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Studies on Morphological Features and Biological Activities of the Genus Annona of Ethiopia, N. E. Africa with a Special Emphasis on Graviola: A Review

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Abstract: The Mother Nature provided us with a huge number of flora and fauna. These flora and fauna show so many characteristic features such as their medicinal value. Medicinal plants are the oldest known healthcare agents. Their importance is still growing although it varies depending on the phyletic, medical and historical background of each place. Medicinal plants are important for pharmacological research and drug development too, not only when plant constituents are used directly as remedial agents, but also when used as basic materials for the synthesis of drugs or as a models for pharmacologically active compounds. Some of the natural medicinal plants are very common, that we use in our daily life without knowing their medicinal importance. Worldwide, Annonaceae family is one of the large families showing many characteristic features such as antitumor, antioxidant, antimicrobial and many other bio-activities. The current review paper mainly focused on various biological activities of different species of the genus Annona of Ethiopia, North East Africa with a special emphasis on graviola.

Keywords: Annona, Graviola, biological activities, Ethiopia and North East Africa.

1. Introduction

The Annonaceae is a very large family of plants comprising about 120 genera and more than 2000 species. Basing on morphology and habitat, it is a very homogenous family producing edible fruits and oils. The Annonaceae family seems to be one of the minutest chemically as well as pharmacologically known families compared with its large size. The edible fruits and oils from seed of this plant have immense economic importance. Seed oils of certain Annonaceae plants are used in soap production and as edible oils. Flowers of some Annonaceae plants are used in perfumery and many members of this family are used in folk medicine to treat various types of tumors and cancers. The earlier studies on Annona species showed that this family is a potent source of a wide variety of secondary metabolites belonging to several categories (Pardhasaradhi, B.V et al., 2005). The most predominant species of the genus Annona includes Annona squamosa, Annona muricata L. (Graviola), Annona reticulata, Annona crassiflora, Annona cherimola, Annona glabra and Annona sylvatica. Whereas, in Ethiopia, North East Africa the commonly found Species of Annona are A. squamosa L., A. senegalensis Pers., A. reticulata L., A. cherimola Mill., and A. muricata. A. squamosa is a cultivated species of Annona which was collected deficiently. It"s quite possible that, A. muricata and A. glabra could also be grown in climatic conditions of Ethiopia. These species most frequently found being sold under the name of GISHTA (Amharic).

Annona L. In general

Trees, shrubs, subshrubs or small tree up to 1.5-10 m tall with mostly ovate or elliptic leaves and simple or stellate

hairs or glabrous. Flowers usually hermaphrodite, solitary, fasciculate or in few-flowered cymes, terminal, leaf-opposed or extra-axillary, pedicellate; buds globose, ovoid or elongate, 3-angled; bracteoles 0-2, persistent. Sepals-3, valvate, free, smaller than the petals. Petals usually 6 in 1-2 whorls, both valvate or the inner one imbricate, subequal or the inner ones reduced or absent, free or united at the base, thick and leathery, mostly suberect or connivent. Stamens are numerous, linear or linear-clavate; filaments short; anthercells sometimes unequal; connective extension obliquely capitate, dilated or ±apiculate. Carpels numerous, ± united, cylindrical, with 1 erect basal ovule; styles clavate; stigmas rough. Fruit is pedicellate, syncarpous, fleshy, indehiscent, ovoid-globose or cylindrical. Fruits 6-25 cm long (cultivated or naturalized plants grown mostly for their edible fruits); Fruits 2.5-4.5cm long (wild plants); Seeds are numerous, irregularly arranged; aril terminal, compressed, annular or grooved and ridged so as to appear as if made of fine thread-like fibres. Annona is a large genus of 110 species mostly available in tropical America, but a few species found in tropical Africa. Several species are widely cultivated for their edible fruits.

Annona squamosa L.

Annona squamosa L., known as custard apple, is often found in deciduous forests, also cultivated in wild in various countries. Literatures of many research works prove that every parts of A. squamosa possess medicinal property (Kirtikar and Basu, 1993). The species of Annona squamosa is a small evergreen tree reaching 6-8 meters (20-26 ft) tall; it is native of West Indies. It was a much branched shrub or small tree and grows up to 3-6m tall. Leaf blades are oblong, elliptic-oblong, oblong-lanceolate or narrowly elliptic, 7-17 x

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3-5.5 cm, obtuse or sub acute at the apex, rounded or cuneate at the base, glabrous above, slightly pubescent or glabrescent beneath; petiole 0.4-1 cm long. Outer petals green, purple at the base, oblong, 16-25 x 6-7.5 mm, keeled inside; inner petals reduced to minute scales or absent. Fruits are globose or broadly conical, 5-10 cm long and wide, formed of loosely cohering or almost free carpels, the rounded ends projecting giving a tuberculate surface; surface greenish-yellow with a powdery bloom; pulp white tinged yellow, sweet. Seeds are shiny and dark brown in colour. Initially a native of the West Indies and South America, but now extensively cultivated throughout the tropics at low to medium altitudes; also naturalized near the coast in East Africa.

Annona squamosa is extensively used as traditional medicine in various cultures. The plant is traditionally used for treating epilepsy, dysentery, cardiac problem, worm infection, constipation, hemorrhage, antibacterial infection, dysuria, fever, and ulcer. It also possesses antifertility, antitumor and abortifacient properties (Asholkar, L.V et al., 1992). Roots are employed internally in depression of spirits and spinal diseases. Bark is known to be an awful astringent. In herbal medicine, fruits are considered as a good tonic, enrich blood, used as expectorant, hikes muscular strength, cooling, decreases burning sensation and tendency to biliousness, sedative to heart and alleviate vomiting (Patel and Kumar, 2008). The plant possesses potent bioactive ingredients in all of its parts. Acetogenins-a class of natural compounds, isolated from members of Annonaceae have potent antineoplastic, parasiticidal, pesticidal and anti-microbial activities (Oberlies, N.H et al., 1997). Acetogenins derived from long chain fatty acids are known to be powerful inhibitors of ubiquinone oxidoreductase in mammalian and insect mitochondrial electron transport systems (Degli, E.H et al., 1994). Two Acetogenins, squamocin and squamostatin are isolated from A. squamosa seeds have shown cytotoxic activity (Fujimoto, Y et al., 1994). Squamocin inhibited proliferation of HL-60 cells and induced apoptosis by the activation of caspase-3. Ascimicin, another acetogenin also exhibited cytotoxic activity against different tumour cells (Ruppercht, J.K et al., 1982). Squamocin-O1 and squamocin-O2 were isolated from the methanolic extracts of the seeds of A.squamosa (Araya, H et al., 2002). Extracts of A. squamosa contains ribosome-inactivating protein (RIP), which acts as an immune-toxin is found to be effective (Sismindari et al., 1998). Leaf extracts of A. squamosa exhibited activity against different tested bacteria such as Bacillus megaterium, B. subtilis, Escherichia coli, Enterobacter faecalis, Proteus vulgaris, Pseudomonas aeruginosa and Staphylococus aureus. (Harikrishna Ramaprasad, 2004). Conventional aqueous extracts of leaves of Annona squamosa possessed antimicrobial activity against selected microbial strains such as B. megaterium, B. subtilis, E. coli, E. faecalis, P. vulgaris, P. aeruginosa, S. aureus, A. niger, A. fumigatus and C. albicans (Harikrishna Ramaprasad S, 2007 and 2012).

Annona senegalensis Pers.

A. senegalensis Pers (1806) is also called as A. senegalensis Roussillon (1969) or A. chrysophylla Boj. (1843) belongs to the family Annonaceae; other common/indigenous names are Af: Gishta, Am: Giishta, Yebere lib, Eng: Wild custard apple Or: Komate, and Wt: Eta. The species is widespread in

tropical Africa, from Senegal to South Africa and Madagascar in semi-arid to subhumid regions. In Ethiopia, it is found in Combretum woodland and wooded grassland in the Moist and Wet Kolla agroclimatic zones of Wolega, Gojam, Ilubabor, Kefa, Gamo Gofa and Bale regions, 400–1,600 m. various parts of this plant possess several uses such as Firewood, timber, poles, tool handles, food (fruit), medicine (roots, gum, fruit), fodder (leaves, fruit), ornamental, windbreak, fibre (bark), yellow-brown dye (bark) (Edwards *et al.*, 2000).

It is a shrub-like tree, 2–10 m. Bark is grey and smooth, thick and folded when old, young stems hairy and orange red. Leaves are broadly oval, 15 x 10 cm, blue green, hairy at the bottom, fragrant when crushed, on a short thick stalk. Shrub or small trees ranges (0.8-)1.5-10 m tall; bark is grey brown, often rough and corrugated; young stems are mostly redbrown velvety to greyish tomentose, at length glabrous; leaf bases and scars are mostly prominent, often with raised lines decurrent beneath them. Leaves are glabrescent or ± hairy above, softly velvety tomentose beneath; consists outer 3 petals linear-oblong, inner scale-like. Fruits are edible appears as smooth to tuberculate. Leaf blades oblong to ovate or elliptic, (4-)6-18.5(-34) x 2.5-11.5 (-26) cm, obtuse or apiculate to rounded or slightly emarginate at the apex, cuneate or truncate to cordate at the base, papery to leathery, glabrous or sparsely pubescent above save for hairy midrib, glabrescent to thickly red-brown velvety beneath; petiole (0.5-)1-2(-2.5) cm long, glabrous to velvety tomentose.

Flowers are solitary or in bunches (fascicles) of 2–4, extra-axillary; pedicels 1-2.7cm long, tomentose; bracteoles 1-2 small or wanting; small flowers hanging down below twigs, yellowish with petals and sepals in threes; petals thick and hard, many stamens. Sepals are ovate-triangular, 3-4 x 4-5 mm, obtuse to acute, pubescent or tomentose outside, glabrous inside. Petals greenish outside, cream or yellowish or pinkish within, sometimes blotched with purple, or crimson-blotched at base inside, fleshy; outer broadly ovate, 8-12(-15) x 9-11 mm, obtuse, adpressed grayish or yellowish pubescent outside, glabrous or minutely papillose within; inner narrowly oblong, 3-sided, 8-10 x 2.5-3 mm. Stamens are linear, 1.7-2.5 mm long; connective extension obliquely capitate, minutely papillose. Carpels are cylindric, 1-1.5 mm long and glabrescent.

Fruiting stalks 1.5-5 cm long; fruits yellow or orange, ovoid or globose, 2.5-5 x 2.5-4 cm; areoles slightly raised, very obtuse, glabrescent. Fruit is round, 2–7 cm, smooth, divisioned and pinkish green in color during un-ripened condition. When it turns into orange yellow and smelling like

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Figure 1: Fruits of different species of the Genus Annona

pineapple, the pulp is sweet and edible. Fruit covered with soft spines about 6 mm or so long or with conical protuberances or Fruit smooth or with areoles raised and rounded but without distinct spines.

Seeds are numerous in number and appear as orange-brown, cylindric, oblong, compressed, $8-14 \times 4-6(-8.5)$ mm, 3.5-5 mm thick; aril of fine threadlike fibres, ± 2 mm long. Generally this plant is propagated in different ways like seedlings (sow seeds in pots), wildings, and root suckers from exposed or injured roots. The number of seeds per kg of

fruits is about 2,500–4,000 depending on their size. Seeds are susceptible to insect damage and lose viability within 6 months. During storage of seeds add ash to reduce insect damage.

Traditionally, parts of this plant are used as medicines like, roots to treat cold; fruit against diarrhoea, dysentery and vomiting. Gum from the bark is used to seal small cuts and wounds, and even to plug leaking pots (Azene Bekele-Tesemma, 2007).

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In general, *A. senegalensis* subsp. *Senegalensis*, the lower surface of adult leaves are sparsely to very densely pubescent with long hairs, but mostly red-brown velvety in those growing in eastern Africa. Usually they grow at wooded grassland on greyish clay, mostly in combretaceous woodland, ranges 500-1500m. Widespread in tropical Africa from Senegal to Cameroon, Zaire, Sudan and Kenya, extending to Zimbabwe and northern parts of South Africa; also in Madagascar, Comoros and Cape Verde; other subspecies extend to Angola.

Annona reticulata L.,

Annona reticulata L. is referred as bullock's heart, which is a slender semi-deciduous tree 6-7.5 m tall. Leaf-blades are oblong-lanceolate to lanceolate, 10-15 cm long, acute, glabrescent. Flowers are borne in small clusters on the new branchlets. Corolla white, the three outer petals linear oblong, \pm 2.5 cm long, the inner small, ovate, scale-like. Fruit having a smooth skin that becomes dull red when ripe,

conical, ovoid or heart-shaped, up to 12.5cm long, smoothsurface divided by impressed lines to form a reticulation, but areoles not much raised, loosely cohering fruiting carpels, which have markedly projecting rounded ends, so that fruit surface is covered with separate rounded lumps. These are cultivated and also persisting in abandoned gardens; originally from the West Indies (Edwards *et al.*, 2000).

Annona reticulata L. Plant extracts consists a few volatile substances such as alkaloids, diterpenoids, and acetogenins (Ogunwande et al., 2006). Phytochemicals are secondary metabolic compounds found in plants. The important bioactive constituents of Annona reticulata are Alkaloids, Tannins, Flavonoids, Cardiac glycosides, Steroids and Saponins (Nithya, T.G et al., 2011). Solvent extracts of Annona reticulata showed antibacterial activity against gram positive and gram negative bacteria (Jansi Rani, D et al., 2013). The leaves are used as insecticides, anthelmintic, and are also used externally as suppurant. The bark is used as a powerful astringent, antidysentric and vermifuge. Root bark, leaves and stem possess isoquinoline alkaloids (Suresh, H.M et al., 2011). The ethanol extract of roots, shows antiproliferative activity of against cancer cell lines in vitro (Xu L et al., 1992).

Annona cherimola Mill.

A small deciduous tree with the lower surface of the leaves covered in a soft brown tomentum. Leaves \pm glabrous or subglabrous or sparsely hairy; outer 3 petals ovate, inner imbricate, somewhat smaller; fruit \pm smooth, edible with fleshy spines; areoles separated by a marked reticulation of, often raised, ridges but not themselves raised. Fruits are heart-shaped, roundish, green, 8-15 cm in diameter, with fingerprint depressions, with or without fleshy spines. Flesh is very sugary. These species are highly found in Ambo at around 2100-2200m. Native of Andean valleys in Ecuador and Peru, it can grow at higher altitudes than other cultivated *Annona* in the tropics (Edwards *et al.*, 2000).

The plant contains biomolecules like carbohydrates, proteins (amino acids) and various secondary metabolites such as alkaloids, flavonoids, glycosides, saponins, tannins, phenolic

compounds and phytosterols. The anti stress activity of cherimoya is mainly attributed to these constituents with established antioxidant activity (Adarsh Verma, M *et al.*, 2011). The chemical composition of the essential oils of leaves, flowers and fruits of *Annona cherimola* were studied for its anti microbial activity (Maria Yolanda Rios *et al.*, 2003; Rajiv Dahiya, 2007), anti viral activity (Betancur-Galvis, L.A *et al.*, 1999), cytotoxicity (Kim, D.H *et al.*, 2001; Bode, A.M and Dong, Z., 2009; Newmann, D.J and Cragg, G.M. 2007).

Annona muricata L.

A small, much branched, perennial tree, 3-8m tall, with terete having reddish-brown glabrous branchlets. Leaves are biseriate, shortly petiolate, pale green to bright green, oblong-obovate or oblongelliptic, 6-18cm by 3-7cm wide, glossy leaves and laurel-like with acuminate apex and cuneate or acute base. Flowers are regular, green to yellowish green (to yellow on a yellow fruited variety), on short axillary, 1-2 flowered branchlets. Flower has three dark green, ovate deltoid, coriaceous sepals which are pubescent and persistent; six broadly ovate, coriaceous, and usually green to subsequently yellowish-green, tomentose, cordate base petals; Petals all ovate, inner smaller but both whorls valvate; having numerous stamens in rows around the gynoecium; numerous ovary, pubescent with styles shaped like soft prickles. Fruit is oblong or somewhat curved, 15-35 cm by 10-15cm wide, beset with soft spines with a thin, green or yellow or orange, inedible, areoles distinct, coriaceous rind. Seeds are numerous, obovoid and flattened, dark brown to black, glabrous and glossy, embedded in firm, white, fleshy, acid-sweet, juicy pulp(Edwards et al., 2000). It's a member of the family of Custard apple trees called Annonaceae. It is a typical tropical tree with heart shaped edible fruits and widely distributed in most of the tropical countries. Annona muricata produces fruits that are usually called soursop due to its slightly acidic taste when ripe. Trees grew natively in the Caribbean and Central America. It has been used in traditional medicine in many countries since long. Fruit extracts show antiviral, antinociceptive, antiinflammatory and antihyperglycemic properties. It is also effective against multidrug resistant cancer cell line (Nicholas H. Oberlies et al., 1997). The leaves are lanceolate with glossy and dark green in color had been traditionally used to treat headaches, hypertension, cough, asthma and used as antispasmodic, sedative and nervine for heart condition. Previous reports about this plant species from different countries have demonstrated that the leaf, bark, root, stem, and fruit seed extracts of Annona muricata are anti-bacterial. Annona muricata is a potent anticancer plant of Annonaceae family. Leaf extracts of A.muricata posses significant anticancer potentials in human cancerous cells. Plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers (Polterait, O., 1997).

Annona glabra

Annona glabra (pond apple), is a tropical tree growing in wild in the countries like America and Asia. Petals are linear or oblong, the inner ones minute or scale-like. Leaves are velvety (Edwards *et al.*, 2000). It is used in traditional medicine against several human ailments, including cancer. To validate the ethno pharmacological claims against cancer,

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the anti cancer effects of alcoholic extracts prepared from pond apple leaves, pulp and seed, were investigated in human leukemia cell lines (Cochrane, C.B et al., 2008). Annona glabra has been reported to have a group of compounds called Acetogenins. Acetogenins are reported to possess potent cytotoxic activity and are potent inhibitors of mitochondrial complex I in respiratory chain (Gallardo, T et al., 1998). The leaves and bark of Annona glabra are used in Chinese medicine against cancer and other ailments. If the extracts of leaf, pulp and seed are compared, the seed extracts are found to be more potent than other extracts. Li et al isolated a cytotoxic cytopeptides from seeds (Li C.M et al., 1999). Cochrane et al has also reported the anti-cancer activity of Annona glabra extracts in human cancerous cell lines. On a preliminary screening, substantial antimicrobial, and cytotoxic activities were observed for the solvent extracts of the stem bark of Annona glabra (Padmaja, V et al., 1995).

Graviola (Soursop)

Graviola is a small, upright evergreen tree, 5–6m high, with large, glossy, dark green leaves. It produces a large, heart-shaped, edible fruit that is 15–20cm in diameter and green in color, with white flesh inside. The leaves, fruit, seeds, bark, root and stem are used to make varieties of medicines (Edwards *et al.*, 2000).

Synonyms: Annona bonplandiana, A. cearensis, A. cherimola, A, macrocarpa, A. muricata, A.senegalensis, Brazilian cherimoya and Guanabanus muricatus (Edwards et al., 2000).

Graviola is a fruit that generally grows in the rain forests of Africa, South America, and Southeast Asia. It has also other local names like thorny custard apple, cherimoya and brazilian pawpaw. In various languages, this fruit is named differently like: Gishta (Ethiopia), guanabana (Spanish), aluguntugui (Ghana), (French), (Papiamento), adunu (Acholi), guyabano, guanavana, durian benggala, nangka blanda, sirsak, toge-banreisi, nangka londa, zuurzak, Brazilian Paw Paw, Corossol, Corossol Épineux, Corossolier, Durian Benggala, Guanabana, Guanavana, Nangka Blanda, Nangka Londa, Soursop, Sour Sop, Toge-Banreisi. guanaba, épineux, huanaba, toge-banreisi, durian benggala and cachiman épineux. In India, it is less known as shul-ram-fal and hanuman fal, and as mullaatha in Malayalam, whereas in Harar (Ethiopia) in Harari language known for centuries as Amba Shoukh (Thorny Mango or Thorny Fruit). "yebere lib" meaning heart of cow in Jimma, Ethiopia (Blackherbals, 2012).

The bark, leaves, root and fruits of this tree are used for traditional remedies in many countries. Graviola extracts are used for treating virus or parasites infections, rheumatism, arthritis, diarrhea, dysentery, depression and sickness. Despite safety concerns, traditional medicinal practitioners claimed that graviola is used to treat infections caused by bacteria and parasites including leishmaniasis, a disease caused by parasites transmitted through the bite of sand fleas; herpes; coughs; and even cancer. It is also used to cause vomiting and to empty the bowels. Some people use graviola to help them relax. Graviola is applied to the skin in

arthritis. As food, the graviola is mostly used in cooking and preparing beverages.

Many active compounds and chemicals have been found in graviola. Most of the research carried out on graviola in different countries focuses on a novel set of chemicals called Annonaceous acetogenins. Graviola produces these natural compounds in its leaf and stem, bark, fruit and seeds. These chemicals have significant antitumor properties and selective toxicity against various types of cancer cells (without harming healthy cells). Annonaceous acetogenins are only found in the Annonaceae family (to which graviola belongs). These chemicals in general have been documented with antitumorous, antiparasitic, insecticidal, and antimicrobial activities (Keinan, E., 1997-1998). These chemicals were less toxic; on considering the antitumor activity and its relative toxicity, they might be used as a lead to develop a potential anticancer agent (Wang, L. Q., 2002).

Graviola contains many chemicals that may be active against cancer (Nicholas H. Oberlies *et al.*, 1997), as well as disease-causing agents such as bacteria, viruses, and parasites. More evidence is needed to rate the effectiveness of graviola for their uses. There is a controversy regarding safety profiling of Graviola. It can kill nerve cells in the brain and other parts of the body. It may cause movement disorders. It is not advisable to use Graviola during pregnancy and breast feeding. Currently, there is no scientific information regarding the interactions of Graviola species found in Ethiopia and its appropriate dosage. Moreover, intense research works need to be undertaken on the Graviola species of Ethiopia, North East Africa to evaluate its medicinally important bioactive molecules.

2. Conclusions

Plants have been a prime source of highly effective conventional drugs for the treatment of many forms of cancer. Plants provide active constituents which acts directly against various ailments or indirectly by providing leads for the development of potential novel agents. Large number of herbal species has been used traditionally or as folk medicines against cancer. Many of them have been studied scientifically and proved to be a beneficial anti-cancer agent. In medicine, particularly in the field of cancer, the use of herbs is increasingly enhanced especially with the excessive use of synthetic drugs and awareness of their toxicity, which contributed in oncology, leading to a favorable reconsideration of the medicinal practices made from natural herbs. Despite the divergent bioactivities of the plant medicines against various diseases, active components of most plant extracts have not been elucidated thoroughly, due their complex mixtures. The ability of agents to attach to carrier molecules directed to specific tumors, shows highly cytotoxic natural products to the tumors. A better understanding of the characteristics of tumor cells has recently led to the development of more target oriented treatments, and therefore found to be less toxic. In conclusion, the use of naturally occurring molecules in the treatment of cancer and other disease has greatly contributed to the improvement of the therapeutic efficacy of drugs used today. In this review, the biological activities of different species of the genus Annona of Ethiopia, North East Africa

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with their phytochemical and pharmacological profile are presented but, some species of the genus Annona specially graviola of Ethiopia does not have scientifically proven information regarding their safety profile and biological efficacy. These plants exhibit good characteristic against leading life style diseases. This article provides some basic knowledge of medicinal plant species of the genus Annona of Ethiopia, North East Africa.

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