



A REVIEW ON ANNONA RETICULATA

Badgajar Pankaj¹, Ahire Rakesh², Bacchav Bhavesh³, Bagad Sanket⁴, Ahire Khushal⁵, Bagul Sarvesh⁶,

UG Students at Kisan Vidya Prasarak Sanstha's Institute of Pharmaceutical Education, (Approved by PCI New Delhi, Govt. Maharashtra, DTE & Affiliated to KBC NMU, Jalgoan & MSBTE Mumbai) NAAC Accredited B+ (CGPA 2.68), An ISO9001:2015 Certified Under The Guide of Dr. Vikas V. Patil⁷ Sir (Principal)

ABSTRACT:

Indian literatures like Ayurveda and various ancient literatures have stated herbal remedies for a number of human ailments. *Annona reticulata* which is commonly known as bullock's-heart in English and Ramphal in Hindi and Marathi is having various pharmacological activities such as antioxidant, anticancer, analgesic and CNS depressant, antimalarial, anthelmintic, in syphilis and few more. Some compounds have been isolated and reported from the extract of various parts of the plant possessing good pharmacological activity. The studies performed on the seed and root extract also evidenced that the same compounds causes cell death in various cancer cell lines. This review article is a sincere effort to put forward the medicinal importance and botanical, phytochemical, pharmacological study, traditional uses of the plant.

INTRODUCTION:

Custard apple is tropical fruit which belongs to genus *Annona* and family Annonaceae and are collectively known as annonaceous fruits. There are over 120 species of genus *Annona* and are commonly found in India as a fruit consuming plant. This plant is commonly known as custard apple in English and Ramphal in Marathi and Gujarati [1]. having flavor sweet and pleasant, which is a very common name, shared with fruits of various other species belonging to same genus: *A. cherimola*, *A. chrysophylla* and *A. squamosa* or sometimes it is called bullock's-heart, wild- sweetsop, or ox-heart. The Custard apple roots are used in the treatment of acute dysentery, spinal marrow diseases and some cases of depression. The leaves of Custard apple are used in cases of prolapse of the anus, sores and swelling. The plant extract is used for the treatment of diarrhoea pediculosis[2].

Plants are recognized as aromatic as well as source of medicine. The extracts obtained from various plant parts possess medicinal properties and are used as colouring agent, preservative, sweetening agent and as an additive in many medicinal formulations. Plants contain abundant amount of secondary metabolites, they are considered to be principal source of therapeutically active compounds. Along with medicinal formulations plants have been successfully utilised for the development of cosmetics and toiletry preparations. Herbal medicines cause lesser side effects. The regular consumption of synthetic drugs may lead to addiction but such effects are not observed for plant based medicines and are relatively safer than synthetic compounds. Also in pharmaceutical companies commercially plants are used as a source for the synthesis of synthetic compounds. Most of population of developing countries utilize plant based traditional medicine for their primary health care needs. Indian traditional system of medicine; Ayurveda is also based on plant. Medicines derived from plants act as first line defence of body and help to restore the health. Extracts from different plant parts hold wide range of medicinal properties and also utilized as raw materials in herbal industry. Exploration of chemical constituents obtained from plants may provide new leads for the development of novel drug[3].

ECOLOGY AND DISTRIBUTION:

History of cultivation:

Plant is native of Caribbean region and has also been spread across Central and South America, Africa and Asia. *Annona* species is cultivated all over India for its edible fruit belonging to custard apple family. All parts of *annona* are used in natural medicine in the tropics. It is considered to be good source of natural antioxidants for various diseases. Therefore, attention in recent times has been focused on the isolation, characterization and utilization of natural antioxidants. In India the tree grows wild in many areas but is cultivated, especially around Calcutta. It was found in tropical Africa in the 17th century and is grown there as a dooryard fruit tree. It has long been naturalized and cultivated as far south as Peru and Brazil and is grown mostly in the Bahamas and occasionally in southern Florida and Bermuda. It is very common on the east coast of Malaysia, and throughout Southeast Asia and the Philippines [1].

Geographic Distribution:

Exotic: Mexico Bahamas, Bermuda, United States of America, Guam, Philippines, Malaysia, Peru, Brazil, South Africa, India. Native: Guatemala, Belize [1].

TAXONOMY OF ANNONA RETICULATA LINN [4,5] -

| Scientific Classification | Synonyms | Botanical, Common and Vernacular Name | Local Name |
|-----------------------------------|-------------------------------|--|------------------------|
| Kingdom: Plantae | <i>Annona excelsa</i> Kunth. | Botanical Name: <i>Annona Reticulata</i> Linn. | Marathi: Ramphal |
| Order: Magnoliids | <i>Annona Laevis</i> Kunth. | Common Name: Netted Custard Apple | Tamil: Ramachita |
| Family: Annonaceae | <i>Annona Longifolia</i> Moc. | English: Bullock's Heart, Corazon | Telegu: Ramasitapalam |
| Genus: <i>Annona</i> | <i>Annona Longifolia</i> sse. | Portuguese: Frutoda condessa | Malayalan: Manilanilam |
| Species: <i>Annona Reticulata</i> | <i>Annona Riparia</i> Kunth | Indonesian: Buah Nona | Kannada: Ramaphala |
| | | India: Ramphal | |

MORPHOLOGY –

The height of *A. reticulata* is near about 6.0 - 7.5 m. It contains numerous lateral branches. It is a small tree with glabrous branches. The stems are cylindrical having lenticels and very short coffee coloured hairs [5]. Leaves are oblong, lanceolate, membranous, acute, and rounded or curvate at the base. The upper surface of leaves is glabrous and on lower surface it contains few

spreading hairs. Two to four flowers may present on lateral pedicel. Fruits are edible, somewhat heart shaped, rough and yellow in colour which change to yellowish red on ripening [4]. Fruits are sweet, astringent and useful in blood complaints [6]. Seeds are smooth and blackish in colour [4].



FIGURE 1 WHOLE PLANT



FIGURE 2 LEAVES

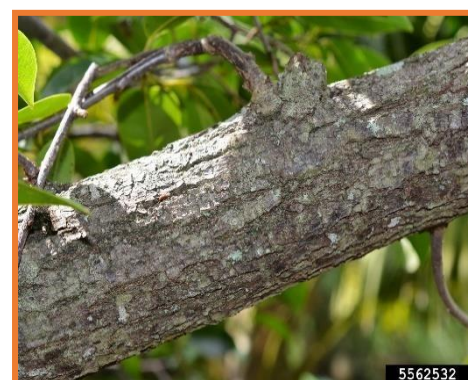


FIGURE 3 STEAM BARK



FIGURE 4 FLOWER



FIGURE 5 FRUIT

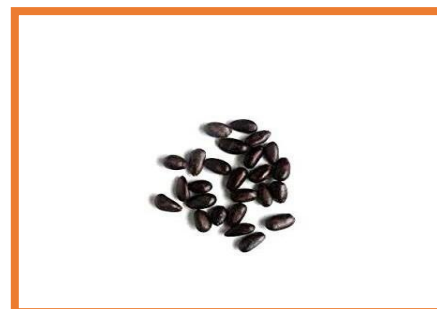


FIGURE 6 SEED

PHYTOCONSTITUENTS -

Annona reticulata L. are known to possess various primary and secondary metabolites, primary metabolites are directly involved in plant growth and development whereas secondary metabolites help indirectly by providing defense mechanism against herbivory, insects and pathogen attack and also help in withstanding adverse stressful climatic conditions. Leaf extract of plant are known to have following phytochemicals alkaloids, amino acids, flavonoids, glycosides, phenolic compound, proteins, steroids, triterpenoids, tannins, starch, saponins, anthraquinones, aleurone grains and inulin [7,8]. Stem bark extract consist of following phytochemicals like alkaloids, fats and oil, lignin, steroid, tannins, phenolic compounds and triterpene. Seed's extract consists of following phytochemicals like alkaloids, carbohydrates, terpenoids, proteins, steroids, and phenols [9]. Fruits consist of following phytochemicals like carbohydrates, terpenoids, proteins, steroids, tannins and phenols [9].

Following compounds were isolated from leaf extracts of *Annona reticulata* L.; Annonaretin A, kaurenoic acid, taraxerol, β sitosterol, 16 α -hydro-19-*al*-ent- kauran-17-oic acid, 6 β hydroxystigmast-4-en-3-one, 17-acetoxy-16 β -ent-kauran- 19-oic acid, 16 α -hydro-ent-kauran-17,19-dioic acid and (2S)-di-Omethylquiritigenin [9] and GC-MS analysis gave following compounds

Piperidine, 2-propyl-, Piracetam, Benzene, (1-methyl butyl), Palmitic acid, Mitoflaxone, Oleic acid, (1,1'bicylopropyl)-2-octanoic acid, 2 hexyl-,methyl ester, 3,4-dihydroxy-1,6-bis (3-methoxy-phenyl)-hexa-2,4-diene-1,6- dione, Cholesta-7,14-diene, 4H-1-Benzopyran-4-one, 2-(3,4 dimethoxyphenyl)-5-hydroxy-3,6,7-trimethoxy, 4-(4- nitrophenylazo)-benzoicacid,

methoxycarbonylmethyl ester, 2,6,10,14-Hexadecatrienoic acid, and N-(4- Hydroxyphenyl)acetamide. Acetogenins is a type of secondary metabolite exclusively found in family Annonaceae. They are characterised by C32 or C34 fatty acid chain with a terminal γ -lactone. Acetogenins shows some pharmacological activities antifeedant, antimicrobial, antiparasitic, antitumor, immunosuppressant, and pesticidal activities. Reticulatacin is a bioactive acetogenin isolated from *Annona reticulata* L. which shows prominent anti- tumour and anti-cancer activity [10]. Stem consists of compounds are N-trans- feruloyltyramine, N-*pc*poumaroyltyramine and N-trans-caffeoyltyramine, lignans, β sterol [11] GC-MS analysis of stem showed following compounds- 2,3- Dihydrobenzofuran (7.910%), Deconoin acid ethyl ester (14.730%), 2,3-

Dimethoxysuccinicacidimethyl ester (4.021%), 3-Hexadecyne (13.035%), Allo-Aromadendrene (1.970%), AlloAromadendrene (6.739%), Megastigmatrienone(1.901%), Arturmerone (3.952%), Oleicacid (10.028%), Gentsic acid (8.496%),

and 13-Docosenamide (23.190%) [12]

PHARMACOLOGICAL STUDIES

A. reticulata L.

A. reticulata leaf extract shows high activities in quenching 1,1-diphenyl-2-picrylhydrazil and superoxide radicals in plant [13]. Annonaceous acetogenins are a group of phytoconstituents obtained from plants, have potent antineoplastic agents. Acetogenins are efficient cytotoxic inhibitors of the mitochondrial nicotinamide adenine dinucleotide: Ubiquinone oxidoreductase (complex I of the respiratory chain). Seeds of *A. reticulata* contains squamocin which has cytotoxic constituent for mostly all the cancer cell lines tested [14,15]. In ethanol extracts, in vitro inhibition toward the vero cell line proliferation was found to be lower when compared with cancer cell lines [16].

A. reticulata leaves show in vitro cytotoxic and human recombinant caspase inhibitory effect [17]. Hence, *A. reticulata* has potent chemopreventive agent in cancer therapy. The aqueous leaf extract has anthelmintic activity [18]. Leaves of *A. reticulata* can be used in the treatment of inflammatory diseases, and potent new anti-inflammatory agents [19]. Leaves of *A. reticulata* possess potent glucose lowering effect. The glucose lowering activity is more of corrective in nature than disruptive [20]. By using roots, tea is prepared and used as a treatment for fevers and the bark is used as a powerful astringent for antidiarrhetic and vermifuge [21]

Pharmacological activities of *Annona Reticulate*

| Name of the plant | Pharmacological Activities |
|--------------------------|--|
| <i>Annona reticulata</i> | Antipyretic activity, anthelmintic activity, antiulcer activity, antinociceptive activity, analgesic and anti-inflammatory, antiproliferative activity, antioxidant and antimicrobial activity |

- Antipyretic Activity [22]** – An examination was conducted on the pain-relieving properties of a crude aqueous leaf extract from *A. reticulata*, administered at doses of 200 mg/kg and 400 mg/kg. Hyperpyrexia was induced in rats by injecting a 20% aqueous suspension of Brewer's yeast subcutaneously. Rats exhibiting a temperature increase of 0.5 °C–1 °C or higher after 18 hours were chosen for the study. The effectiveness of the extract was compared to that of the standard drug, paracetamol, administered at a dose of 150 mg/kg. The findings suggest that the *A. reticulata* leaf extract possesses significant antipyretic properties.
- Anthelmintic Activity [4]** - The effectiveness of *A. reticulata* leaves in treating worms was tested using Indian earthworms, *Perentima posthuma*. The leaves were ground and soaked in ethanol to make an extract. Vacuum distillation was used to concentrate the extract, yielding 15.83 g. The extract was then separated into fractions using petroleum ether, chloroform, ethyl acetate, and ethanol. Each fraction was concentrated, yielding 3.39 g, 0.15 g, 0.13 g, and 1.51 g respectively. Earthworms of specific dimensions were selected for the study, with Albendazole serving as the control. The ethanol fraction showed faster paralysis onset, indicating it had stronger anthelmintic activity compared to the other fractions.
- Antilulcer Activity [23]** - The potential of the aqueous extract from *A. reticulata* leaves to treat ulcers was explored using ethanol and indomethacin to induce ulcers in rats. The extract, obtained through Soxhlet extraction and vacuum concentration, was administered to different groups of rats alongside a vehicle-treated group and a group treated with famotidine as a reference drug. Significant reductions in ulcer index, acid volume, and total acidity were observed in rats treated with both the extract and famotidine. Additionally, the extract showed improvements in glutathione levels and pH compared to the vehicle-treated group. These findings suggest that the antiulcer activity of the extract may be attributed to its cytoprotective, antisecretory, and antioxidant properties.
- Antinociceptive Activity [24]** - A model using acetic acid-induced gastric pain was employed to evaluate the potential pain-relieving effects of methanolic extract from *A. reticulata* leaves in Swiss albino mice. The leaves were dried, powdered, and soaked in methanol for 48 hours. Swiss albino male mice weighing 20–25 g were divided into groups. The control group received a vehicle, while another group received aspirin at doses of 200 and 400 mg/kg. The remaining groups were given different doses of the extract (50, 100, 200, and 400 mg/kg). After 60 minutes, the mice were injected intraperitoneally with 1% acetic acid to induce writhing, and the number of writhings was recorded for 10 minutes. The extract reduced the number of writhings by 47.0%, 55.1%, 67.3%, and 69.4% at doses of 50, 100, 200, and 400 mg/kg, respectively, indicating a significant dose-dependent effect and suggesting the presence of potent pain-relieving compounds in the leaves.
- Analgesic and Anti-inflammatory [25]**- The sesquiterpene portion of *A. reticulata* bark underwent testing for its pain-relieving and anti-inflammatory effects, both centrally and peripherally. The study utilized a sesquiterpene fraction extracted from unsaponified petroleum ether, containing a mix of three primary sesquiterpenes, constituting 71.66% of the fraction. Analysis via GC/MS revealed copaene (35.40%), patchoulane (13.49%), and 1H-cycloprop(e)azulene (22.77%) within the fraction. Central and peripheral pain relief was assessed using the Eddy's hot plate and acetic acid-induced writhing methods, while anti-inflammatory properties were evaluated through the carrageenan-induced paw edema method. Significant pain relief was observed with the sesquiterpene fraction at doses of 12.5 and 25 mg/kg, and with the unsaponified petroleum ether extract at 50 mg/kg. Pentazocin and aspirin served as standard analgesics. The inhibition of carrageenan-induced paw edema was dose-dependent in groups treated with the extract and fraction, comparable to aspirin's effects.
- Antiproliferative Activity [26,27]** - The research explored the antiproliferative abilities of aporphine alkaloids liriodenine, norushinsunine, reticuline, and acetogenin neoannonin, sourced from *A. reticulata* roots, against various cancer cell lines (A-549, K-562, HeLa, MDA-MB) and normal Vero cells using MTT assay. The compounds were identified structurally through ¹H NMR, ¹³C NMR, and mass spectroscopic techniques. Aporphine alkaloids were extracted via column chromatography (neutral alumina) from the root's ethanolic extract using a toluene:ethyl acetate:diethyl amine solvent system, while acetogenin was isolated via ethanol partitioning and column chromatography with n-hexane, ethyl acetate, and methanol. The activity was assessed using isolated compounds at concentrations of 5, 10, and 20 µg respectively. Neoannonin demonstrated significant cytotoxicity (IC₅₀: 5.8 to 6.9 µg/ml) against all cancer cell lines, whereas norushinsunine showed moderate cytotoxicity (IC₅₀: 7.4 to 8.8 µg/ml). The compounds exhibited lower cytotoxicity (IC₅₀: 13.8 to 26.0 µg/ml) on normal Vero cells compared to cancer cell lines. The study concluded that the pronounced cytotoxicity of the isolated aporphine alkaloids is attributed to the isoquinoline moiety, the presence of a hydroxyl group, and the apoptosis-inducing ability of these compounds in

cancer cell lines.

- **Antioxidant and Antimicrobial Activity [28]** - The study focused on exploring the antioxidant and antimicrobial properties of *A. reticulata* root extract. Antioxidant screening involved DPPH free radical scavenging and hydrogen peroxide assays, while antimicrobial analysis utilized agar cup and poison plate methods for bacteria and fungi, respectively. The roots were processed, dried, powdered, and extracted using a Soxhlet apparatus. Antioxidant activity was assessed at various concentrations, and antibacterial efficacy was tested against both gram-negative and gram-positive bacteria. Similarly, antifungal activity was evaluated against several fungi strains. The extract demonstrated significant scavenging activity comparable to ascorbic acid, notably inhibiting *B. cereus* and showing substantial effectiveness against all bacteria strains. It also exhibited notable antifungal activity, particularly against *T. viride* and *C. albicans*. These findings underscore the potent antimicrobial potential of *A. reticulata* root extract.

TRADITIONAL USES -

Traditionally the plant has been employed for the treatment of cardiac problem, dysentery, epilepsy, parasite and worm infestations, constipation, haemorrhage, bacterial infection, dysuria, fever, ulcer and as insecticide. Bark is a powerful astringent and used as a tonic whereas leaves used for helminthiasis treatment.[29,4,31]

CONCLUSION –

Mother Nature has given us many plants and animals. Some plants have healing powers, like the *Annona reticulata*. It's a common plant, but it's important for medicine. Scientists found that some chemicals in this plant can fight cancer, especially bladder cancer. It could be useful in cancer treatment. We still need to study more about this plant to understand its full potential for helping people with illnesses.

REFERENCES –

1. Jigesh P., Prमित K. P., Ramchandra S., Kamlesh R.S. Identification of phytochemicals from seed extract of custard apple, *Annona Squamosa*.
2. Bioscience Biotechnology Research Communications. 2021 14(1):397-402
3. Shital S. C, Prashant B.S., Manoj G. D., Deepak P.P. A Comprehensive review on *Annona Reticulata*. International Journal of Pharmaceutical Science and Research 2013 5(1):45-50
4. P. G. Jamkhande, A. S. Wattamwar. *Annona Reticulata* Linn.(Bullock's Heart): plant profile, phytochemistry and pharmacological properties. Journal of Traditional and complementary Medicine 5.3 (2015):144-152
5. Nirmal S.A., Gaikwad S.B., Dhasade V.V., Dhikale R.S., Kotkar P.V., Dighe S.S. Anthelmintic activity of *Annona reticulata* leaves. Res J Pharm Biol Chem Sci. 2010;1:115–118.
6. Pinto A.C., Cordeiro M.C.R., Andrade S.R.M. University of Southampton; Southampton UK: 2005. *Annona Species*. International Centre for Underutilized Crops; pp. 3–24
7. Savithamma N., Linga R.M., Suhrulatha D. Screening of medicinal plants for secondary metabolites. Middle East J Sci Res. 2011;8:579–584.
8. Mallick S, Chandra G. "Biochemical Profiling of Primary and Secondary Metabolites of *Annona reticulata* Leaf with their Seasonal Fluctuation", Recent trends in biochemistry, 2020.
9. Jayaprakash A. "Phytochemicals, Antimicrobial and Antioxidant Properties of *Annona reticulata* Linn." Journal of Academia and Industrial Research, 2017;6(6):90-95.
10. Reddy MP, Shantha TR, Bharathi V, Kumar RK, Venkateswarlu G. "Pharmacognostical Evaluation On The Medicinal & Nutritive Fruits Of Raamphal- *Annona Reticulata* L.", Journal of Pharmacognosy and Phytochemistry, 2015;4(1):21-28.
11. Saad JM, Hui Yu-hua, Rupprecht JK, Anderson JE, Kozłowski JF, Zhao Geing-xian et al. "Reticulatacin: A New Bioactive Acetogenin from *Annona Reticulata* (Annonaceae)" Tetrahedron, 1991;47(16/17):2751-2756.
12. Barbalho M Sandra, Alvares Goulart, Ricardo de, Maria Vasques Farinazzi-Machado, Flavia da Silva Soares de Souza, Maricelima, Cincotto dos Santos Bueno et al. "Annona sp: Plants with Multiple Applications as Alternative Medicine - A Review", Current Bioactive Compound, 2012;8:277-286.
13. Wen W, Lin Y, Ti Z. "Antidiabetic, Antihyperlipidemic, Antioxidant, Anti-inflammatory Activities of Ethanolic Seed Extract of *Annona reticulata* L. in Streptozotocin Induced Diabetic Rats", Frontiers in Endocrinology, 2019;10:716.
14. Baskar R, Rajeswari V, Kumar TS. In vitro antioxidant studies in leaves of *Annona* species. Indian J Exp Biol 2007;45(5):480-5.
15. Yuan SS, Chang HL, Chen HW, Yeh YT, Kao YH, Lin KH, et al. Annonacin, a mono-tetrahydrofuran acetogenin, arrests cancer cells at the G1 phase and causes cytotoxicity in a Bax- and caspase-3-related pathway. Life Sci 2003;72(25):2853-61
16. Yuan SS, Chang HL, Chen HW, Kuo FC, Liaw CC, Su JH, et al. Selective cytotoxicity of squamocin on T24 bladder cancer cells at the S-phase via a Bax-, Bad, and caspase-3-related pathways. Life Sci 2006;78(8):869-74.
17. Suresh HM, Shivakumar B, Shivakumar SI. Inhibitory potential of the ethanol extract of *Annona reticulata* Linn against melanoma tumor. J Nat Pharm 2011;2:168-72.
18. Mondal S, Mondal N, Mazumder U. In vitro cytotoxic and human

23. recombinant caspase inhibitory effect of *Annona reticulata* leaves. *Indian JPharmacol* 2007;39:253-4.
24. Kaleem M, Asif M, Ahmed QU, Bano B. Antidiabetic and antioxidant activity of *Annona squamosa* extract in streptozotocin-induced diabetic rats. *Singapore Med J* 2006;47(8):670-5.
25. Thang TD, Kuo PC, Huang GJ, Hung NH, Huang BS, Yang ML. Chemical constituents from the Leaves of *Annona reticulata* and their inhibitory effectson no production. *Molecules* 2013;18:4477-86.
26. Rout SP, Kar DM, Mohapatra SB, Swain SP. Anti-hyperglycemic effect *Annona reticulata* L. leaves on experimental diabetic rat model. *Asian J PharmClin Res* 2013;6(1):56-60.
27. Jyothi BA, Venkatesh K, Chakrapani P, Rani AR. Phytochemical and pharmacological potential of *Annona cherimola*-A review. *Int J Phytomed*2011;3:439-47.
28. Patil S.B., Chavan G.M., Ghodke D.S., Naikwade N.S., Magdum C.S. Screening of some indigenous plants for their antipyretic activity. *Res J Pharmacol Pharmacodyn.* 2009;1:143.
29. Singh J., Kumar S.V., Kadam V. Antiulcer activity of *Annona reticulata* leavesextract in rats. *Int J Pharm Pharm Sci.* 2012;4:412–414.
30. Islam R.M., Rahman S.M., Ahmed M. Antinociceptive activity studies with methanol extract of *Annona reticulata* L. (annonaceae) and *Carissa carandas* L.(Apocynaceae) leaves in Swiss albino mice. *Adv Nat Appl Sci.* 2012;6:1313– 1318.
31. Islam R.M., Rahman S.M., Ahmed M. Antinociceptive activity studies with methanol extract of *Annona reticulata* L. (annonaceae) and *Carissa carandas* L.(Apocynaceae) leaves in Swiss albino mice. *Adv Nat Appl Sci.* 2012;6:1313– 1318.
32. Suresh H.M., Shivakumar B., Shivakumar S.I. Phytochemical potential of *Annona reticulata* roots for antiproliferative activity on human Cancer cell lines. *Adv Life Sci.* 2012;2:1–4.
33. Suresh H.M., Shivakumar B., Hemalatha K., Heroor S.S., Hugar D.S., Rao
34. K.R. In vitro antiproliferative activity of *Annona reticulata* roots on humancancer cell lines. *Pharmacogn Res.* 2011;3:9–12.
35. Jamkhande P.G., Wattamwar A.S., Pekamwar S.S., Chandak Antioxidant, antimicrobial activity and in silico PASS prediction of *Annona reticulata* Linn.root extract. *Beni-Suef Univ J Basic Appl Sci.* 2014;3:1–9.
36. 6. Zaman K., Pathak K. Pharmacognostical and phytochemical studies on the leaf and stem bark of *Annona Reticulata* Linn. *J Pharmacogn Phytochem.*2013;1:1–8.
37. Wele A., Mayer C., Dermigny Q., Zhang Y., Blond A., Bodo B. Sequence and three-dimensional structure of cycloreticulins A and B new cyclooctapeptides from the seeds of *Annona reticulata*. *Tetrahedron.* 2008;64:154–162.