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HPLC profiles of some bioactive compounds in *Annona muricata* L. and *Garcinia mangostana* L. peels

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Abstract: Alkaloids, antioxidants, and phenols compounds were investigated in peel of two tropical medical plants were Annona muricata L. and Garcinia mangostana L. by High Performance liquid chromatographic (HPLC). Spectrophotometer results showed the biggest rate of Alkaloids compounds was Annomuracin in both peels plants were 499.40, 523.62 µg/ml respectively, but Muricatain vanished in A.muricata peels. Analysis of HPLC confirm difference among antioxidant compounds, while Quercetin recorded highest concentration in A.muricata was312.53µg/ml, rise Luteolin as a highest concentration was 368.20 µg/ml in G. mangostana peels, Homoorientin disappear in G. mangostana, but it had a strong present in A.muricata was171.16 µg/ml. The outcome of phenols compounds showed variation in contents, average of phenols in G. mangostana were more than A.muricata except Caffeic acid, p- coumaric acid had a highest concentration in both peels plants were 359.54, 441.89 µg/ml respectively. This work aims to make HPLC fingerprints of standard of some bioactive compounds could be used as benchmarks for comparison as a chemotaxonomic key, and provides pharmaceutical data.
***Keywords**: bioactive compounds compounds, HPLC, Annona muricata L. and Garcinia mangostana L.*

Introduction

Plant use as a major source of medicinal drugs is considered to be effective in many diseases as well as environmentally friendly and inexpensive [1]. Several active compounds have been identified in plant foods, including phenolic and alkaloids, which are widely distributed in the plant kingdom as secondary products of photosynthesis. Fruit is one of the richest sources of these compounds, as well as the fact that these compounds are a food source, Such as anti-oxidant antioxidants as they reduce blood cholesterol and anti-anticoagulant anticoagulant and stimulate the immunity of the body and against fungal, bacterial and viral diseases [2].

Annona muricata L. genus belongs to the Annonaceae (custard apple family), a family included about 26 genera and approximately 260 species, cultivated in all over tropical rainforest areas of South-Asian countries, All parts of this genus have been used as a natural remedy for a variety of illnesses because owning allegedly medical and immunological properties as antiparasitic, antispasmodic, antidiarrheal, antiulcer, sedative, analgesic, hypotensive, and vermifugal effects[3]. Its leaf, fruit and stem constituents exhibit antioxidant activity in different in vitro models due to the presence of flavonoids like rutin and hyperoside [4], It is also a wonderful fruit that plays an important role in the treatment of cancer and prevent the spread of

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cancer cells faster and more effective than chemical treatment, which results in many side effects, in addition to being very expensive [5],[6].

Garcinia mangostana L. afamous tree of Clusiaceae family, Globally grown in tropical climate [7] and known as "Mangostin" Historically, mangosteen fruit has been used medically in Southeast Asia and is fast becoming a popular dietary supplement and juice beverage. For these reasons, products containing α-mangostin have been receiving increased attention by scientists and consumers for its potential health promoting properties [8]. More recently, a variety of biological activities were reported with from the mangosteen fruit or standardized extracts that included antioxidant [9], anti-inflammatory [10],[11] anti-bacterial [12], and anti-cancer related effects [13],[14]. Many preclinical pharmacokinetic studies worked possibly due to the bioactive compounds that are mangostin directly associated with the prevention of diseases. The plant kingdom distributed throughout the bioactive compounds such us alkaloids, antioxidants, phenolic compounds, steroids, etc. The medical activities of any plant specimen are due to the presence of secondary metabolites products in it. Literature is rich in studies explained the pharmacognostical, phytochemical, toxic and biological properties of bioactive compounds . In particular, the qualitative and quantitative analysis of the phenolic compounds found in any plant part following systematic scientific methodology and its comparison with standard phenolic compounds. The main objective of this research was to determine the chromatograms of standard Alkaloids, antioxidant ,and phenolic compounds which are commonly found in *A. muricata*, and *G. mangostana* samples by HPLC. Therefore, These HPLC fingerprints of standard bioactive compounds could be used as chemical marks for a Pharmacia revision/taxonomic study of genera under study for comparison purpose

Material and Methods

Plants samples

Peels of were collected from super market and they were dried and ground into powder for chemical analysis. This study was conducted at the laboratory of the ministry of science and technology.

Alkaloids extraction and Analysis

1 gram was homogenized, grinding to fine powder, dissolved in 3% H_2SO_4 for 2h at room temperature, filtrations on 2.5 µm filter paper. The extract of alkaloids according to enclosed procedure were separated on FLC (fast liquid chromatographic) column, 3um particle size, phenomenexC-18(50x 4.6 mm ID) column. Mobile phase were 0.01M phosphate buffer, pH 6.2; acetonitrile, flow rate 1.4 ml/ min. the supernatants were made alkaline with 25% NH2OH (pH 9.5) and applied to extrelut (Merck) columns , the alkaloids were eluted by CH2CL2 (6ml/1g) extrelut, and the extracts were evaporate to dryness by using stream of nitrogen , thus obtained residues were resolved in 1 ml $CH_3OH$for the further analysis by HPLC according the optimum separation of anthentic standard , the concentration were determined by standard with that of sample under the same separation condition. The separation occurred on liquid chromatography Shimadzu 10AV-LC equipped with binary delivery pump model LC-10A Shimadzu, the eluted peaks were monitored by UV-VIS 10A-SPD. [15]. The table (1) shows the standard alkaloids compounds used in the study.

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Antioxidant analysis

According to [16] the standard of active material were separated on FLC column under the optimum condition. Column: Zorbax clipse XXDB_C-18 ,3 µm particle size (50x 2.0 mm LD) column, Mobile phase: 0.1% acetic acid (A) methanol (B) using linear gradient program from 0%B to 100%B for 8 minutes, flow rate: 1.0 ml/min; detection: UV set at 370nm; temperature: ambient; injection volum : 20 µl. Standard antioxidant compounds data shows in table (3) .

Phenols extraction and analysis

The main compounds were separated on FLC column under the optimum condition. Column: phenomenex C-18 ,3 µm particle size (50x 2.0 mm LD) column, Mobile phase: linear gradient of solvent A 0.1% phosphoric acid; solvent B was (6:3:1, v/v) of acetonitrile: methanol: 0.1% phosphoric acid, linear gradient program from 0%B to 100%B for 15 minutes, flow rate: 1.0 ml/min; detection: UV 280nm, 1 gm of sample powder were dissolved in 20 ml of hexane to remove fat, then 100 ml of 80:20 (methanol: water) added, the extract was subjected to ultra-sonication(Brabson sonifier, USA) at 60% duty cycles for 25 minutes at 25ºC followed by centrifugation at 7,500 rpm for 15 minutes. The clear supernatant of each sample was subjected to evaporation under vacuum (Buchi Rotavapor Re Type) . Dried samples were re-suspended in 1.0ml HPLC grade methanol by overtaxing, the mixture were passed theough 2.5 µm disposable filter and stored at 4ºC for further analysis, then 20µl of the sample injected in to HLPC system according the optimum condition. Method is used as stated in the [17] with some modification. standard phenols compounds that used in the study shows in table (2) .The calculation of the concentration of bioactive compounds in the sample µg/ ml= area of sample/ area of standard x cons. of standard x dilution factor [18].

Table 1. standard Alkaloids in study

S	Compound(20µm)	R.T.	A.µ volt
1	Annomuricin A	2.18	246559
2	Annomuricin B	3.02	225495
3	Annomuracin	4.68	185455
4	Annonacin-A	7.08	190211
5	Muricapentocin	5.66	236670
6	Muricatain	6.80	24890

Table 2. Standard antioxidant in study

S	Compound (20µm)	R.T.	A.µ volt
1	Coumaric acid	8.66	332105
2	Emodin	7.50	351355
3	Homoorientin	4.60	331379
4	3 Luteolin	2.25	28165
5	Quercetin	6.44	256065
6	Tangeretin	5.27	353967

Table 3. Standard phenols in study

S	Compound(20µm)	R.T.	A.µ volt
1	Caffeic acid	4.66	335875
2	Chlorogenic acid	3.72	291653
3	Cinnamic acid	5.37	351355
4	Gallic acid	2.47	273062
5	p- coumaric acid	6.93	286694
R.T.= Retention time (minute), A=area			

Results

The results of spectrophotometer spectroscopy showed the variability of peels of the two plants under study in their content of alkaloids. Figure 1 clears these differences. The alkaloid rate of plant peels was the highest concentration of the compound Annomuracin at 499.40 And 523.62 µg / ml respectively. Muricatain disappeared in *A.muricata*, while Muricapentocin was the least concentrated in *G. mangostana*, It was 114.18 µg / ml. The results of the HPLC analysis confirmed the divergence in the content of the both plants from the antioxidants. Quercetin recorded the highest concentration of *A.muricata* peels at 312.53 µg / ml, but the concentration of the luteolin increased to 368.20 µg / ml in the *G. mangostana* peels, the homooriorin disappeared in *G. mangostana* while having a clear presence in *A.muricata* peels with a concentration of 171.16 µg/

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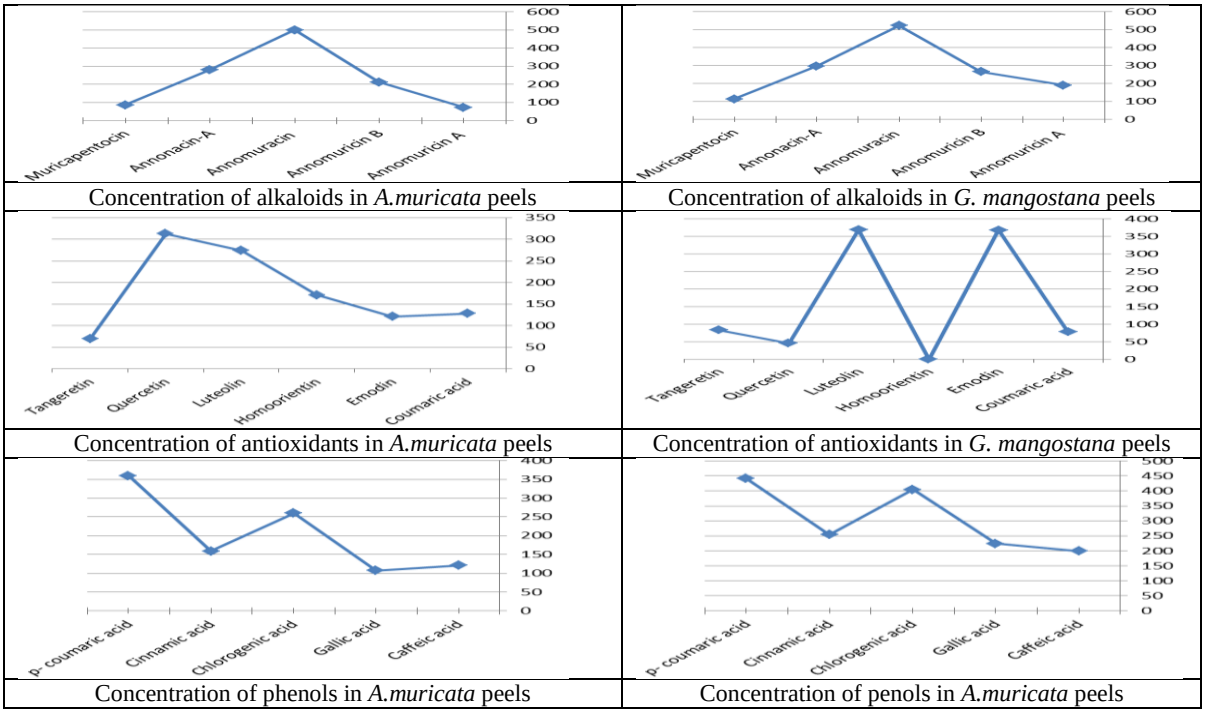
ml. The results in figure 1 show differences between the content of *A.muricata* and *G. mangostana* peels phenols, while p-coumaric acid was the highest in both plants, it recorded 359.54 and 441.89 µg / ml respectively. Galic acid was isolated with lowest concentration in *A.muricata* peels with 107.34 µg/ ml. The Caffeic acid had the lowest concentration of 199.44 µg / ml in *G. mangostana*. The results showed that the percentage of phenolic compounds studied was higher in *G. mangostana* compared to *A.muricata* peels, except Caffeic acid.



Concentration of alkaloids in *A.muricata* peels | Concentration of alkaloids in *G. mangostana* peels
Concentration of antioxidants in *A.muricata* peels | Concentration of antioxidants in *G. mangostana* peels
Concentration of phenols in *A.muricata* peels | Concentration of penols in *A.muricata* peels

Fig1. Bioactive compounds concentration in *A.muricata* and *G. mangostana* peels

Discussion

The outcome of study shows divergence in the content of the both plants from concentration of alkaloids, antioxidants, and phenols may be due to concentrations of compounds obtained due to different environmental conditions where plants grow and their ability to produce and accumulate anodic alkaloids in cells, this outcome conform with [19] results. This may be due to the properties of this compound as a secondary metabolite as it protects cells from cancerous effects and anti-burns. This is consistent with [20], [21], and [22] they explained that the presence of high levels of antioxidants leads to corroborate antifungal activity multiplication of cancer cells.

The results showed that the percentage of phenolic compounds studied was higher in *G. mangostana* compared to *A.muricata* peels, except Caffeic acid, this results which confirmed by [23] That extract of mature plants peel's contains larger amounts of flavonoids and α-mangostin and also showed higher activity against acne-producing bacteria than other plant species. The high concentrations of Annomuricin A, Annomuricin B, and Annonacin-A in both plants confirm their role in resistance cancer disease and health promotion [24].

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Conclusion

The HPLC fingerprints of qualitative and quantitative alkaloids, antioxidants and phenolic compounds obtained using the methods described above would serve the pharmaceutical purposes as this can provide beneficial sources for future studies and identified the significance of some bioactive compounds and established benchmarks for future plant research.

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