



Annona reticulata L. phytoconstituents and pharmacology: A review

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Abstract

Annona reticulata L. belongs to family Annonaceae, which consists of 130 genera and 2500 species and commonly known as Bullock's heart, custard apple, Ramphal etc. It is mainly found in tropical and sub-tropical areas of world and is native to West Indies. Plant is mainly grown for its delicious sweet fruit. Fruits are rich in potassium, phosphorous, calcium, magnesium, iron, vitamin C, which helps in boosting immunity. It is use in traditional system of medicine for treatment of various diseases diarrhea, dysentery, fever, cough and cold, and as tonic, expectorant, analgesic, astringent etc. It possesses pharmacological property like antimicrobial, antioxidant, anticancer, antidiabetic, anthelmintic etc.; acetogenins and reticulenins are the compounds isolated from *Annona reticulata* L. which are known to be responsible for the various pharmacological properties of the plant.

Keywords: *Annona reticulata* L., phytoconstituents, pharmacology

Introduction

Family Annonaceae consists of 130 genera and 2300 to 2500 species, and the genus *Annona* consists of 119 species (Chatrou *et al.* 2018; Jasmine *et al.* 2020; Pineda-Ramirez *et al.* 2020) [7, 18, 6]. The commonly cultivated *Annona* species for domestic and commercial use are *Annona squamosa* (sitaphal/saripha/custard apple) which is the most commonly cultivated for its delicious fruit, *Annona reticulata* (ramphal/bullock's heart), *Annona muricata* (soursop), *Annona glabra*, *Annona cherimola* (cherimola), *Annona chrysophylla*, *Annona purpurea* (Chavan *et al.* 2014; Jasmine *et al.* 2020) [9, 18]. All these plant species possess various pharmacological property like antimicrobial, antioxidant, anticancer, larvicidal etc. (Kaladhar *et al.* 2015) [20], these plants are also economically important for their nutritious fruits, also they are used as bio fence, also used to make herbal tea from their leaves and dried fruits, which makes these plants valuable in every aspect (Nirala *et al.* 2019; Minh *et al.* 2019) [27, 23]. Variation in number of chromosomes in members of family annonaceae is reported, the number of chromosomes ranges from $2n = 14$ to 22 , the basic number of chromosomes is $X=7$. Number of chromosomes in *Annona squamosa* and *Annona reticulata* is $2n=14$ (Anuragi *et al.* 2016; Datta *et al.* 1990) [1, 10].

Annona reticulata L. is a small fast-growing tree, grows up to a height of 10 meter. Trees are deciduous in nature. Branching is extensive and canopy is dense covering a large area. Trees are mainly known to grow in tropical and sub-tropical areas of the world and pantropical in distribution. It is native to West Indies and South and Central America (Padhi *et al.* 2011; Jamkhamde *et al.* 2015; Pathak, Zaman 2013; Renu *et al.* 2020) [31, 15, 34, 40]. It is mainly cultivated for its fleshy, sweet, pleasant and nutritious fruit. Globally it is cultivated in India, Bangladesh, Pakistan (Jamkhamde *et al.* 2015) [15], Brazil, Malaysia, Philippines (Chavan *et al.* 2014) [9], Bhutan, China, Nepal, Africa, North America, Costa Rica, Mexico, Belize, Caribbean Island, South America, Bolivia, Ecuador, Peru, Venezuela (Renu *et al.* 2020) [40]. In India it is inhabited in states like Andhra Pradesh,

Arunachal Pradesh, Assam, Bihar, Chhattisgarh, Delhi, Jharkhand, Meghalaya, Orissa, Rajasthan, Tamil Nadu, Tripura, Uttar Pradesh, West Bengal, Punjab, Gujarat, Maharashtra, Karnataka, and Kerala (Pathak, Zaman 2013; Renu *et al.* 2020) [34, 40].

Annona reticulata L. is commonly known as Bullock's heart, Jamaican apple, custard apple, sugar apple, netted custard apple in English, in Hindi as Ramphal, in Tamil as Ramachita, in Telegu as Ramasitaphalam, in Malayalam as Manilanilam, in Kannada as Ramphala, in orriya as Ramopholo (Jamkhamde *et al.* 2015; Renu *et al.* 2020; Orwa *et al.* 2009) [15, 40, 30]. The word Annona is derived from Latin word 'Anon' meaning yearly produce (Jayaprakash 2017) [19].

Leaves are simple, dark green, oblong-lanceolate, margin entire, and alternate, petiolate, leaf tip acute, mid rib strong, lateral veins 15-17 in number, thin and pungent odor (Zaman, Pathak 2013; Switu *et al.* 2012) [34, 45]. Stems are hard, woody and brown in colour. Flowers are complete, bisexual, trimerous and pale green in colour. Pollination occurs by insects i.e., entomophilous. Fruits are smooth skinned; heart shaped and becomes dull red when ripe. Seeds are dark brown in colour and smooth. Flowering occurs in month of May and fruiting occurs between September to November (Gadani 2020) [11]. Fruits are sweet in taste and eaten raw or after ripening.

Trees are known to grow at the altitude of 0 to 1500 meter from sea level (Orwa *et al.* 2009) [30]. It is not drought tolerant, prefers moist, sandy loam soil with a pH around 6, humid atmosphere and can tolerate light night frost. It is found in lowland rainforest, component of scrub forest and growing on roadside. The annual produce of mature tree is around 35-45 Kg of fruits.

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. (Mallick, Chandra 2020; Jayaprakash 2017) ^[21, 19].

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 (Reddy *et al.* 2015) ^[39].

Fruits consist of following phytochemicals like carbohydrates, terpenoids, proteins, steroids, tannins and phenols (Reddy *et al.* 2015) ^[39].

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-*O*-methylquiritigenin (Reddy *et al.* 2015) ^[39] 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)-benzoic acid, 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 (Saad *et al.* 1991) ^[42].

Stem consists of compounds are N-trans-feruloyltyramine, N-p-coumaroyltyramine and N-trans-caffeoyltyramine, lignans, β -sterol (Barbalho 2012) ^[31] GC-MS analysis of stem showed following compounds- 2,3-Dihydrobenzofuran (7.910%), Deconoin acid ethyl ester (14.730%), 2,3-Dimethoxysuccinic acid dimethyl ester (4.021%), 3-Hexadecyne (13.035%), Allo-Aromadrene (1.970%), Allo-Aromadrene (6.739%), Megastigmatrienone (1.901%), Arturmerone (3.952%), Oleic acid (10.028%), Gentic acid (8.496%), and 13-Docosamide (23.190%) (Wen *et al.* 2019) ^[47].

Ethnomedicinal uses

Almost every part of *Annona reticulata* L. are used in traditional system of medicine for treatment of various diseases, parts used are leaves, stem bark, fruit peel, fruits, root and root bark. Leaves are used as astringent, anthelmintic, antidiarrheal, antidiabetic and anti-diarrhea. Decoctions of leaves are given in treatment of worm infestations, gastritis and poultice of leaf were applied for abscesses, boils, ulcers, external and internal wounds (Nugraha

2019) ^[29]. Crushed leaves used in treatment of hysteria, dizziness and nausea. Bark is used as astringent and tonic. Seeds are used as astringent, vermifuge, and in treatment of diarrhea and dysentery. Seed oil is used to reduce inflammation. Unripe fruit is used as astringent, anthelmintic, anti-dysenteric, anti-diarrhea and used to treat fever and enlarged spleen, juice of unripe fruit used to treat insect bite. Ripe fruit used to kill lice, as expectorant, as coolant, as stimulant, as hair tonic and stop vomiting. Decoction of root is used as febrifuge and to relief toothache (Reddy *et al.* 2015) ^[39].

Pharmacological Activities

Antioxidant activity

Plants, plant derived products and compounds are known to mimic antioxidants to protect the cells from oxidative damage, treatment with plant derived compounds has increased the activity of naturally occurring antioxidant enzymes like catalase, superoxide dismutase, glutathione, peroxidase etc. (Banerjee *et al.* 2018) ^[2]. Increase in free radical concentration results in higher oxidative stress leading to various health complications like diabetes, cancer etc.

According to experimental study conducted by Parthiban *et al.* (2019) ^[32] on chloroform extract of *Annona reticulata* L. leaves for testing their antioxidant activity by using DPPH and ABTS free radical. The chloroform leaf extract showed free radical quenching activity with value of 99.2% and 71.0% at the concentration of 1000 μ g/ml and IC₅₀ value of 137.8 and 722.8 μ g/ml for DPPH and ABTS respectively (Pratibhan *et al.* 2019) ^[32]. Narayana *et al.* (2020) tested the antioxidant property of ethanolic extract of *Annona reticulata* L. leaves by OH, ROO and O₂⁻ free radical scavenging assay, leaf extract showed no result for O₂⁻ free radicals but showed significant result for OH and ROO free radicals with an IC₅₀ value of 126.98 μ g/ml and 13.13 μ g/ml respectively (Pineda-Ramirez *et al.* 2020) ^[36]. Jamkhande *et al.* (2016) ^[16] tested the antioxidant activity of methanolic leaf extract of *Annona reticulata* L. by DPPH and H₂O₂ free radicals and the IC₅₀ value recorded were 52.08 μ g/ml and 70.06 μ g/ml respectively. Mondal *et al.* (2008) ^[26] evaluated the free radical scavenging property by using free radical DPPH, NO, and Superoxide at the concentration ranging from 10-100 μ g/ml, the leaf extract showed the free radical scavenging property in dose dependent manner. The IC₅₀ value for DPPH, NO, and Superoxide were 3.22, 170.01 and 25.12 μ g/ml respectively. Biswas *et al.* (2012) ^[6] observed the antioxidant capacity of leaves extract with different solvents that is n-hexane, petroleum ether, methanol and chloroform. The antioxidant activity was tested by phosphomolybdenum method and Cupric Reducing Antioxidant Capacity (CPRAC) methanolic extract showed maximum antioxidant activity and petroleum ether showed the least activity.

Wen *et al.* (2019) ^[47] tested the ethanolic seed extract of *Annona reticulata* L. which resulted in improved oxidative status in Streptozotocin induced diabetic rats by decreasing level of Malondialdehyde (MDA) and Nitric Oxide (NO) and increase activity of Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GPx) by administration of ethanolic seed extract at the dose of 50 or 100 mg/kg for 45 days.

Jamkhande *et al.* (2014) ^[17] had experimentally evaluated the antioxidant activity of methanol extract of roots by using DPPH free radical scavenging assay and H₂O₂ radical scavenging

activity assay at the concentration ranging from 20-100 µg/ml. The highest free radical scavenging activity was reported at concentration of 80µg and 100µg with activity percent of 42.60 and 47.92 respectively and IC₅₀ value was 108.71µg/ml. Whereas, high activity percent was reported for H₂O₂ at the concentration of 80µg/ml and 100µg/ml with percent activity of 51.19 and 55.95 respectively and IC₅₀ value of 80.08µg/ml.

Antimicrobial activity

Padhi *et al.* (2011) [31] tested the methanolic and aqueous extract of *Annona reticulata* L. leaves for their antibacterial potential against different bacterial strains, extract showed zone of inhibition against *Bacillus subtilis*, *Staphylococcus aureus*, *Vibrio alginolyticus* but no zone of inhibition reported for *Escherichia coli* and *Pseudomonas aeruginosa*.

Jamkhande *et al.* (2014) [17] studied the antibacterial activity of methanolic root extract of *Annona reticulata* L. using agar cup diffusion method against gram negative bacteria *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*. Root extract showed antibacterial activity against all the above-mentioned bacteria with zone of inhibition ranging from 20 mm to 29 mm and maximum zone of inhibition was obtained for *Bacillus cereus*. For antifungal test poison plate method was used against *Aspergillus niger*, *Penicillium chrysogenum*, *Fusarium moneliforme*, *Trichoderma viride*, and *Candida albicans*. Root extract showed antifungal activity against all the tested fungi but were highly effective against *Trichoderma viride* and *Candida albicans*.

Sangeetha V.S. *et al.* (2016) [43] performed antimicrobial studies on hexane, ethyl acetate, acetone, ethanol and water extract of pericarp of mature and immature fruits of *Annona reticulata* L., extracts were tested against *Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*. Among various extracts ethanolic extracts were found to be most effective whose extract with unripe pericarp showed efficacy against all bacteria except *Staphylococcus aureus* whereas, ethanolic extract of ripe pericarp were effective against all bacteria. Fungal strains used were *Aspergillus fumigatus*, *Aspergillus niger* and *Penicillium chrysogenum*. Ethanolic extract of unripe pericarp were effective against all fungi but ethanolic extract of ripe fruit pericarp were not effective against *Aspergillus fumigatus*.

Jamkhande *et al.* (2016) [16] tested the leaf extract against gram negative bacteria *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Proteus vulgaris*, and gram-positive bacteria *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi* for its antibacterial property by agar well diffusion method. Methnolic extract were effective against all strains but not against *Staphylococcus epidermidis*, n-butanol fractions were effective against all the strains except *Staphylococcus epidermidis*, *Escherichia coli*, and *Pseudomonas aeruginosa*, chloroform fractions were effective against all the strains, Acetone fractions were not effective against *Proteus vulgaris* and *Pseudomonas aeruginosa*. The maximum zone of inhibition was shown by chloroform fraction against *Bacillus subtilis*. All extracts showed zone of inhibition against fungal strains *Candida albicans*, *Saccharomyces cerevisiae*, *Candida blanki*, and no zone of inhibition were reported for *Aspergillus niger*, *Aspergillus flavus*, *Vestilago*

myditis and *Microsporum canis*. Maximum zone of inhibition shown by acetone fraction against *Aspergillus niger*.

Parthiban *et al.* (2018) [33] had synthesized silver nanoparticles from the aqueous extract of *Annona reticulata* L. leaves, which were tested for its antibacterial activity by agar well diffusion method against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Escherichia coli* and antifungal activity against *Candida albicans*, silver nanoparticle showed significant zone of inhibition and hence their antibacterial and fungal property.

Extract of leaf and pericarp showed antimicrobial activity against wide range of gram positive and gram-negative bacteria and fungi also.

Analgesic and CNS depressant

The analgesic activity was studied using hot-plate method in swiss albino mice, mice were injected with 100mg/kg of petroleum ether extract, ethyl acetate extract and methanol extract of *Annona reticulata* L. bark and Pentazocin (20mg/kg) was used as standard drug. All the extract showed significant result for its analgesic activity. Petroleum ether extract showed its peak activity between 60-120 minutes (Bhalke, Chavan 2011) [4].

CNS depressant activity of *Annona reticulata* L. bark was tested by studying the locomotors activity and pentobarbitone sleeping time. Locomotor activity was studied by injecting 100mg/kg of bark extract and standard drug used was Diazepam (2mg/kg). All the extracts were successful in decreasing locomotor activity, decrease in locomotor activity started at 30 minute and continued up to 180 minutes. For testing pentobarbitone sleeping activity animals were injected with extract at the dose of 100mg/kg, all the extracts significantly increased the sleeping time and compared with standard drug pentobarbitone sodium (40mg/kg). Increase in sleeping time and decrease in locomotor activity shows that the bark extracts are effective CNS depressant.

Larvicidal activity

Parthiban *et al.* (2018) [33] had synthesized silver nanoparticles from the aqueous extract of *Annona reticulata* L. leaves, which were tested for its larvicidal activity against early instar larvae of *A. aegypti* at the concentration of 3-20µg/ml with an exposure for 24hr. Maximum mortality rate was obtained at concentration of 15µg/ml (Pratibhan *et al.* 2018) [32].

Mohankumar *et al.* (2016) [24] screened nine methanolic plant extract against *Aedes aegypti* and *Anopheles stephensi* which includes *Passiflora foetida*, *Annona glabra*, *Cosmos bipinnatus*, *Laurus nobilis*, *Abutilon indicum*, *Gossypium herbaceum*, *Annona reticulata*, *Hyptis suaveolens* and *Thespesia populnea*, among all the tested plants *Annona reticulata* showed most significant larvicidal activity with LC₅₀ value of 95.24ppm against *Aedes aegypti* and 262.71 against *Anopheles stephensi*.

Antiplasmodial activity

Yamthe *et al.* (2015) [48] tested the crude ethanol, H₂O and CH₂Cl₂ extract of *Annona muricata* (pericarp, root and stem bark) and *Annona reticulata* (leaf, twig, root, stem bark, root and fruit) for antiplasmodial activity against *Plasmodium falciparum* with an IC₅₀ value ranging from 0.29-1.90 µg/ml (Yamthe *et al.* 2015) [48].

Antidiabetic activity

Many plants are known to possess antidiabetic property which thought to be because of presence of more than one bioactive compound that help to improve blood glucose level along with other problems associated with it like hyperlipidemia, improved insulin secretion, exerted antioxidant effect and improve renal dysfunction (Pereira *et al.* 2019) [35].

Rahman *et al.* (2011) [38] has experimentally evaluated the crude methanolic extract of *Annona reticulata* Linn. leaf for its antidiabetic activity by their application on diabetes induced Swiss albino male mice for treatment of diabetes at the dose of 50mg/kg, 100mg/kg, 200mg/kg and 400mg/kg resulted in decrease of serum glucose level by 34.8%, 37.0%, 49.6% and 56.1% respectively in a dose dependent manner.

Treatment of Streptozotocin induced wistar albino rats by Hydro-Alcoholic extract of *Annona reticulata* Linn. (HAAR) leaf showed a significant decrease in glucose level at the dose 200mg/kg HAAR, 400mg/kg HAAR and Metformin by percent decrease of glucose level 16.08%, 30.47%, and 36.33% respectively. At start of treatment the percent decrease of blood glucose level was 1.94%, 6.43% and 25, 31% for doses 200mg/kg HAAR, 400mg/kg HAAR and Metformin respectively but it increased to 40.05%, 47.36% and 43.06%. It showed that the antidiabetic effect of 400mg/kg HAAR is similar to the standard antidiabetic drug Metformin. 400mg/kg HAAR has significantly decreased the triglyceride and cholesterol level (Rout *et al.* 2013) [41].

Streptozotocin induced wistar male rats were treated with ethanolic extract of *Annona reticulata* Linn. seeds for 42 days have given significant results in decreasing the glucose level at two doses i.e., 50mg/kg and 100mg/kg. It has proven effective in treating diabetes by improving insulin secretion, glucose tolerance and reduced blood glucose level. It helps in restoring the shape and size of islet cells of pancreas thus increasing insulin secretion. Islet cells consist of α -cells, β -cells, and γ -cells, they secrete glycogen, insulin and somatostatin, these three hormones help in glucose metabolism. It also improves kidney histology and function by healing glomerular injury and significant decrease in urine volume and urinary protein, reduced serum creatinine, and uric acid (Wen *et al.* 2019) [47].

Anti-cancer activity

Acetogenins are the secondary metabolite which are mainly found in members of family Annonaceae which prominent anticancer activity which have given significant results in in-vivo model. [15] Isolated acetogenins are found to be effective even at low concentration doses in tumour causing cells and hence can prove effective in cancer treatment (Nugraha 2019; Quilez *et al.* 2018) [29, 37].

In-vitro cytotoxic and recombinant caspases inhibitory activity

Shade dried leaves of *Annona reticulata* were powdered and extracted using Soxhlet apparatus with solvents like petroleum ether, chloroform and methanol. Methanol extract of *A. reticulata* leaves were used to investigate its cytotoxic activity against three cell lines Caco-2 (human colorectal adenocarcinoma), Hep G2 (human hepatocellular carcinoma) and HEK (human kidney carcinoma). Caspases inhibitory activities were studied for initiator caspase (caspase-9) and executor caspase (caspase-3

and 6). Methanol extract showed significant cytotoxic activity against Caco-2 and Hep G2 cell lines in a dose dependent manner while no effect for HEK up to a concentration of 20 μ g/ml. Methanol extract showed inhibition against caspase-6 and 9 with inhibition percent of 66.64% and 87.03% respectively for concentration of 10 μ g/ml but failed to show inhibition against caspase-3 (Mondal *et al.* 2007) [25].

Anti-proliferative activity against cancer cell lines

To test the anti-proliferative activity of Annonin-I MTT assay was performed against HCT-16 cell lines (colorectal cancer cells) it inhibited the cell growth by least value of 46% and maximum value of 90%, GI50 value was recorded at 76.13 μ M. The mode of mechanism of action of acetogenins was considered as inhibition of mitochondrial NADH dehydrogenase enzyme which regulates the process of electron transport chain thus inhibiting the energy synthesis leading to cell death (Ghosh, Mohideen 2020) [12].

Ethanol extract of *Annona reticulata* roots were tested by MTT assay for their antiproliferative activity on different cancer cell lines A-549 (Human lung carcinoma), K-562 (Human chronic Myelogenous Leukemia Bone Marrow), HeLa (Human cervix) and MDA-MB (Human Adenocarcinoma Mammary Gland). Three aporphine alkaloids, liriodenine, norshinsunine, reticuline and one acetogenin, neoannonin were isolated from ethanol extract of root. Isolated aporphine alkaloids and acetogenin compounds showed cytotoxicity against different cancer cell lines in dose dependent manner, out of all isolates neoannonin showed maximum cytotoxicity activity with IC₅₀ value ranging between 5.8 to 6.9 μ g/ml. It has been concluded that the cytotoxic activity of these compounds is due to presence of hydroxyl group in their structure (Suresh *et al.* 2012) [44].

Activity against skin cancer

The main cause of skin cancer is estimated to be exposure to UV rays but other than UV radiation there are some other factors which results in induction of skin cancer these are polycyclic aromatic hydrocarbons, arsenic, tar, raw paraffin and some viruses. To test the activity of *Annona reticulata* methanol root extract against skin cancer swiss albino mice were induced with cancer using chemical carcinogen DMBA with concentration of 25 μ g in 0.1mL of Acetone for a period of six weeks (twice in a week). For treatment of mice a topical was prepared loaded with methanol root extract, mice were treated with the topical gel for 4weeks once in a day with amount 20g/kg of body weight. Animals treated with topical gel depicted a decrease in the size of keratinocyte pearl which shows that methanolic root extract was effective in treatment of skin carcinoma (Bharadwaj *et al.* 2019) [5].

Wound healing activity

The wound healing property of the *Annona reticulata* leaves were tested *in vitro* by cell proliferation assay (MTT assay), cell migration assay (scratch assay), western blot to test the expression of various growth factors (TGF- β , VEGF, and CTGF), proteins (SMAD2, SMAD3, α -SMA), phospho-AkT and phospho-FAK and gelatin zymography to detect MMP-2 and MMP-9 activity. And for *in vivo* evaluation streptozotocin induced diabetic swiss albino mice were used. Ethanol extract of leaves were effective in cell proliferation and migration of cells

associated with wound healing, highest proliferation was reported at concentration 20µg/ml. Results were significant and positive for MTT assay and scratch assay. Ethanol extract has stimulated the expression of phospho-AKT, phospho-FAK, MMP-2 and MMP-9. Animals treated with plant extract at the dose of 150mg/kg body weight for 14 days had shown wound closure up to 98.7%. The recordings showed that the plant extract has promising wound healing property (Mazumdar *et al.* 2019) [22].

Anthelmintic activity

Nirmal *et al.* (2010) [28] used Indian adult earthworm (*Pheretima prosthuma*) for evaluating the anthelmintic property of ethanol extract of *Annona reticulata* leaves. The extracts were further fractionated with ethanol, petroleum ether, chloroform and ethyl acetate, ethanol extract showed maximum anthelmintic activity at concentration 20mg/ml. Column chromatography of ethanol fraction was performed which gave three fractions out of which fraction 2 took least time to paralyze the earthworm.

Chavan *et al.* (2020) [8] used vermicompost earthworm (*Eisenia fetida*) to test anthelmintic property of *Annona reticulata* leaves. Saline water was used to prepare ethanolic extract of concentration 10mg/ml, 20mg/ml and 30mg/ml. Extract showed the anthelmintic activity in dose dependent manner, time required to cause paralysis ranges from 15.1 to 7.5 minutes and for death 18.2 to 9.15 minutes. Least time was required by extract concentration 30mg/ml to cause paralysis and death.

Inhibitory effect on NO production

Leaves were extracted with methanol and crude extract was suspended with n-hexane, purification of n-hexane fraction by column chromatography yielded annonaretin, kaurenolic acid, taraxerol, β-sitosterol, 16α-hydro-19-al-ent-kauran-17-oic acid, 6β-hydroxystigmast-4-en-3-one, 17-acetoxy-16β-ent-kauran-19-oic acid and purification of ethyl acetate fraction gave 16α-hydro-ent-kauran-17,19-dioic acid and (2S)-di-O-methylquiritigenin. The compounds kaurenolic acid, taraxerol, 16α-hydro-19-al-ent-kauran-17-oic acid, 6β-hydroxystigmast-4-en-3-one, 17-acetoxy-16β-ent-kauran-19-oic acid and 16α-hydro-ent-kauran-17, 19-dioic acid significantly decreased NO production in dose dependent manner in RAW 264.7 cells, the IC₅₀ value of these compounds ranges from 48.6 to 99.8 µM (Thang *et al.* 2013) [46].

Antinociceptive activity

Swiss albino male mice were used to test the antinociceptive activity of *Annona reticulata* leaves methanolic extract, mice were administrated with dose of 50, 100, 200, and 400 mg extract per kg body weight they reduced the number of writhing by 47.0, 55.1, 67.3 and 69.4% respectively. Leaf extract exhibited the antinociceptive activity in dose dependent manner and significant analgesic activity is expected from the plant extract of *Annona reticulata* (Islam *et al.* 2012) [13].

Conclusion

Annona reticulata L. member of family Annonaceae is ethnomedicinally very important which is being used as medicine for treatment of various diseases by traditional practitioners which has been seeking the attention of researcher to evaluate these ethnomedicinal properties scientifically. This plant has been explored widely for their pharmacological properties which have been proven scientifically by many workers. The plant is

now known to possess many pharmacological properties like antimicrobial, antioxidant, anticancer, antidiabetic, anthelmintic, larvicidal etc. The acetogenin derived from *Annona reticulata* L. is known to possess potent anticancer potential which has been proved in in-vivo models and cancer cell lines, and found to be effective at low dose concentration. Since plant derived compounds are safe for use as drug, they can be preferred over synthetic medicines, although natural compounds can also be proved toxic so it is suggested to go through toxicity test of the plants.

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