IRA-International Journal of Applied Sciences
ISSN 2455-4499; Vol.04, Issue 03 (2016)
Institute of Research Advances
Pg. no. 471-481
http://research-advances.org/index.php/IRAJAS

Screening of antinutrients in leaves, fruit pulp and seeds of Gishta (Annona spp.) of Ethiopia, North East Africa

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Type of Review: Peer Reviewed.
DOI: http://dx.doi.org/10.21013/jas.v4.n3.p11

How to cite this paper:
Saripalli, H., & Dixit, P. (2016). Screening of antinutrients in leaves, fruit pulp and seeds of Gishta (Annona spp.) of Ethiopia, North East Africa. *IRA-International Journal of Applied Sciences* (ISSN 2455-4499), 4(3), 471-481.
doi:http://dx.doi.org/10.21013/jas.v4.n3.p11

ABSTRACT

Graviola or Gishta (Annona spp. of Ethiopia) are generally useful for human consumption were analysed for the presence of potentially harmful chemicals (antinutrients) and for their toxicity. The purpose of the study was to determine whether the Graviola or Gishta (Annona spp. of Ethiopia) leaves, fruit pulp and seed extracts were safe for human consumption. Chemical analysis showed that none of tested parts contained cyanogenic glycosides, however all the three tested plant materials contained oxalic acid in high concentrations and also contained negligible amounts of phytic acid, saponins and alkaloids. Tested plant samples also found to inhibit trypsin activity. These chemical analyses were carried out in duplicate.

KEY WORDS: Gishta, Graviola, Annona spp., and Antinutrients.

INTRODUCTION

Plants consumed by human beings should be free of toxicity or other adverse effects on their consumer. Common indicators of unsafe plants include their toxic, cytotoxic and mutagenic potential. These harmful properties of plants are produced by the antinutrient chemicals that are produced in certain plants as defense mechanisms. Antinutrient are chemical compounds produced in plants as a defense mechanism that inhibits the action of digestive enzymes in insects that attack plants (Lea and Leegood, 1999). These compounds may decrease the nutritional value of a plant food, usually by making an essential nutrient unavailable or indigestible when consumed by humans or animals. A common strategy of plants is to produce foul-tasting compounds such as many bitter nitrogen containing alkaloids, glucosinolates and cyanogenic glucosides. Plants are capable of bioconverting promutagens into toxic metabolites (Grant, 1998). Experimental studies found many 'edible' and medicinal plants to be mutagenic *in vitro* (Higashimoto *et al*., 1993; Kassie *et al*., 1998). According to statistics of the Poison Unit of the Johannesburg General Hospital, about 6.5% of all poisoning cases are plant related (Van Wyk *et al.*, 2002). Antinutritional components such as oxalic acid, nitrate and erucic acid that are present in many plants (Guil *et al*., 1996; Siddhuraju *et al.*, 2002)) increase the toxicity of edible plants. These substances may express cytotoxic and genotoxic activities upon consumption and show correlation with tumour development (Yen *et al*., 2001). This raises concern about the potential hazards resulting from the long-term use of such plants. Some of the common groups of antinutrients are phytic acid, saponins, alkaloids, cyanogenic gycosides, trypsin inhibitors and oxalic acid.

Graviola is a fruit bearing tree, flowering, broadleaf and evergreen which is native to South America, Central America and the Caribbean. The tree is also found in Colombia, Brazil and even the sub-Saharan countries of Africa. Graviola is adaptive to areas with high humidity and also relatively warm temperatures. The laboratory and field research suggests soursop extract substance has potential to various future applications such as, they have shown cytotoxic and anti-leishmanial also anti-diabetic, anti-cancer, anti-inflammatory and anti-nociceptive activities. Soursop (scientific name - Annona muricata), also called Graviola (Portuguese), is a fruit that generally grows in the rain forests of Africa, South America, and Southeast Asia. It has other names like thorny custard apple, cherimoya and brazilian pawpaw. In various languages, this fruit is referred as: guanabana (Spanish), corossol (French), gishta – Scientific name – *Annona senegelensis* (Ethiopia), aluguntugui (Ghana), sorsaka (Papiamento), adunu (Acholi), guyabano, guanavana, durian benggala, nangka blanda, sirsak, toge-banreisi, nangka londa and zuurzak. In India, it is less known as shul-ram-fal and hanuman fal, and as mullaatha in Malayalam (Harikrishna Ramaprasad Saripalli and Prasanna Kumar Dixit, 2016; 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 infections of viruses or parasites, rheumatism, arthritis, diarrhea, dysentery, depression and sickness. In foods, graviola is used in cooking and beverages. More evidence is needed to rate the effectiveness of graviola for these uses (Harikrishna Ramaprasad Saripalli and Prasanna Kumar Dixit, 2016; Blackherbals, 2012).

The use of Soursop can have certain adverse effects in some people, especially movement disorders and nerve damage results movement disorders similar to Parkinson's disease, which is due to the very high concentration of annonacin. Consumption of Graviola is unsafe. It can kill nerve cells in the brain and other parts of the body. The Memorial Sloan-Kettering Cancer Center cautions, "alkaloids extracted from graviola may cause neuronal dysfunction and degeneration. Graviola also has few other side effects like lowering the blood pressure, so it should not be taken by people with low blood pressure or heart complications. The antimicrobial properties of Soursop can also kill beneficial bacteria on the skin, in the vagina and gut, which can lead to infections in long term use (Cancer Center, 2013).

In 2010 the French food safety agency (Agence française de sécurité sanitaire des aliments) concluded that, it is not possible to confirm the presence of antinutrients (toxic metabolites) in the genus Annona spp. so, calling for further study on potential risks (Avis de, 2010).

This practically indicates that parts of the same plant contain both toxic and well being substances. There is, however, no experimental evidence that it consists of toxic metabolites(Cancer Research UK, 2013). In view of the above controversial claims, it becomes imperative to carry out conclusive biochemical investigations on Graviola or Gishta (Annona spp. of Ethiopia) leaves, fruit pulp and seed extracts to find out the presence of antinutrients/toxic substances.

OBJECTIVE OF THE STUDY

To investigate the presence of antinutrients in leaves, fruit pulp and seed extracts of Graviola or Gishta (Annona spp. of Ethiopia) using chemical analysis methods.

MATERIALS AND METHODS

Collection of Plant Material and Sample preparation

Graviola or Gishta (Annona spp. of Ethiopia) leaves, fruit pulp and seeds were collected from Oromeo region, Ethiopia, in January 2014. The plant was identified, authenticated and supplied by Prof Behailu Etana Disasa of Natural Resource Management, College of Agriculture and Veterinary Medicine, Jimma University, Jimma, Ethiopia. The plant materials were shade-dried and coarsely powdered. The coarse powder was used for antinutrients studies.

Tested material

The dried materials of leaves, fruit pulp and seeds of Graviola or Gishta (Annona spp. of Ethiopia) were tested for antinutrients screening.

Chemicals used

Fe (III)- sulphosalicylate, trichloroacetic acid, FeCl3, NaOH, dH2O, HNO3, potassium thiocyanate (KSCN), 80% methanol, blood agar, 10% acetic acid in ethanol, chloroform, tyrosine, casein bovine substrate, Folin Ciocalteau (FC) reagent, Tyrosine (Sigma, USA), 80:20 HPLC grade methanol: 0.4% acetic acid *v/v,* 0.025 M HCl was used during experimental protocol.

Instruments used

Shaking incubator, centrifuged, Whatman No. 2 filter paper, Spectrophotometer, Whatman no.1, rotary evaporator, sodium picrate paper, isocratic reverse phase high performance liquid chromatography (HPLC), Phenomenex C18 solid-phase extraction cartridge was used during chemical analysis.

Table. 1. Antinutrients (toxic substances) and their analytical approach with references.

S.No	Antinutrient	Method and Technique	Reference
1.	Phytic acid	Spectrophotometric Method	Omotoso (2006); Wheeler and Ferrel (1971)
2.	Saponins	Disc Diffusion Method (Haemolytic Property)	Makkar (2004)
3.	Alkaloids	Precipitation method	Harikrishna Ramaprasad Saripalli (2012); Edeoga *et al*. (2005; 2000); Harborne (1984);
4.	Cyanogenic glycosides	Picrate-Impregnated Paper Technique	Tan and Yeoh (1997).
5.	Trypsin inhibitors	Activity Determined By Fc Reagent	Jayaraman (1981).
6.	Oxalic acid.	Isocratic Reverse Phase HPLC Analysis	Miller and Woodrow (2004).

Antinutrients studies (Toxic metabolites)

The solvent extracts of leaves, fruit pulp and seeds of Graviola or Gishta (Annona spp. of Ethiopia) were assayed for the Antinutrients such as phytic acid, saponins, alkaloids, cyanogenic glycosides, trypsin inhibitors and oxalic acid. In all antinutrient assays, 5g of oven dried, crushed material was used, except for oxalic acid analysis where 0.5g of crushed material was used. Special methods used for each antinutrient are clearly given in the table.1.

RESULTS

Phytic Acid

The concentration of phytic acid was determined spectrophotometrically in three tested plant materials of Gishta as shown in table 2. The concentrations of phytic acid were obtained from 5 g dried leafy material. All the leaf samples contained phytic acid (detection limit 0.025 mg/ml) in this study. This may have been due to the fact that there was a limitation in terms of the detection concentration since concentrations less than mg was not tested. Seed extracts was found to contain the highest concentration of phytates with a concentration of 0.39mg/ml. leaf extracts contained phytates even though it was detected at a low concentration of 0.06mg/ml. whereas fruit pulp extracts had a concentration of 0.17mg/ml.

Saponins

Saponins were detected by the presence of haemolytic zones on blood agar plates using the disc diffusion method (Figure 1). The concentrations of saponins were obtained from 5g dried plant material. A standard curve was drawn and used to determine concentration of saponins in the three types plant material extracts of Gishta. These zones were used to draw up a standard curve and the unknown concentrations of saponins in the samples were determined. Saponins were not found in all the testes samples of Gishta. ranging from 0.59 to 1.7mg/ml. Seeds contained the highest concentration (1.7mg/ml) which is very close to the saponin standard (2mg/ml). Whereas leaves and fruit pulp contained 0.59, 0.97mg/ml respectively.

Figure 1. Haemolysis produced on blood agar plates by saponins from seeds of Gishta (Annona spp. of Ethiopia)

Table. 2. Antinutrients profile in leaves, fruit pulp and seeds of Gishta (Annona spp. of Ethiopia) determined in this study.

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S.No.	Tested material (mg)	Phytates (mg/ml)	Saponins (mg/ml)	Alkaloids (g/5g)	Cyanogenic glycosides	Trypsin inhibitors	Oxalic acid. (mg/ml)
1.	Standard	-	2	1.5	0	1931.2	-
2.	Leaves	0.06	0.59	0.14	0	358.3	44.6
3.	Fruit pulp	0.17	0.97	0.24	0	918.5	68.8
4.	Seeds	0.39	1.7	0.95	0	2872	24.4

Alkaloids

Alkaloid precipitates measured in the test samples of Gishta are shown in table 2. Seeds had fairly high alkaloid concentration 0.95mg/ml. Low levels (less than 0.5g/5g) were found in leaves and fruit pulp. Their concentrations ranged from 0.14 and 0.24/5g respectively.

Cyanogenic Glycosides

In this study, all the test samples of Gishta did not release hydrogen cyanide gas and thus the test samples were negative. Picrate paper remained yellow in all extracts, thus none of the sample contained cyanogenic glycosides as indicated in Figure 2.

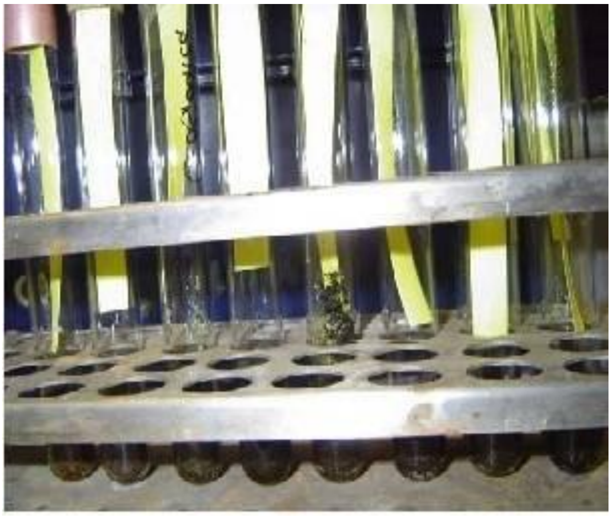

Figure 2. Detection of cyanogenic glycosides in seeds of Gishta (Annona spp. of Ethiopia) using sodium picrate paper

Trypsin Inhibition

Table 2 illustrates the results of the trypsin inhibitors. All the tested plant materials of Gishta had reduced activity of trypsin (inhibition effect) compared to the tyrosine standard. Seeds had high trypsin activity even when compared to the standard, they had activity which indicates the absence of trypsin inhibitors their activity was 2872. Whereas for leaves it was 358.3 and fruit pulp activity was 918.5.

Oxalic Acid

Oxalic acid was quantified using the HPLC. The concentrations of oxalic acid were obtained from 0.5g dried plant material. All the samples were positive for this antinutrient with fruit pulp having the highest concentration followed by leaves and seeds with concentrations ranging from 68.8, 44.6 to 24.4mg/ml respectively (Table 2).

DISCUSSION

There are more than 900 different phytochemicals that have been identified as components of food. It is estimated that there may be more than 100 different phytochemicals in one vegetable (Fowler, 2002). Some of these phytochemicals are antinutrients, and are said to reduce the bioavailability of essential nutrients (Aletor and Adeogun, 1995) and also cause toxicity and mutagenicity.

Phytic acid is a natural organic plant compound (Harland and Oberleas, 1986) that is a simple ringed carbohydrate with six phosphate groups attached to each carbon. It is a major phosphate storage compound in plants and can account for 80% of total phosphorous (Lopez *et al*., 2002). The presence of phytic acid in plants consumed may inhibit iron absorption in infants to an extent similar to that in adults. Iron deficiency in infants can lead to reduced psychomotor and mental development with long term negative consequences on school performance (Davisson *et al*., 1994). All the plants were found to have low concentrations of phytic acid. Seeds of Gishta contain more Phytates compared with leaves and fruit pulp. There is no information in literature regarding the relationship between the plants in this study and phytic acid, however, Sirkka (1997) reported that phytic acid markedly reduced calcium bioavailability and formed calciumphytate complexes that inhibit Fe and Zn. Phytic acid intake of 4-9 mg/100g DM is said to decrease absorption of iron by 4-5 fold in human (Hurrel *et al*., 1992). Depending on the amount of plant-derived foods in the diet and the grade of food processing, the daily intake of phytic acid can be as high as 4 500 mg (Reddy, 2002). In average, Reddy *et al.* (1982) found the daily intake of phytic acid to be an estimated 2000-2600 mg for vegetarian diets as well as diets of inhabitants of rural areas of developing countries, and 150-1400 mg for mixed diets.

Saponins are glycoside compounds often referred to as “natural detergents” because of their foamy texture. They are chemical structures consisting of triterpenoidal or steroidal aglycones with various carbohydrate moieties that are found in many plants. On injection, saponin, like all detergents cause lysis of the red blood cells, called haemolysis and are therefore toxic. Seeds of Gishta had the highest concentrations of saponin, it was found to have concentrations of 1.7 mg/ml. Steroid and triterpenoid saponins with a single sugar chain were found to have strong haemolytic activity (Fuduka *et al*., 1985).

Leaves of Gishta had the lowest alkaloid concentration of 0.14g/5g of extract while fruit pulp and seeds had fairly the high concentration of 0.24, 0.95 respectively. The fatal dose of alkaloids in humans has been reported as 50mg/kg (Van Wyk *et al.,* 2002).

Cyanide is one of the most rapidly poisons known with a lethal dose of 0.5-3mg/kg body weight. This is due to its ability of linking with metals (Fe2+, Mn2+, and Cu2+) that are functional groups of many enzymes, inhibiting processes like the reduction of oxygen in cytochrome respiratory chain, electron chain transport in photosynthesis and the acting of enzymes such as catalase and oxidase (Cheeke, 1997). All the tested materials of Gishta investigated did not have any cyanogenic glycosides. The reason may be that the plant material was dried and other researchers such as Tan and Yeoh (1997) used fresh plant material.

Trypsin inhibitors, according to Venter and Van Eyssen (2001) are compounds that interfere with protein digestion, they cause pancreatic enlargement and enhance chemically induced pancreatic tumors. All the tested samples were found to have trypsin activity. However the trypsin inhibitors are heat-labile in nature and this suggests that they can be inactivated by cooking (Prathibha *etal*., 1995). And this also shows that if the plant material is properly cooked, the trypsin inhibitors may not interfere with digestion (Bhandari and Kawabata, 2003).

Oxalic acid is one of the antinutritional factors which are widely distributed in plant foods (Gupta, *et al*., 2005). All the tested samples were had oxalic acid in little amounts. A lethal dose of oxalic acid has been reported in *D. teltoida* (high oxalate containing yams) as 1kg of fresh material (at once) as it contains 2g oxalic acid, which is thought to be a lethal dose in humans (Libert, *et al*., 1987). Massey *et al*., (2001) advised patients who suffer from kidney stones to limit their oxalates intake to 50-60mg per day.

RESEARCH HIGHLIGHTS

- Identified presence or absence of antinutrient compounds in leaves, fruit pulp and seeds of Graviola/Gishta (Annona spp. of Ethiopia) by using different chemical methods.
- Discovered safety profiling of tested plant materials of Gishta (Annona spp. of Ethiopia).
- No research report published on Graviola (Gishta) cultivar of Ethiopia in terms of antinutrient profiling.

LIMITATION

The following limitation was identified by the researchers in the present experimental study: Anti-nutrient assay methods are basically chemical reactions, multi-component analysis of metal ions with mixed reagents.

RECOMMENDATIONS

After obtaining the results discussed above the answer to the problems presented in the antinutrient profiling of leaves, fruit pulp and seeds of Gishta (Annona spp. of Ethiopia) by using different chemical methods, the researchers have arrived at the following recommendations to further improve the study:

- The study has focused only on the antinutrient leaves, fruit pulp and seeds of Gishta (Annona spp. of Ethiopia) by using different chemical methods. In lined with this, the researchers highly recommend the phytochemical screening of pulp extracts of Graviola (Gishta) cultivar of Ethiopia for the identification of its specific active bio ingredient.
- Future research can be encouraged to determine the effect of different plant parts of Graviola (Gishta) of Ethiopia, in the metabolism and other physiological function.

CONCLUSION

The leaves, fruit pulp and seeds of Gishta (Annona spp. of Ethiopia) evaluated in this study are well endowed with all of the essential nutrients required for human nutrition. Should also be cautioned as they have indicated high antinutrient content especially trypsin inhibitors and alkoloids. All the tested samples contained antinutrients in varying concentrations. Consumption of this plant with low concentrations of antinutrients may appear safe at face value, but it should be noted that consumption of these plant materials over a long period as may contribute to adverse accumulative effects in the consumer. Thus further investigation with respect to the accumulative effect and toxic doses need to be established.

ACKNOWLEDGEMENT

The authors which to appreciate the effort of Prof Behailu Etana Disasa of Natural Resource Management, College of Agriculture and Veterinary Medicine, Jimma University, Jimma, Ethiopia-307 and University of Oslo, Faculty of Mathematics and Natural Science, Centre for Ecological and Evolutionary Synthesis (CEES), University of Oslo, Oslo, Norway for identification, authentication and supplying experimental plant material; Lydia Swapna Nandam of Department of Microbiology & Genetics, Institute of Fundamental Sciences, Massey University, Palmerston North-4474, New Zealand; Management of Aksum University, Axum, Ethiopia-1010, North East Africa for providing the basic facilities required for completing the work and especially the corresponding author highly grateful to Prof K R S Sambasiva Rao, Prof D M Prasad, Dr K Ammani for their constant support and encouragement extended.

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