

ANTIDIABETIC, ~~antioxidant~~ AND ANTI-INFLAMMATORY POTENTIALS OF *SIDA ACUTA* LEAF ETHANOLIC EXTRACT

Running title: Antidiabetic ~~antioxidant~~ and anti-inflammatory potentials of *sida acuta* leaf ethanolic extract

Type of study: Original study

ABSTRACT:

Introduction: *Sida acuta* is known as common wireweed, and has a pantropical distribution. Antioxidants fight against free radicals and prevent a variety of diseases. Anti-inflammatory reaction is the ability of a substance to reduce swelling or inflammation. Antidiabetic substances are defined as agents which help in maintaining the level of glucose in the blood.

Comment [a1]: Add -

Aim: Present study was aimed at assessing the *in-vitro* antidiabetic and anti-inflammatory potentials of *Sida acuta* leaf ethanolic extract.

Formatted: Font: Italic

Methods: *Sida acuta* was purchased and its ethanolic extract was prepared. Protein denaturation inhibition, DPPH radical scavenging, alpha amylase and alpha glucosidase inhibitory assays were carried out in order to check *in-vitro* anti-inflammatory, antioxidant and antidiabetic activity spectrophotometrically. The data were analysed using one-way analysis of variance ~~(ONE WAY ANOVA)~~ to see the statistical significant among the different concentrations of extract. ~~P<0.05 levels were considered as statistical significance.~~

Formatted: Strikethrough

Formatted: Strikethrough

Results: The extract of *Sida acuta* was compared with aspirin and an anti-inflammatory reaction was analysed. Statistically significant at the levels of $p < 0.05$. The extract proved to contain anti-inflammatory and antioxidant reactions. Alpha amylase and alpha glucosidase activity was analysed by comparing the extract with metformin.

Conclusion: ~~*Sida acuta* can be used for the management and treatment of various diseases and disorders. It can be used for the treatment of diabetes and cancer.~~ [Rewrite the conclusion.](#)

Formatted: Strikethrough

Formatted: Not Strikethrough

Formatted: Strikethrough

Keywords: *Sida acuta*, anti-inflammatory, antidiabetic, cancer, ~~Innovative technology,~~ ~~Novel method~~

INTRODUCTION:

Sida acuta, is known as the common wireweed, is a species of flowering plant in the mallow family, Malvaceae. It originated in Central America, but has a pantropical distribution. It is considered a weed in some areas. *Sida acuta* is viewed as an astringent, tonic which is used in treating urinary diseases and blood disorders, bile, liver and as treatment for nervous diseases (1). The economic importance of the plant is it is the source of natural fibres, it also used in food, beverages, timber, traditional medicine and in horticulture. The plant is also used in stomachic, diaphoretic and antipyretic. The plant causes illness like fever, headache and infections. Phytochemical analysis of the plant revealed the presence of saponins, tannins, alkaloids and cardiac glycosides (2). *Sida Acuta* shortens the duration of hexobarbitone-induced narcosis. Since *sida Acura* has a narrow spectrum of activity it is useful in treating infections of Gram positive bacteria.

Antioxidants are substances that protect the cell from the damage caused by free radicals which play a major role in cancer, heart diseases, stroke and diseases of aging. Vitamins C and E, selenium, and carotenoids, such as beta-carotene, lycopene, lutein, and zeaxanthin are some of the examples of free radicals. Fruits and vegetables are very rich in free radicals (3). Antioxidants are divided into two classes, primary or chain-breaking antioxidants and secondary or preventative antioxidants. Natural antioxidants like tocopherols and polyphenols are found in spices, herbs, fruits, vegetables, cereals, grains, seeds, teas and oils. Synthetic antioxidants like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and *tert*-butylhydroquinone (TBHQ), have been used as potent compounds in food preservation for decades due to their low cost and bland flavour (4). The effectiveness of antioxidants is characterised by its structural features, concentration, temperature, type of oxidation substrate and physical state of the system and presence of pro-oxidants and synergists. The chemical structure of an antioxidant is used for determining its intrinsic reactivity towards free radicals and ROS (5).

Anti-inflammatory is the ability of a substance to reduce swelling or inflammation. Anti-inflammatory agents block substances in the body that are responsible for inflammation. Green leafy vegetables like spinach, kale, nuts like almonds and walnuts and certain fruits like strawberries, blueberries, cherries are rich in anti-inflammation (6). Plants like Mimosaceae, Caesalpinaceae, Poaceae, Euphorbiaceae are rich in anti-inflammatory

Formatted: Font: Not Bold

properties. Anti-inflammatory agents are primarily used for prevention and management of cancer (7)(8). Diabetes mellitus is a concerned health issue with a progressing rate of incidence and mortality. It is marked by increased plasma glucose concentration from insufficient insulin and insulin resistance. Anti-diabetic agent is defined as the substance that helps in maintaining the level of glucose in the blood. Antidiabetic agents include insulin and oral hypoglycemic agents. Plants like Babul, garlic, onion, ash gourd and beetroot show hypoglycemic effects thereby they are rich in anti-diabetic properties. Our team has extensive knowledge and research experience that has translate into high quality publications (9),(10),(11),(12),(13),(14),(15), (16),(17), (18), (19), (20),(21),(22),(23),(24),(25),(26),(27),(28). The aim of the study is to estimate the antidiabetic and antiinflammatory potentials of *Sida acuta* leaf ethanolic extract.

Formatted: Font: Italic

Comment [a2]: Only latest reference are added

Formatted: Font: Italic

Formatted: Font: Italic

MATERIALS AND METHODS:

Preparation of plant extract:

Sida acuta was purchased from an ~~organic~~ supermarket, Chennai. It was weighed for and ~~was crushed to a fine powder with the help of mortar and pestle and 50% of the ethanolic extract was prepared by solvent extraction method.~~ Rewrite

Formatted: Strikethrough

Formatted: Underline

In-Vitro anti-inflammatory and antidiabetic activity Write the methodology of antidiabetic

Formatted: Font: Italic

Formatted: Underline

Protease inhibition assay

Inhibition of trypsin was evaluated by the method of ~~Oyedepo and Femure was (1965) and Sakat et al. (2010).~~ 100 µL of bovine serum albumin was added to 100 µl of plant extracts (0.1 to 0.5mg/ml) with increase in concentrations (100-500 µg/ml). This was incubated at room temperature for 5 minutes. Reaction was inhibited by the addition of 250 µl of trypsin followed by centrifugation. The supernatant was collected, and absorbance was observed at 210 nm. Acetyl salicylic acid was used as a positive control. The experiment was carried out in triplicates and percent inhibition of protease inhibition was calculated. In this study, Aspirin was used as a standard anti-inflammatory drug.

Formatted: Underline

Calculations:

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

In-vitro antioxidant activity by potential of *Sida Acuta*

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Comment [a3]: Write as per guideline

DPPH radical assay The DPPH free radical scavenging assay was performed by LiyanaPathirana and Shahidi method [Kikuzaki and Nakatan, 1993]. 200 µL of 0.1 mM DPPH prepared in methanol was added to 100 µL of the plant extract with increase in

concentration (100-500µg/ml). The resulting mixture was incubated at room temperature in the dark for 15 minutes. Absorbance was observed at 517 nm. BHT was taken as a positive control. The experiment was carried out in triplicates and percentage inhibition of the DPPH radical scavenging activity was calculated.

$$\% \text{ Inhibition} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where A₀ is the absorbance of the control and A₁ is the absorbance of the sample.

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 $p < 0.05$.

Comment [a4]: Rewrite as per scientific language

RESULTS:

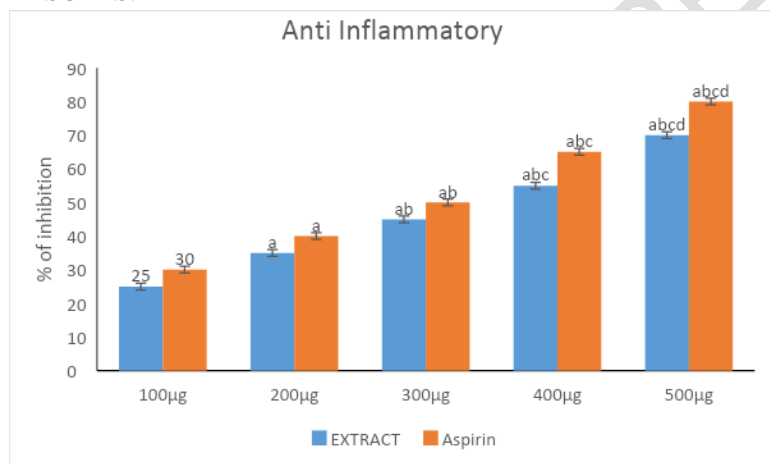


Figure:1 Represents anti inflammatory activity of *sida acuta* extract compared with the standard(Aspirin)..” X” axis represents the different concentrations of the *sida acuta* extract and the “Y” axis represents the % of inhibition. Orange colour denotes standard drug(Aspirin), blue colour represents *sida acuta* extract. 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, d-compared with 400µg.

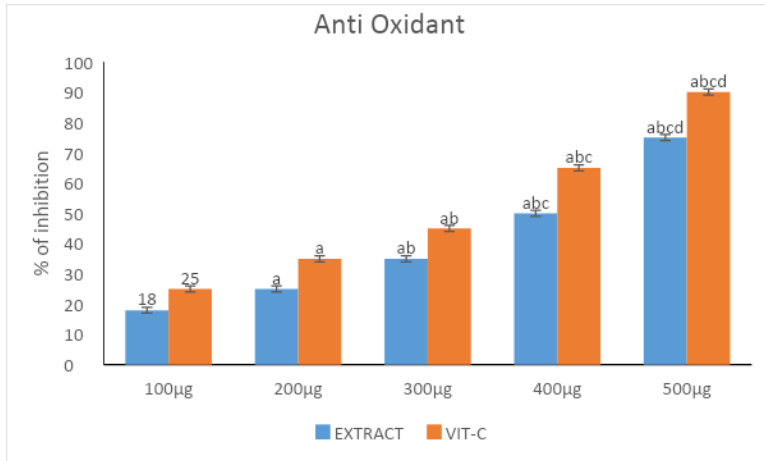


Figure:2 Represents antioxidant activity of *sida acuta* extract compared with standard(Vitamin C). "X" axis represents the different concentrations of the *sida acuta* extract and the "Y" axis represents the % of inhibition. Orange colour denotes standard (Vitamin C), blue colour represents *sida acuta* extract. Each bar represents 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 , d-compared with 400 μg .

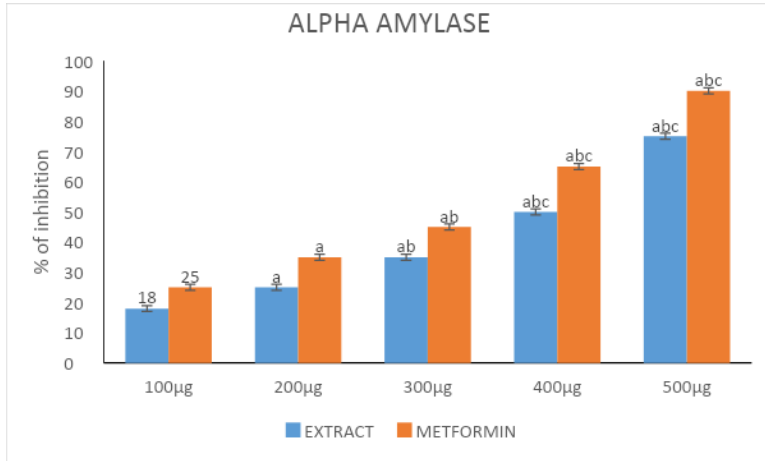


Figure:3 Represents antidiabetic potential (Alpha amylase activity) of *sida acuta* extract compared with the standard (Metformin). "X" axis represents the different concentrations of the *sida acuta* extract and the "Y" axis represents the % of inhibition. Orange colour denotes standard drug (Metformin), blue colour represents *sida acuta* extract. Each bar represents 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 .

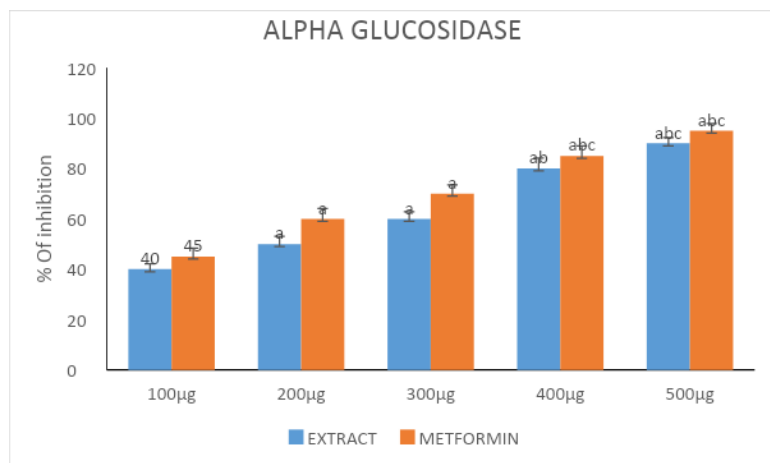


Figure 4: Represents antidiabetic potential (Alpha glucosidase activity) of *sida acuta* extract compared with the standard (Metformin).” X” axis represents the different concentrations of the *sida acuta* extract and the “Y” axis represents the % of inhibition. Orange colour denotes standard drug(Metformin), blue colour represents *sida acuta* extract.Each bar represents mean \pm SD of 6 observations. Significance at the levels of $p < 0.05$.a-compared with 100 μ g; b-compared with 200 μ g; c-compared with 300 μ g.

Formatted: Strikethrough

RESULTS AND DISCUSSION:

Comment [a5]: Rewrite the result and discussion
There are lack of scientific information with flow

On analysing the ethanolic extract of *Sida acuta*, the plant shows anti inflammatory and antidiabetic properties. *Sida acuta* anti-inflammatory property was analysed compared with aspirin and antidiabetic property was assessed using alpha amylase and alpha glucosidase tests compared with metformin.

By comparing the article of Marimuthu, it was seen that crude extract of *Sida acuta* exhibits larvicidal and repellent activities, having the ability to control *Culex quinquefasciatus*, *aedes aegypti* and *anopheles* mosquitoes. In comparison, the article of MA Ekpo and PC Etim shows that the plant shows the presence of various phytochemicals like saponins, tannins, cardiac glycosides and alkaloids (4). Study of CD Sreedevi et al. stated that *Sida acuta* shows antimicrobial and hepatoprotective properties, the plant is traditionally used for the treatment of liver diseases. The alkaloids present in *Sida acuta* contribute to a potent antimicrobial activity against microorganisms (29) (30). Due to the phenolic content present in *Sida acuta*, it shows a good level of antiinflammatory and antioxidant properties (31). Due to the presence of the active component, cryptolepine, *Sida acuta* has antiplasmodial activities. *Sida acuta* is used as an astringent, tonic and is used in treating blood disorders, nervous and urinary diseases. *Sida acuta* is known to have side effects like dizziness, restlessness, nausea, vomiting, pounding heartbeat and so on. Moreover, *Sida acuta* has the potential to produce silver nanoparticles and is stable in solution. *Sida acuta* is easily available, safe to handle and possess a wide range of metabolites.

In the growing world of medical advances, with the use of traditional plants and natural herbs a variety of diseases and disorders can be cured without any side effects. From the study of AS Johnson et al. concluded that the *Sida acuta* shows potent antioxidant properties of free radical scavenger due to the presence of flavonoids present in the extract (32). Since the plant possesses antioxidant property, it has the ability to destroy

Comment [a6]: What?

free radicals and has the ability to prevent diabetes and cancer. The methanol extract of *Sida acuta* produces positive response for excision and incision wounds. The extract helps in epithelialise faster and contraction of wounds in higher rate. In future, the phytochemical screening and total phenolic content of *Sida acuta* can be assessed.

CONCLUSION:

The plant exhibits antidiabetic, antioxidant and anti-inflammatory properties. The plant can be used in the management and prevention of cancer. The plant has anti diabetic and antioxidant properties which shows the plant can be used in the treatment of diabetics and various disorders. Further research on *Sida acuta* can be done to analyse the various inherited properties and use of them in treating various ailments.

Comment [a7]: Rewrite

REFERENCES:

1. Karou SD, Nadembega WMC, Ilboudo DP, Ouermi D, Gbeassor M, De Souza C, et al. *Sida acuta* Burm. f.: a medicinal plant with numerous potencies. *Afr J Biotechnol* [Internet]. 2007;6(25). Available from: <https://www.ajol.info/index.php/ajb/article/view/58270>
2. Prakash A, Varma R, Ghosal S. Alkaloid Constituents of *Sida acuta*, *S. humilis*, *S. rhombifolia* and *S. spinosa** [Internet]. Vol. 43, *Planta Medica*. 1981. p. 384–8. Available from: <http://dx.doi.org/10.1055/s-2007-971529>
3. Reische DW, Lillard DA, Eitenmiller RR, Others. Antioxidants. *Food lipids: chemistry, nutrition, and biotechnology*. 1998;423–48.
4. Nwankpa P, Chukwuemeka OG, Uloneme GC, Etteh CC, Ugwuezumba P, Nwosu D. Phyto-nutrient composition and antioxidative potential of ethanolic leaf extract of *Sida acuta* in wistar albino rats. *Afr J Biotechnol*. 2015;14(49):3264–9.
5. Umavandhana R, Jayanthi S. Phytochemical Screening and Free Radical Scavenging

Comment [a8]: Write the reference as per journal guideline

Formatted: Font color: Red

Formatted: Font: Italic

Activity on Some Selected Seaweeds from Gulf of Mannar, India. Research Journal of Pharmacy and Technology. 2018;11(8):3385–8.

6. Konaté K, Souza A, Coulibaly AY, Meda NTR, Kiendrebeogo M, Lamien-Meda A, et al. In vitro antioxidant, lipoxygenase and xanthine oxidase inhibitory activities of fractions from *Cienfuegosia digitata* Cav., *Sida alba* L. and *Sida acuta* Burm f. (Malvaceae). Pak J Biol Sci. 2010 Nov 15;13(22):1092–8. Formatted: Font: Italic
7. Semwal A, Senthil Kumar M. Development of quality control parameters for the standardization of Leaves and bark of *Sida acuta* Burm.f. Indian Journal of Pharmaceutical and Biological Research. 2014 Dec 31;2(04):89–93. Formatted: Font: Italic
8. Pradhan DK. Phytochemical screening of the root of *Sida acuta* Burm. F. Int J Life Sci Pharma Res [Internet]. 2012 May 17 [cited 2021 Mar 19];3(1). Available from: <https://arklib.com/index.php/irjps/article/view/32> Formatted: Font: Italic
9. 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.
10. 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.
11. 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>
12. Babu S, Jayaraman S. An update on β -sitosterol: A potential herbal nutraceutical for diabetic management. Biomed Pharmacother. 2020 Nov;131:110702.
13. 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.
14. 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.

15. 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.
16. 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>
17. 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.
18. 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.
19. 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.
20. Barma MD, Muthupandiyam 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.
21. 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.
22. 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.
23. 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>
24. 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.

25. 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.
26. 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.
27. 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>
28. 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>
29. Sreedevi CD, Latha PG, Ancy P, Suja SR, Shyamal S, Shine VJ, et al. Hepatoprotective studies on *Sida acuta* Burm. f. *J Ethnopharmacol.* 2009 Jul 15;124(2):171–5.
30. Arciniegas A, Pérez-Castorena AL, Nieto-Camacho A, Kita Y, de Vivar AR. Anti-hyperglycemic, antioxidant, and anti-inflammatory activities of extracts and metabolites from *Sida acuta* and *Sida rhombifolia* [Internet]. *Química Nova.* 2016. Available from: <http://dx.doi.org/10.21577/0100-4042.20160182>
31. Tcheghebe OT, Seukey AJ, Tatong FN. Ethnomedicinal uses, phytochemical and pharmacological profiles, and toxicity of *Sida acuta* Burm. f.: A review article. *The Pharma Innovation.* 2017;6(6, Part A):1.
32. Kumar R, Ambasht RS, Srivastava A, Srivastava NK, Sinha A. Reduction of nitrogen losses through erosion by *Leonotis nepetaefolia* and *Sida acuta* in simulated rain intensities. *Ecol Eng.* 1997 Jul 1;8(3):233–9.

Formatted: Underline

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic