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Review article

Annona reticulata Linn. (Bullock's heart): Plant profile, phytochemistry and pharmacological properties

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ABSTRACT

From the beginning of human civilization plants and plant based chemicals are the most important sources of medicines. Phytochemical and different products obtained from plant are used as medicines, pharmaceuticals, cosmetics and food supplements. *Annona reticulata* Linn. (牛心果 niú xīn guǒ; Bullock's heart) is a versatile tree and its fruits are edible. Parts of *A. reticulata* are used as source of medicine and also for industrial products. It possesses several medicinal properties such as anthelmintic, analgesic, anti-inflammatory, antipyretic, wound healing and cytotoxic effects. It is widely distributed with phytochemicals like tannins, alkaloids, phenols, glycosides, flavonoids and steroids. Present article is an attempt to highlight over taxonomy, morphology, geographical distribution, phytoconstituents and pharmacological activities of *A. reticulata* reported so far.

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1. Introduction

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.¹ Plants restrain 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.² 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.³

Most of population of developing countries utilize plant based traditional medicine for their primary health care needs.⁴ 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.⁵ Exploration of chemical constituents obtained from plants may provide new leads for the development of novel drug.⁴

Annona reticulata Linn. (牛心果 niú xīn guǒ; Bullock's heart) is one of the traditionally important plant used for the treatment of various ailments.⁶ It belongs to family Annonaceae.⁷ The synonyms (Table 1) of plant are Ramphal, Bullock's heart and Custard apple.⁸ Near about 119 different species of the *Annona* genus (Annonaceae) are identified among which most of them are shrubs and trees. Traditionally the plant extract is used for the treatment of diarrhoea¹⁰ and pediculosis.¹¹

1.1. Geographical distribution

A. reticulata (Table 1) is widely distributed in tropical and subtropical regions.⁹ The plant is indigenous to the West Indies. In India it is widely cultivated and naturalized as a fruit consuming plant and deciduous tree. It is distributed in Bengal, Burma and Southern regions of India. It is native to tropical regions of America,

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Table 1
Taxonomy of *Annona reticulata* Linn. (牛心果 niú xīn guǒ; Bullock's heart).^{8,9}

Scientific classification	Synonyms	Botanical, common and vernacular names	Local names
Kingdom: Plantae Order: Magnoliids Family: Annonaceae Genus: Annona Species: <i>Annona reticulata</i>	<i>Annona excelsa</i> Kunt. <i>Annona laevis</i> Kunth. <i>Annona longifolia</i> Moc. <i>Annona longifolia</i> Sesse. <i>Annona riparia</i> Kunth.	Botanical name: <i>Annona reticulata</i> Linn. Common name: Netted Custard apple English: Bullock's heart, Corazon Portuguese: Frutada-Condessa Indonesian: Buah nona India: Ramphal	Tamil: Ramachita Telegu: Ramasitapalam Malayalan: Manilanilam Kannada: Ramaphala

particularly in West Indies and South America. The plant is widely cultivated in Bangladesh and Pakistan.^{6,12,13}

1.2. Morphology

The height of *A. reticulata* is near about 6.0–7.5 m. It contains numerous lateral branches. It is a small tree (Fig. 1) with glabrous branches. The stems are cylindrical having lenticels and very short coffee coloured hairs.⁹ Leaves are oblong, lanceolate, membranous, acute, and rounded or curate 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.⁸ Fruits are sweet, astringent and useful in blood complaints.¹⁴ Seeds are smooth and blackish in colour.⁸

1.3. Traditional uses

Traditionally the plant has been employed for the treatment of epilepsy, dysentery, cardiac problem, parasite and worm infestations, constipation, haemorrhage, bacterial infection, dysuria, fever, ulcer and as insecticide. Bark is a powerful astringent and



Fig. 1. Parts of *Annona reticulata* Linn. plant.

used as a tonic whereas leaves used for helminthiasis treatment.^{6,8,15}

2. Phytoconstituents

Several phytoconstituents have been identified from different parts of *A. reticulata* (Tables 2 and 3). Stem bark contains tannins, alkaloid and phenolic compounds. Leaves contain wide range of chemicals like alkaloids, amino acids, carbohydrates, steroids, flavonoids, proteins, tannins, glycosides and phenolics. Root has been identified for the content of acetogenin, alkaloid, carbohydrates, proteins, flavonoids, tannins. The plant also found to be rich in minerals such as Ca, P, K, Mg, Na, Cl, S, Mn, Zn, Fe, Cu, Se, Co, Ni and Cr.^{6,28,29}

3. Pharmacological properties

Several parts of *A. reticulata* (牛心果 niú xīn guǒ; Bullock's heart) were assessed for their biological potential (Table 4). The extracts and phytoconstituents such as cytotoxic acetogenins isolated from various parts showed diverse pharmacological properties.

3.1. Antipyretic activity³⁰

Crude aqueous extract of leaves of *A. reticulata* has been screened for analgesic activity at dose of 200 mg/kg and 400 mg/kg. Hyperpyrexia was induced by injecting 20% aqueous suspension of Brewer's yeast subcutaneously in rats. Rats showing 0.5 °C–1 °C rise or more in rectal temperature after 18 h of injection were separated and selected for the study. The results produced by the extract were compared to the standard drug, paracetamol at a dose 150 mg/kg of body weight. Overall study showed that extract of leaves of *A. reticulata* has significant antipyretic activity.

3.2. Anthelmintic activity⁸

The anthelmintic activity of leaves of *A. reticulata* was screened using Indian earthworm, *Pherentima posthuma*. Leaves were powdered and extracted using ethanol by cold maceration. Vacuum distillation was used to concentrate extract and 15.83 g yield was obtained. The total ethanolic extract was fractionated using petroleum ether, chloroform, ethyl acetate and ethanol in separating funnel. Fractions were concentrated. 3.39 g, 0.15 g, 0.13 g and 1.51 g

yield was obtained for ether, chloroform, ethyl acetate and ethanol respectively. *Pheretima posthuma* of size 3–5 cm in length and 0.1–0.2 cm in width were considered for the study. Albendazole was used as standard. Ethanol fraction showed less time to produce paralysis which indicates ethanol fraction has more pronounced activity than other fractions.

3.3. Antihyperglycemic activity¹²

The antihyperglycemic effect of methanolic extract of *A. reticulata* L. leaves were investigated using oral glucose tolerance tests in glucose loaded mice. Leaves were powdered and extracted with methanol (1:5 W/v). Male swiss albino mice of weight 18–22 g were used. Mice were divided into different groups each containing six mice. Control group was treated with 1% tween-80 at 10 ml/kg body weight in water whereas standard drug, glibenclamide at 10 mg/kg body weight was used for standard treated group. Other four groups were orally treated with 50, 100, 200 and 400 mg per kg body weight of methanol extract. After one hour of administration all mice were treated with 2 g glucose/kg of body weight and blood samples were collected. The dose-dependent and statistically significant antihyperglycemic activity was observed. Serum glucose level was reduced to 34.8, 37.0, 49.6 and 56.1% at the dose 50, 100, 200 and 400 mg/kg body weight. Glucose oxidase method was used to estimate serum glucose levels. Overall results showed that leaves of *A. reticulata* possess significant and potent antihyperglycemic activity.

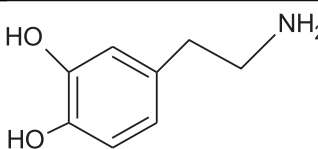
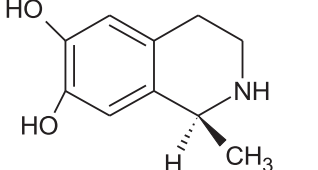
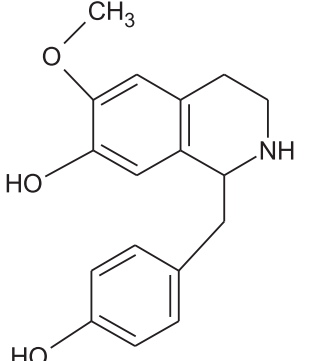
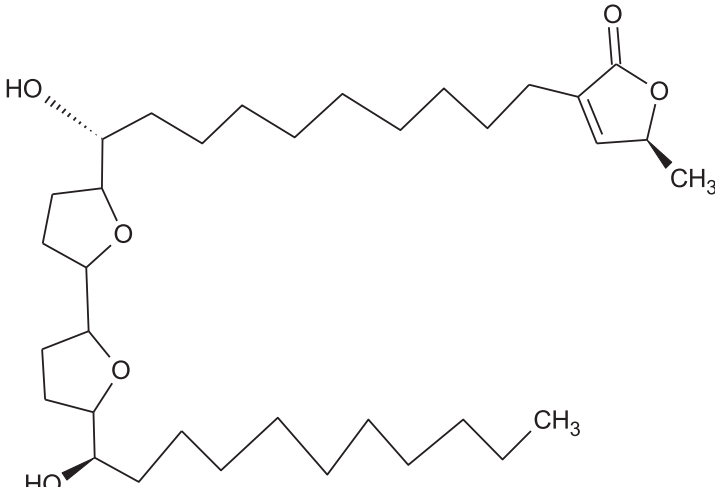
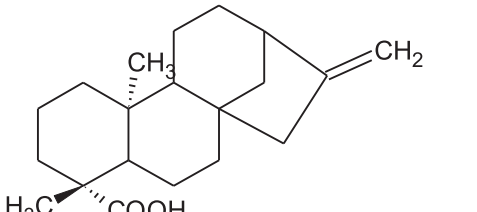
3.4. Antiulcer activity³¹

Antiulcer potential of aqueous extract of *A. reticulata* leaves was investigated using ethanol and indomethacin induced ulcer model in rats. Extract was prepared by Soxhlet extraction method and concentrated in vacuum. Rats were divided into four groups each containing six rats as vehicle treated group, famotidine (3 mg/kg) as reference drug treated group, and two extract (100 mg/kg and 200 mg/kg) treated groups respectively. Extract was administered to fasted rats and after 30 min ulcer was induced using 50% alcohol. Indomethacin (10 mg/kg, p.o.) was used to induce ulcer in another group. All treated rats were sacrificed after 1 h and ulcer index, acid volume, pH and total acidity were determined. Significant dose dependent reduction in ulcer index was observed in rats treated with extract and reference standard drug, famotidine. The extract

Table 2
Phytochemicals of *Annona reticulata* Linn. plant.

Plant part	Phytochemicals	References
Leaf	Dopamine, Salsolinol, Coclaurine, Sesquiterpenes mainly Spathenelol, Muurolene, Copaene, Eudesmol, Acetogenin – Squamone, Solamin, Annonomicin, Rolliniastatin 2, Anoreticu-9-one. Triterpenoid – annonaretin A	8,13,16–18
Bark	Monotetrahydrofuron acetogenins, Reticulatacin, Diterpenes: (–) kau-M-en-19-oic acid and methyl 1β, 17-dihydro-(–)-kauran-19-oate, Alkaloids: Liriodenine, Copaene, Patchoulane and 1H-cycloprop (e) azulene, (–)Kau-16-en-19-oic acid, Bistetrahydrofuron acetogenin, Bullatacin.	7,19,20
Stem bark	Dopamine, Salsolinol, Coclaurine, Diterpenes (–)-kaur-16-en-19-oic acid, 16-α-hydroxy-(–)-kauran-19-oic acid, Methyl-17-hydroxy-16-β-(–)-kauran-19-oate, Reticullacinone, Rolliniastatin-2 (=bulatacin = annonin-VI), Molvizarin.	8,13,21
Root	Aporphine alkaloids Liriodenine, Norushinsunine, Reticuline, Acetogenin neoannonin, Sesquiterpenes mainly Spathenelol, Muurolene, Copaene, Eudesmol.	13,22
Root bark	Anonaine, Michelalbaine, Oxoushinsunine, Reticuline, Unknown phenolic comp.	8
Seed	Series of N-fatty acyl tryptamine where acyl portion ranged from hexadecanoyl to hexacosanoyl. Cytotoxic acetogenins as Squamocin, cis-/trans-isomurisolenin, Anoreticu, Anoreticu-9-one, Bullatacin, cis-/trans-bullatacinone, cis-/trans-murisalinone, Solamin, Annonomicin, Rolliniastatin-1, 2 squamone and isoannonareticin. Volatile oil constituents like α-pinene, β-pinene, Myrcene, Limonene, Terpinen-4-ol, and Germacrene D. Cycloreticulic A, Cycloreticulic B, Acetogenins mainly cis and transisomurisolenin, Anoreticu, Bullatacin, Squamosine and Rolliniastatin. Aminoacyl triesters of Squamocin 1, N-fatty acyl tryptamines. Annonaceous acetogenins (polykelides): Annonaretic, 2,-4-cis-isoannonaretic, 2, 4-trans-isoannonaretic, Solamin, Murisolin, Reticullacinone, Anoreticu, Annonomicin, Sitosterol, Daucosterol, Sucrose, Palmitic acid and Stearic acid. Annonaceous acetogenin: 2, 4-cis-isoan-nonaretic.	8,13,15,23–25
Fruit	Pinene, Myrcene, Limonene, Terpinen-4-ol, Germacrene D	12

Table 3
 Phytochemical structures of *Annona reticulata* Linn.

Name of phytochemical	Structure	References
Dopamine		26
Salsolinol		26
Coclaurine		
Acetogenin		27
Diterpenes (–)-kau-16-en-19-oic acid		

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Table 3 (continued)

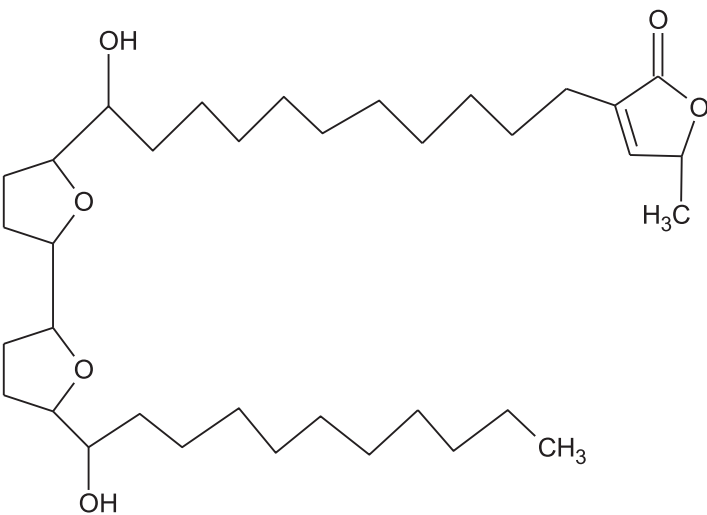
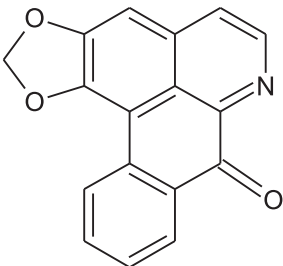
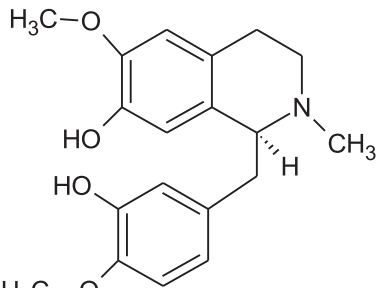
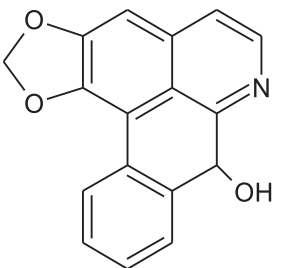
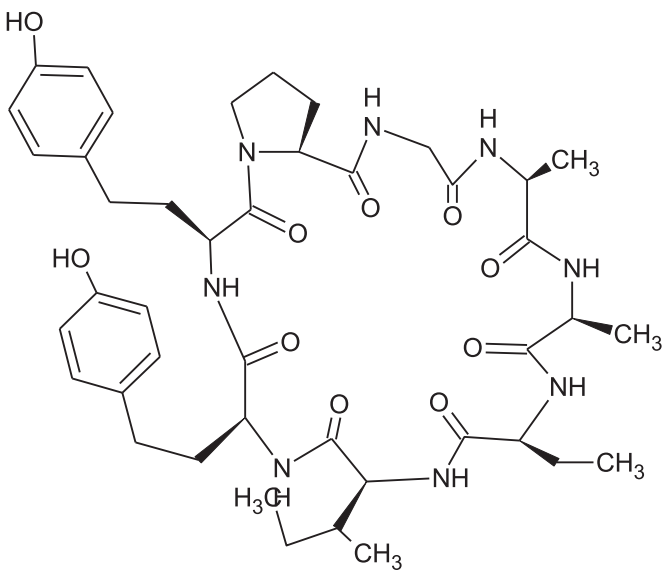
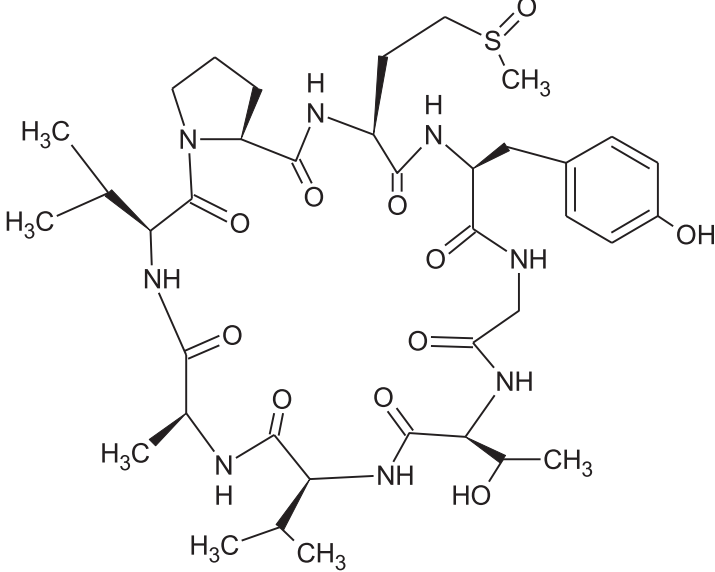
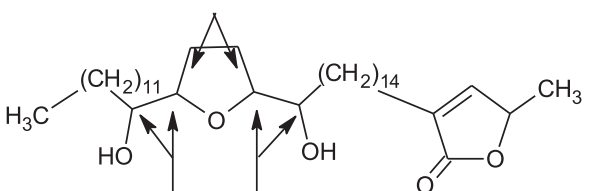
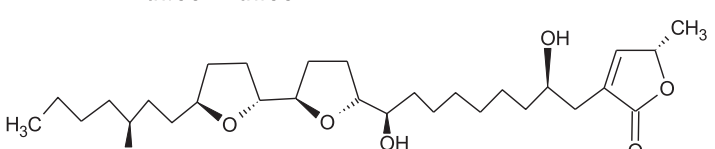
Name of phytochemical	Structure	References
Acetogenin neoannonin	 <p>The structure of Acetogenin neoannonin consists of two tetrahydrofuran rings connected by a long, zigzag aliphatic chain. The upper tetrahydrofuran ring has a hydroxyl group (-OH) attached to its 2-position. The lower tetrahydrofuran ring has a methyl group (-CH₃) attached to its 2-position. The aliphatic chain extends from the 5-position of the upper ring to the 5-position of the lower ring, ending in a methyl group (-CH₃). The chain is substituted with a 2-methyl-2-butenoate group at the 1-position of the upper ring.</p>	22
Liriodenine	 <p>The structure of Liriodenine is a complex polycyclic system. It features a central benzene ring fused to a pyridine ring and a quinoline ring. A benzofuran moiety is attached to the benzene ring, and a phenyl ring is attached to the quinoline ring. A carbonyl group (-C=O) is also present on the quinoline ring.</p>	22
Reticuline	 <p>The structure of Reticuline is a complex polycyclic system. It features a central benzene ring fused to a piperidine ring and a quinoline ring. A methoxy group (-OCH₃) is attached to the benzene ring, and a hydroxyl group (-OH) is attached to the piperidine ring. A methyl group (-CH₃) is attached to the nitrogen atom of the piperidine ring. A phenyl ring is attached to the quinoline ring.</p>	22
Norushinsunine	 <p>The structure of Norushinsunine is a complex polycyclic system. It features a central benzene ring fused to a pyridine ring and a quinoline ring. A benzofuran moiety is attached to the benzene ring, and a hydroxyl group (-OH) is attached to the quinoline ring.</p>	22

Table 3 (continued)

Name of phytochemical	Structure	References
Cycloreticuliculin-A		15
Cycloreticuliculin-B		15
Reticulatacin	<p data-bbox="751 1498 810 1523"><i>trans</i></p>  <p data-bbox="703 1740 858 1768"><i>threo threo</i></p>	7
Bullatacin		

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Table 3 (continued)

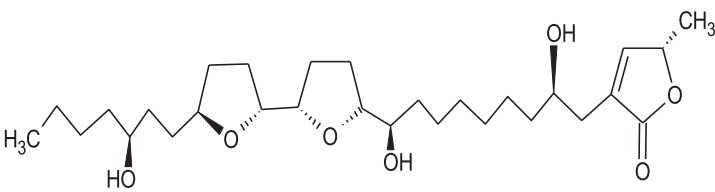
Name of phytochemical	Structure	References
Rolliniastatin		

Table 4
Plant part, extraction procedure and method/model used for screening of pharmacological activities and their results obtained.

Plant part	Extraction procedure	Activity	Screening method/model	Results	References
Leaves	–	Antipyretic	Injecting aqueous suspension of Brewer's yeast.	Proved	30
	Cold maceration	Anthelmintic	Using Indian earthworm (<i>Pherentima posthuma</i>)	Proved	8
	–	Antihyperglycemic	Oral glucose tolerance tests in glucose loaded mice.	Proved	12
	Soxhlet	Antiulcer	Ethanol and indomethacin induced ulcer model.	Proved	31
	Soxhlet	In vitro cytotoxic	Caco-2, Hep G2, HEK cell lines.	Proved	32
	–	Recombinant caspase inhibitory activity	Caspase inhibitory assay using Caspase-6, Caspase-9, Caspase-3	Proved Failed	
Bark	–	Antinociceptive	Acetic acid induced gastric pain.	Proved	16
	Soxhlet	Analgesic and CNS depressant	Analgesic activity by Hot plate method. CNS depressant activity by Photoactometer.	Proved	13
	Maceration	Analgesic and anti-inflammatory	Analgesic activity by hot plate method and acetic acid-induced writhing. Anti-inflammatory activity by carrageenan induced rat paw oedema.	Proved	19,20
Root	Soxhlet	Antiproliferative	A-549, K-562, HeLa, MDA-MB cancer cell lines and normal cell lines (Vero cells) by MTT assay	Proved	22,33
	Soxhlet	Anticancer	In vivo anticancer activity against melanoma cells in mice and in vitro inhibitory activity on MDA-MB-435 human melanoma cells by the MTT colorimetric assay	Proved	28
	Soxhlet	Antioxidant and antimicrobial	Antioxidant activity by DPPH free radical scavenging assay and hydroxyl (H ₂ O ₂) radical scavenging activity assay. Antibacterial activity by agar cup method and antifungal activity by poison plate method		34
Stem bark	Refluxed with distilled water	Analgesic and Anti-inflammatory	Analgesic activity by tail flick test, tail immersion test, writhing test. Anti-inflammatory by histamine and carrageenan induced paw oedema	Proved	35
Seed	Soxhlet	Wound healing and antimarkng activity	Creating wounds in paravertebral area	Proved	36

and famotidine also showed significant decrease in acid volume and contents. The extract showed significant improvement in glutathione and pH level as compared to vehicle treated rats. The study suggested that the significant antiulcer activity of aqueous extract of *A. reticulata* leaves may be due to cytoprotective, anti-secretory and antioxidant potential of phytoconstituents present in the extract.

3.5. In vitro cytotoxic and recombinant caspase inhibitory activity³²

Cell lines were used to investigate in vitro cytotoxic activity of methanol extract of *A. reticulata* leaves. Caspase inhibitory assay was performed using recombinant caspase inhibitory initiator capsase (Caspase-9) and executioner capsase (Caspase-3 and 6). Leaves were powdered by using mechanical grinder and dried under shade. Petroleum ether (60–80 °C), methanol and chloroform extract was obtained by Soxhlet extraction process. Cytotoxic property of extract was examined against Caco-2 (human colorectal adenocarcinoma), Hep G2 (human hepatocellular carcinoma) and HEK (human kidney carcinoma) cell lines. Doxorubicin 10 µM was used as a standard and maintenance media treatment was considered as a control. The extract showed dose dependent cytotoxic activity against Caco-2 and Hep G2. At concentration 5 µg/

ml and 10 µg/ml extract showed 56.02 and 66.64% inhibition against caspase-6 and 76.35 and 87.03% inhibition against caspase-9 respectively. Such effects were not observed against caspase-3. The study concluded that the extract is effective against colon and liver cancer and might be effective in the treatment of degenerative disorders.

3.6. Antinociceptive activity¹⁶

Acetic acid induced gastric pain model was used to screen antinociceptive potential of methanolic extract of *A. reticulata* leaves using in Swiss albino mice. The air-dried leaves were powdered and extracted with methanol (1:5 w/v) for 48 h. Swiss albino male mice of 20–25 g weight were selected and divided into different groups each containing six mice. Control group was treated with vehicle whereas standard drug treated group received aspirin at doses of 200 and 400 mg per kg body weight. Mice of remaining groups were treated with extract at the doses 50, 100, 200 and 400 mg per kg body weight. After 60 min of extract treatment the mice were intraperitoneally injected with 1% acetic acid at a dose of 10 ml per kg body weight to induce writhings. Number of writhings induced by acetic acid was counted for 10 min. The extract treated mice at 50, 100, 200 and 400 mg per kg body

weight showed reduced number of writhings by 47.0, 55.1, 67.3 and 69.4% respectively. The extract exhibited significant dose-dependent effect which indicates presence of phytoconstituents in the leaves having potent antinociceptive activity.

3.7. Analgesic and CNS depressant¹³

Petroleum ether, ethyl acetate and methanol extracts of *A. reticulata* bark showed significant analgesic activity. Extracts were prepared by successive solvent extraction process. The percentage yields of extracts obtained were petroleum ether 2.3% w/w, ethyl acetate 5.58% w/w and methanolic 13.13% w/w. Analgesic activity was carried out by the hot-plate method whereas central nervous system depressant activity was assessed using locomotor activity assay and pentobarbitone sleeping time test. For both the studies swiss albino mice of either sex weighing 20–25 g were selected. Extract at a dose of 100 mg/kg was used for both studies. Pentazocin lactate injection 20 mg/kg intraperitoneally used as standard for analgesic activity. Locomotor activity was evaluated using actophotometer where diazepam 2 mg/kg intraperitoneally was used as standard. Sleep was induced by pentobarbitone sodium at 40 mg/kg in the mice and the time interval between losing and regaining of righting reflex was measured. The phytochemical study showed presence of terpenes and steroids in petroleum ether extract, alkaloids and flavonoids in ethyl acetate extract while tannins, flavonoids and glycosides were observed in methanol extract. The petroleum ether extract treated mice showed highest increase in reaction time and significant reduction in the locomotor activity. Also petroleum ether extract potentiated pentobarbitone sodium induced sleeping time. Significant central analgesic activity was exhibited by the extracts in hot plate method. All extracts exhibited mild to moderate central nervous system depressant activity which might be due to increased concentration of GABA in brain.

3.8. Analgesic and anti-inflammatory^{19,20}

The sesquiterpene fraction of *A. reticulata* bark was screened for central as well as peripheral analgesic and anti-inflammatory activities. Study was carried out using sesquiterpene fraction obtained from unsaponified petroleum ether extract which contains mixture of three major sesquiterpenes. The percentage of sesquiterpene present in the fraction was 71.66%. Sesquiterpene fraction was studied by GC/MS which showed presence of copaene (35.40%), patchoulane (13.49%) and 1H-cycloprop(e)azulene (22.77%). Eddy's hot plate test and acetic acid-induced writhing method was used to screen central as well as peripheral analgesic activity whereas carrageenan-induced paw oedema method was used to evaluate anti-inflammatory activities. Significant central as well as peripheral analgesic activity was observed for sesquiterpene fraction at doses 12.5 and 25 mg/kg and for unsaponified petroleum ether extract at a dose of 50 mg/kg. Pentazocin and aspirin were used as standard for analgesic activity. The significant dose-dependent inhibition of carrageenan-induced paw oedema was found in the groups treated with unsaponified petroleum ether extract and sesquiterpene fraction. The effects shown by extract and fraction were comparable with that of standard drug, aspirin.

3.9. Antiproliferative activity^{22,33}

Antiproliferative potential of aporphine alkaloids liriodenine, norushinsunine, reticuline and one acetogenin neoannonin isolated from the roots of *A. reticulata* has been investigated against A-549, K-562, HeLa, MDA-MB cancer cell lines and normal cell line (*Vero* cells) by MTT assay. The compounds were structurally identified by

¹HNMR, ¹³CNMR and mass spectroscopic methods. Aporphine alkaloids were obtained from ethanolic extract of roots by column chromatography (neutral alumina) using solvent system toluene: ethyl acetate: diethyl amine (70:20:10). Similarly acetogenin was isolated by partitioning of ethanol extract with ethyl acetate and column chromatography using n-hexane, ethyl acetate and methanol as solvent system. Activity was carried out using 100 µl of isolated compounds, each at the concentration 5, 10 and 20 µg respectively. Untreated micro titre plates of cell lines containing DMSO (0.3 % v/v in water) was considered as proliferative control. Neoannonin showed potent cytotoxicity (IC₅₀ value from 5.8 to 6.9 µg/ml) against all cancer cell lines whereas norushinsunine exhibited moderate cytotoxicity (IC₅₀ value from 7.4 to 8.8 µg/ml). Test compound showed less cytotoxicity (IC₅₀ value from 13.8 to 26.0 µg/ml) on normal cell line (*Vero* cells) as compared to cancer cell lines. The study concluded that prominent cytotoxicity of isolated aporphine alkaloids is because of isoquinoline moiety, presence of hydroxyl group and apoptosis inducing ability of these isolated compounds in cancer cell lines.

3.10. Wound healing and antimarking activity³⁶

Ethanolic seed extract of *A. reticulata* was investigated for antimarking and wound healing potential in combination with neem oil, honey and ghee. Seeds were dried, powdered and extracted in soxhlet extractor using methanol as a solvent. Ointment was formulated containing *A. reticulata* seed extract (10 g), grape seed extract (3 g), ghee (4 g), honey (2 g) and neem oil (2 g). For the study 24 male Wistar Albino rats weighing 150–200 gm were used. Rats were anesthetized by intraperitoneal injection of ketamine (50 mg/kg) and back surface was shaved to create wounds. Paravertebral area was selected and wounds of thickness 500 mm² were created in the rats. Rats were divided in to control group treated with simple ointment B.P, standard drug treated group, 5% w/w test ointment treated group and 10% w/w test ointment treated group. All rats were treated from day 0 to day 27 once in a day. Wound area was observed for the progress in the wound healing and percentage reduction in original wound size was determined by measuring the wound area on graph paper.

Also one incision of thickness 6 cm was made on paravertebral area and stitched with nylon thread. No antimicrobial drugs were used during this period. Rats were treated with test formulation (5% w/w and 10% w/w ointment), standard drug (nitrofurazone ointment) and simple ointment B.P. for twice daily, until complete recovery is obtained. On day 8 sutures were removed. The complete healing strength or tensile strength of healing of incision wound was measured on day 10. The test formulation treated rats showed faster wound closure and wound contraction as compared to other rats. Significant increase in tensile strength was observed in formulation treated rats. The tensile strengths for 5% w/w ointment treated group and 10% w/w ointment treated group were 579 ± 22.7 and 673 ± 15.9 respectively which were comparable with that of Standard ointment treated group (659 ± 27.1). Study suggested that the test formulation is equally effective as that of standard drug formulation.

3.11. Antioxidant and antimicrobial activity³⁴

The root extract of *A. reticulata* was investigated for antioxidant and antimicrobial potential. DPPH free radical scavenging and hydrogen peroxide assay were employed for antioxidant screening. Antibacterial and antifungal study was performed using agar cup method and poison plate method. Roots were dried, powdered and extracted by Soxhlet apparatus. Antioxidant activity was determined by DPPH free radical scavenging assay and hydrogen

peroxide (H₂O₂) assay at 20, 40, 60, 80 and 100 µg/ml concentrations of extract and absorbance was measured at 517 nm and 230 nm respectively. Antibacterial activity was carried out against three gram negative (*Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*) and gram positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*) strains of bacteria using nutrient agar media. The antifungal activity of the extract was carried out against *Aspergillus niger*, *Penicillium chrysogenum*, *Fusarium moneliforme*, *Aspergillus flavus*, *Trichoderma viride*, and *Candida albicans* using potato dextrose agar media. For antibacterial study 100 ml of DMSO was used as negative control and antibiotic disk, penicillin as standard reference antibiotic (positive control). Zone of inhibition of extract sample was measured by antibiotic scale and compared with standard. Similarly for antifungal study DMSO was employed as negative control and 1% griseofulvin as positive control. Increase or decrease in growth of fungi was considered to evaluate antifungal activity. Extract exhibited dose dependent scavenging as that of standard, ascorbic acid. Extract was found to have pronounced ability to inhibit *B. cereus* and also exhibited significant activity against all strains of bacteria. Predominant antifungal activity was showed against *T. viride*, and *C. albicans* fungi. The results obtained from this study revealed that root extract of *A. reticulata* has remarkable antimicrobial activity.

4. Conclusion

Over the last several years plants have been recognized as an imperative source of medicines. Exploration of phytochemicals derived from different plant parts as potential bioactive agent has become a fascinating strategy. Present study has been conducted in attempts to focus multiple aspects of *A. reticulata* Linn. (牛心果 niú xīn guǒ; Bullock's heart). Traditionally it was used to treat several diseases. It contains wide range of secondary metabolites and minerals which could be responsible for different therapeutic activity. Acetogenins is one of them. Phytopharmacological properties indicated that *A. reticulata* Linn. had vital ability to manage different disease conditions. The present study ascertains the value of *A. reticulata* Linn. plant which could be of considerable interest in the development of plant based new drugs. This review also explores entire information of *A. reticulata* Linn. which might be helpful to researcher and scientists working on plant based bioactive agents.

Conflict of interest statement

The authors declare no conflict of interest.

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