosclerosis, diabetes and arthritis. There had been an increasing interest in plant-derived antioxidants, because it could protect our body from free radical damage, diabetes and age-related disorders (25) (26).

Phytochemicals derived from plant products function as a prototype to develop less toxic and simpler medicines in controlling the expansion of microorganisms. These active compounds either might act alone or in combination to inhibit bacterial growth (27). Many previous studies indicated that phenolic compounds stop many diseases from pathogenic invasion (20). Nowadays efforts are focused on plants because of their usage of historical times and the world's population rely on plants for the treatment of infections and noninfectious diseases(28). However, isolation of specific phytoconstituents and screening for biological activities will definitely give better results(29). From this present study, it might be concluded that *A. echioides* contains various bioactive compounds hence, it can be considered as a plant of phytopharmaceutical importance. Therefore, these plants seem to serve as prospective material for advance development of plant-based anti-inflammatory agents.

Phytochemical investigation of the entire plant of *Andrographis echioides* leads to the presence of two new oxygenated flavonoids, **feryl** **phenyl** glycosides alongside 30 non-structures(30)(31). These bioactive compounds offer the plant a good anti-inflammatory activity. The extract of the plant *Andrographis echioides* was rarely available and costly. Hence it was hard to conduct and complete the research.

CONCLUSION:

In conclusion, different extracts of *A. echioides* can be very effective anti-inflammatory and it could protect biological systems against the oxidative stress including aging, cancer, diabetes and cardiovascular disorders. Further investigations that can identify and characterise the specific active components of the plant extract can offer better explanations for its anti-inflammatory potential.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the

advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

NOTE:

The study highlights the efficacy of "herbal medicine" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

REFERENCE:

1. Barabadi H, Mojab F, Vahidi H, Marashi B, Talank N, Hosseini O, et al. Green synthesis, characterization, antibacterial and biofilm inhibitory activity of silver nanoparticles compared to commercial silver nanoparticles [Internet]. Vol. 129, Inorganic Chemistry Communications. 2021. p. 108647. Available from: <http://dx.doi.org/10.1016/j.inoche.2021.108647>
2. Bharath B, Perinbam K, Devanesan S, AlSalhi MS, Saravanan M. Evaluation of the anticancer potential of Hexadecanoic acid from brown algae *Turbinaria ornata* on HT-29 colon cancer cells [Internet]. Vol. 1235, Journal of Molecular Structure. 2021. p. 130229. Available from: <http://dx.doi.org/10.1016/j.molstruc.2021.130229>
3. Kelmanson JE, Jäger AK, van Staden J. Zulu medicinal plants with antibacterial activity [Internet]. Vol. 69, Journal of Ethnopharmacology. 2000. p. 241–6. Available from: [http://dx.doi.org/10.1016/s0378-8741\(99\)00147-6](http://dx.doi.org/10.1016/s0378-8741(99)00147-6)
4. Saraswathi I, Saikarthik J, Senthil Kumar K, Srinivasan KM, Ardhanaari M, Gunapriya R. Impact of COVID-19 outbreak on the mental health status of undergraduate medical students in a COVID-19 treating medical college: a prospective longitudinal study [Internet]. Vol. 8, PeerJ. 2020. p. e10164. Available from: <http://dx.doi.org/10.7717/peerj.10164>
5. Clarizia G, Bernardo P. Diverse Applications of Organic-Inorganic Nanocomposites: Emerging Research and Opportunities: Emerging Research and Opportunities. IGI Global; 2019. 237 p.
6. Tahmasebi S, Qasim MT, Krivenkova MV, Zekiy AO, Thangavelu L, Aravindhan S, et al. The effects of oxygen-ozone therapy on regulatory T-cell responses in multiple sclerosis patients. *Cell Biol Int*. 2021 Jul;45(7):1498–509.
7. Egbuna C, Mishra AP, Goyal MR. Preparation of Phytopharmaceuticals for the Management of Disorders: The Development of Nutraceuticals and Traditional Medicine. Academic Press; 2020. 570 p.
8. Sridharan G, Ramani P, Patankar S, Vijayaraghavan R. Evaluation of salivary metabolomics in oral leukoplakia and oral squamous cell carcinoma. *J Oral Pathol Med*. 2019 Apr;48(4):299–306.

9. Cragg G, Newman D. Natural Product Drug Discovery in the Next Millennium [Internet]. Vol. 39, *Pharmaceutical Biology*. 2001. p. 8–17. Available from: <http://dx.doi.org/10.1076/phbi.39.7.8.5868>
10. Wahab PUA, Madhulaxmi M, Senthilnathan P, Muthusekhar MR, Vohra Y, Abhinav RP. Scalpel Versus Diathermy in Wound Healing After Mucosal Incisions: A Split-Mouth Study. *J Oral Maxillofac Surg*. 2018 Jun;76(6):1160–4.
11. Wink M. Introduction: Biochemistry, Role and Biotechnology of Secondary Metabolites (From APR Volume 3) [Internet]. *Annual Plant Reviews online*. 2018. p. 1–17. Available from: <http://dx.doi.org/10.1002/9781119312994.apr0018>
12. Ezhilarasan D. Critical role of estrogen in the progression of chronic liver diseases. *Hepatobiliary Pancreat Dis Int*. 2020 Oct;19(5):429–34.
13. Shen D-Y, Juang S-H, Kuo P-C, Huang G-J, Chan Y-Y, Damu A, et al. Chemical Constituents from *Andrographis echioides* and Their Anti-Inflammatory Activity [Internet]. Vol. 14, *International Journal of Molecular Sciences*. 2012. p. 496–514. Available from: <http://dx.doi.org/10.3390/ijms14010496>
14. Santhakumar P, Roy A, Mohanraj KG, Jayaraman S, Durairaj R. Ethanolic Extract of *Capparis decidua* Fruit Ameliorates Methotrexate-Induced Hepatotoxicity by Activating Nrf2/HO-1 and PPAR γ Mediated Pathways [Internet]. Vol. 55, *Indian Journal of Pharmaceutical Education and Research*. 2021. p. s265–74. Available from: <http://dx.doi.org/10.5530/ijper.55.1s.59>
15. Gowhari Shabgah A, Ezzatifar F, Aravindhana S, Olegovna Zekiy A, Ahmadi M, Gheibihayat SM, et al. Shedding more light on the role of Midkine in hepatocellular carcinoma: New perspectives on diagnosis and therapy. *IUBMB Life*. 2021 Apr;73(4):659–69.
16. Qadrie ZL, Jacob B, Anandan R, Rajkapoor B, Rahamathulla M. Anti-bacterial activity of ethanolic extract of *Indoneesiella echioides* (L) nees. evaluated by the filter paper disc method. *Pak J Pharm Sci*. 2009 Apr;22(2):123–5.
17. Leong LP, Shui G. An investigation of antioxidant capacity of fruits in Singapore markets [Internet]. Vol. 76, *Food Chemistry*. 2002. p. 69–75. Available from: [http://dx.doi.org/10.1016/s0308-8146\(01\)00251-5](http://dx.doi.org/10.1016/s0308-8146(01)00251-5)
18. Halliwell B. The wanderings of a free radical. *Free Radic Biol Med*. 2009 Mar 1;46(5):531–42.
19. Majithia V, Geraci SA. Rheumatoid Arthritis: Diagnosis and Management [Internet]. Vol. 120, *The American Journal of Medicine*. 2007. p. 936–9. Available from: <http://dx.doi.org/10.1016/j.amjmed.2007.04.005>
20. J PC, Marimuthu T, C K, Devadoss P, Kumar SM. Prevalence and measurement of anterior loop of the mandibular canal using CBCT: A cross sectional study. *Clin Implant Dent Relat Res*. 2018 Aug;20(4):531–4.
21. Hemamalini K, Bhargava A. EVALUATION OF PHYTOCHEMICAL AND PHARMACOLOGICAL ACTIVITY OF METHANOLIC EXTRACT OF SOLANUM

PUBESCENS [Internet]. Vol. 4, INTERNATIONAL RESEARCH JOURNAL OF PHARMACY. 2013. p. 138–42. Available from: <http://dx.doi.org/10.7897/2230-8407.04825>

22. Kamath SM, Manjunath Kamath S, Jaison D, Rao SK, Sridhar K, Kasthuri N, et al. In vitro augmentation of chondrogenesis by Epigallocatechin gallate in primary Human chondrocytes - Sustained release model for cartilage regeneration [Internet]. Vol. 60, Journal of Drug Delivery Science and Technology. 2020. p. 101992. Available from: <http://dx.doi.org/10.1016/j.jddst.2020.101992>
23. Mudigonda SK, Murugan S, Velavan K, Thulasiraman S, Krishna Kumar Raja VB. Non-suturing microvascular anastomosis in maxillofacial reconstruction- a comparative study. *J Craniomaxillofac Surg*. 2020 Jun;48(6):599–606.
24. Nambi G, Kamal W, Es S, Joshi S, Trivedi P. Spinal manipulation plus laser therapy versus laser therapy alone in the treatment of chronic non-specific low back pain: a randomized controlled study. *Eur J Phys Rehabil Med*. 2018 Dec;54(6):880–9.
25. Solai Prakash AK, Devaraj E. Cytotoxic potentials of *S. cumini* methanolic seed kernel extract in human hepatoma HepG2 cells. *Environ Toxicol*. 2019 Dec;34(12):1313–9.
26. Sun T, Xie W, Xu P. Superoxide anion scavenging activity of graft chitosan derivatives [Internet]. Vol. 58, Carbohydrate Polymers. 2004. p. 379–82. Available from: <http://dx.doi.org/10.1016/j.carbpol.2004.06.042>
27. Ruban P, Gajalakshmi K. In vitro antibacterial activity of Hibiscus rosa-sinensis flower extract against human pathogens. *Asian Pac J Trop Biomed*. 2012 May;2(5):399–403.
28. Rajakumari R, Volova T, Oluwafemi OS, Rajesh Kumar S, Thomas S, Kalarikkal N. Grape seed extract-soluplus dispersion and its antioxidant activity. *Drug Dev Ind Pharm*. 2020 Aug;46(8):1219–29.
29. Vivekanandhan K, Shanmugam P, Barabadi H, Arumugam V, Daniel Raj Daniel Paul Raj D, Sivasubramanian M, et al. Emerging Therapeutic Approaches to Combat COVID-19: Present Status and Future Perspectives. *Front Mol Biosci*. 2021 Mar 8;8:604447.
30. R H, Hannah R, Ramani P, Ramanathan A, Jancy MR, Gheena S, et al. CYP2 C9 polymorphism among patients with oral squamous cell carcinoma and its role in altering the metabolism of benzo[a]pyrene [Internet]. Vol. 130, Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology. 2020. p. 306–12. Available from: <http://dx.doi.org/10.1016/j.oooo.2020.06.021>
31. Wadhwa R, Paudel KR, Chin LH, Hon CM, Madheswaran T, Gupta G, et al. Anti-inflammatory and anticancer activities of Naringenin-loaded liquid crystalline nanoparticles in vitro. *J Food Biochem*. 2021 Jan;45(1):e13572.