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Preliminary Phytochemical Investigations with Quantitative Fractionation of Orange Pulp (*Citrus Aurantium Var.* Dulcis L.): Natural Product Waste as Medicine

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Keywords: citrus aurantium var. dulcis L pulp, preliminary phytochemical analysis, quantitative fractionation.

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Preliminary Phytochemical Investigations with Quantitative Fractionation of Orange Pulp (*Citrus Aurantium Var.* Dulcis L.): Natural Product Waste as Medicine

Rufai Y [α] & Fatimah S [σ]

Abstract- Day by day, faith of people on herbal medicine increases due to the side effect of synthetic drugs; this has resulted into people falling back to the traditional knowledge of plant for their health care. Certain local practitioner and traditional healers use the fruits of *Citrus aurantium var.* Dulcis L pulps in various disease management and so, they advise to eat the pulps along with the drinking of the juice. The present study deals with preliminary phytochemical analysis of the fruit of *Citrus aurantium var.* Dulcis L pulp using 95% ethanol for its extraction. The fruits of *Citrus aurantium var.* Dulcis L pulp ethanolic extract revealed the presence of all tested phytochemical compounds except protein and glycoside. These include Alkaloids, Tannins, Phenolic, Quinine, Reducing Sugar, Coumarins, Flavonoids, Saponins, and Steroids. During the analysis, the quantitative fractionation of the ethanolic extract showed a reasonable amount of saturated hexane fraction (40g), unsaturated hexane fraction (2.0g), methanolic fraction (1.3g), acidic fraction (1.2g) and basic fraction (0.3g). These results from the fruit of *Citrus aurantium var.*Dulcis L. pulps revealed their ignored medicinal importance by throwing it away to domestic animals, contributing to environmental de-sanitation and a natural product waste as medicine. And it's a needful help for the scientific documentation and standardization of row fruits waste material as to be used in medicine and recommended for worldwide acceptance.

Keywords: citrus aurantium var. dulcis L pulp, preliminary phytochemical analysis, quantitative fractionation.

I. Introduction

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body [1]. The most important of these bioactive constituents of plants are Alkaloids, Tannins, Flavonoids, and Phenolic compounds [2]. Many of these indigenous medicinal plants are used as spices and food plants. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes [3;4]. This field of natural products research is currently being carried out intensively though it remains far from exhaustion. An attempt to obtain bioactive agents from plants is a worthwhile exercise since only 10% of all plants have been investigated in detail [5]. However, as at the time of this study, a higher percentage of bioactive compounds could have been discovered. The majority of these bioactive compounds are Sesquiterpenes, Diterpenes, Triterpene Saponins, Triterpene Aglycones, and Monoterpenes. It is imperative that ethnobotanical researches and phytochemical tests have led to some patent-able and industrially exploitable compounds for drug development. Plants fulfill the needs of not only human being but also entire animal kingdom.

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Man as a unique creation of God[6] is but a part of the universe that relates domestically with other living creatures, man has been provided with food, water, shelter and herbal medicine around his habitat. However, the orange pulps of the fruits of *Citrus aurantium var.* Dulcis L. which is part of man's food are more beloved to domestic animals like goat and sheep for reason not yet proven scientifically. Domestic animal kept looking at human when eating or drinking such food and most times compete with them self in eating the thrown away part by human.

The popular orange tree (*Citrus aurantium var.* Dulcis L.*)* belongs to the plant family *Rutaceae*. It is a small tree with grayish-brown branches that are widely spread. The petioles of the leaves are winged and the leaves are ova, alternate, and have a deep green colour. The calyx is bell-shaped and bisexual flowers are pure white. The fruit is round and green and yellow when ripe. It is widely used for it juice which is sweet [7].

Table 1 : Showing uses for the various parts of *Citrus aurantium var.* Dulcis L.

Part	Medicinal Uses
Leave	The infusion of the leaves, mixed with a little honey, is used for controlling cough.
Pulp	The pulp should be eaten instead of drinking only the juice as it ease bowel movement.
Fruit	The fruit in general is good for cases of arthritis, asthma, respiratory problems, pneumonia, hysteria, neurasthenia, neuralgia, headache, colds, cough, fevers and influenza. It is highly recommended for scurvy.
Rind	Fresh rind rubbed on the face is a good remedy for acne.
Juice	The consumption of orange juice strengthens the stomach, increases variation, and is refreshing. It purifies the blood, increases thirst in fever, heals inflammation of the mucus membrane, and improves appetite. Orange juice is useful in liver problems. The rind expels gas and is a tonic
Flower	The infusion of the dried flowers is recommended for stress or nervousness.

Source: J.C. Kurian (2010). Healing Wonders of Plant. Vol. 2, Pp 40.ISBN: 978-1-907456-05-3. Zambia Adventist press, P.O. Box 31309, Lusaka, Zambia.

II. Materials and Methods

a) Chemical Used

Ethanol, Methanol, Chloroform, Ethyl Acetate, Hydrochloric Acid, Sodium Hydroxide, Hexane and all others solvents (Analytical grade) from Merck Co. (Darmstadt; Germany), and Distilled Water.

b) Sample Collection

Fresh fruits of *Citrus aurantium var.* Dulcis Lwere collected at Nagazi central market around Federal College of Education Okene, Kogi State, Nigeria. The plant was identified and confirmed at Ahmad Bello University, Zaria, Kaduna; ABU Herbarium (Botany Unit, Department of Biological Science) by Mr. Muhammad Musa, The back of the oranges were pealed, the pulps were collected after juice extraction and washed with pure water, air dried as shown below and pulverized into a fine powder using a commercial blender.

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III. Extraction and Fractionation Procedure

Extraction and fractionation of the pulp ethanolic extract was carried out by bioassay guided fractionation protocol [8]. The procedure was carried out using ethanol-water (95:5v/v) and different organic solvent in order of polarity (Hexane, chloroform and Methanol) using separatory funnel to fractionate them into different fractions. One thousand grams of the powdered fruits of *Citrus aurantium var.* Dulcis L pulp materials (20 mesh~1g) were extracted using percolation process in a mixture of 95ml of distilled ethanol and 5 ml of distilled water at ambient temperature overnight. The extractives was filtered and re-extracted three times. The combined extract were filtered through a Whatman No. 1 paper and then concentrated invacuo at 40^0C using a rotary evaporator, model W2-100 SENCO® @ rpm of 100; Shanghai SENCO technology Co, Ltd Japan. The various extractive concentrates were evaporated to dryness using water bath for some days and residues were obtained in gram for basic, acidic, polar and non-polar fraction as 0.3g, 1.2g, 1.3g, and 40g.

IV. Phytochemical Screening of the Ethanolic Extract

Preliminary Phytochemical screening was done using standard procedures to identify constituents, as described [9;10]as follows. It involves testing of different

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classes of compounds. The methods used for detection of various phytochemical were followed by qualitative chemical test to give idea regarding the nature of constituents present in the fruit of *Citrus aurantium var.* Dulcis L pulp ethanolic extract.

Tests for carbohydrates Fehling's test: 1 ml Fehling's A solution and 1 ml of Fehling's B solution were mixed and boiled for one minute. Now the equal volume of test solution added to the above mixture. The solution was heated in boiling water bath for 5-10minutes. First a yellow, then brick red precipitate was observed.

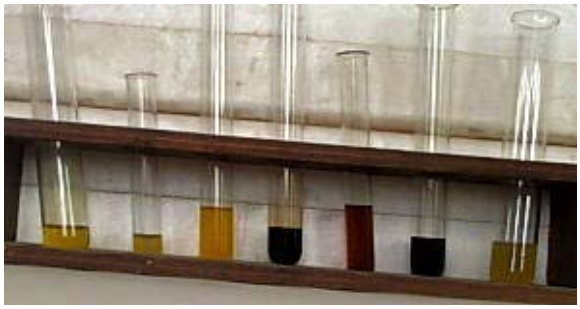

Figure (a)

a) *Benedict's Test*

Equal volumes of Benedict's reagent and test solution were mixed in a test tube. The mixture was heated in boiling water bath for 5 minutes. Solutions appeared green showing the presence of reducing sugar.

b) *Molisch's Test*

Equal volumes of Molisch's reagent and test solution were mixed in a test tube. The mixture was heated in boiling water bath for 5 minutes. Appearance of violet or purple colour ring showing the presence of reducing sugar.

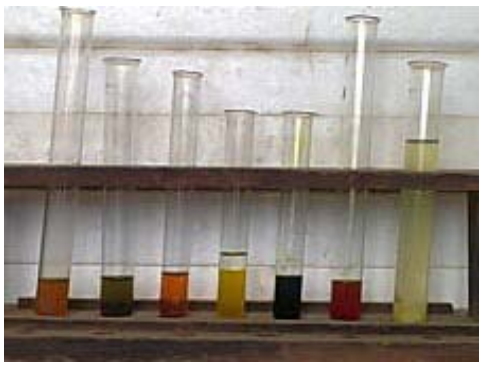

Figure (b)

c) *Test for Proteins (Biurret Test)*

To the small quantity of extract 1-2 drops of Biurret reagent was added. Formation of violet colourprecipitate showed presence of proteins.

d) *Million's Test*

To the small quantity of extract 1-2 drops of Million's reagent was added. Formation of white colour precipitate showed presence of proteins.

e) *Test for Anthraquinone glycosides*

f) *Borntrager's Test*

To the 3ml of extract, dil. H_2SO_4 was added. The solution was then boiled and filtered. The filtrate was cooled and to it equal volume of benzene was added. The solution was shaken well and the organic layer was separated. Equal volume of dilute ammonia solution was added to the organic layer. The ammonia layer turned pink showing the presence of glycosides.

g) *Test for Cardiac glycosides (Keller-Killiani Test)*

To the 5ml of extract, 1ml of conc. H_2SO_4, 2ml of Glacial acetic acid and 1 drop of $FeCl_3$solutions was added. Appearance of Brown ring shows the presence of cardiac glycosides.

h) *Test for Coumarins*

To the 2ml of extract 10% NaOH was added and shake well for 5mm shows the yellow colour.

i) *Tests for Quinone*

To the 2ml of extract conc. H_2SO_4 added and shake well for 5 mm shows the Red colour.

j) *Test for steroids (Salkowski Test)*

To 2 ml of extract, 2 ml of chloroform and 2 ml of conc. H_2SO_4 was added. The solution was shaken well. As a result chloroform layer turned red and acid layer showed greenish yellow fluorescence.

Figure (c)

k) *Test for alkaloids(Hager's Test)*

To the 2-3 ml of filtrate, 1ml of dil. HCl and Hager's reagent was added and shake well. Yellow precipitate was formed showing the presence of alkaloids.

l) *Mayer's Test*

To the 2-3 ml of filtrate, 1 ml of dil. HCl and Mayer's reagent was added and shake well. Formation of yellow precipitate showed the presence of alkaloids.

m) *Dragendroff's Test*

To the 2-3ml of filtrate, 1ml of dil. HCl and Dragendroff's reagent was added and shake well. Formation or orange-brown precipitate showed the presence of alkaloids.

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n) Wagner's reagent test

To the 2-3ml of filtrate, 1ml of dil. HCl and Wagner's reagent was added and shake well. Formation of reddish-brown precipitate showed the presence of alkaloids.

o) Test for Flavonoids (With Lead Acetate)

To the small quantity of extract lead acetate solution was added. Formation of yellow precipitate showed the presence of flavonoids.

p) Test for Tannins and Phenolic compounds ($FeCl_3$ Solution Test)

On addition of 5% $FeCl_3$ solution to the extract, deep blue black colour appeared.

q) Lead Acetate Test

On addition of lead acetate solution to the extract white precipitate appeared.

r) Test for Saponins (Foam Test)

To 1mol extract 20ml distilled water was added and shakes well in measuring cylinder for 15min. Then 1cm layer of loam was formed. Above phytochemical analysis will be carried out using standard procedure [11;12].

Figure (d)

V. Result and Discussion

The Phytochemical analysis for ethanolic extract was determined. It revealed the presence of all tested phytochemical compounds except protein and glycoside. Fig (a), (b), (c), & (d) show the phytochemical analysis bench work photograph. The (a), (b), (c) are the qualitative test for the majority of the secondary metabolites while the (d) shows the test for Saponins in particular. The sharp colour changes during the analysis showed the level of the quantity of such phytochemical compounds present. However, isolation of each component is in progress for further analysis with their quantitative test.

Table 2 : Preliminary screening of *Citrus aurantium var.* Dulcis L. pulp

S/NO	Constituent	Chemical	Observation
1	Alkaloids	Hager's Reagent	+
		Dragendroff's Reagent	+
		Mayer's Reagent	+
		Wagner's Regent	+
2	Carbohydrate & reducing sugar	Fehling's Regent	+
		Benedict's Regent	+
		Molisch's Regent	+
3	Steriods	Salkowski Regent	+
4	Saponins	Foam	+
5	Phenolics& Tannin	$FeCl_3$ Sol.	+
		Lead Acetate	+
6	Fixed oil & fats	Spot test	+
7	Proteins	Biurret Reagent	-
		Million's Regent	-
8	Anthraquinone glycosides	Borntrager's Reagent	-
9	Cardiac glycosides	Keller-Killiani Reagent	-

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10	Flavonoids	Lead Acetate	+
		Extract + NH_3	+
11	Quinone	Extract + Conc. H_2SO_4	+
12	Coumarins	Extract + 10% NaOH	+

Key: + = Present - = Absent

These include Alkaloids, Saponins, Steroid, Carbohydrate, Tannins, Quinone, Coumarins, Phenolics, Terpenoids Fixed Oil, Fat and Flavonoids as shown in Table 2. As it is expected for ethanolic solvent used being an active component extractor [13]. Therefore, the presence of these secondary compounds validates the use of oranges pulps as herbal drugs anywhere they are found. On carrying out phytochemical analysis, crude extracts were fractionated into acidic, basic, polar and nonpolar fractions as shown in Table 3. The highest quantity of phytochemical was found to be oil from hexane fraction thereby indicating steroidal properties responsible in the hormonal production and enhancement. Most Alkaloid fraction is known to be poisonous. Thus, it was the least fraction obtained from the fruitof *Citrus aurantium var.* Dulcis L. pulp showing their friendly and less harmful as to be used in medicine.

Each fraction obtained through the bioassay fractionation protocol showed fluorescence under the UV observation. Thus, wavelength between 254–365nm has indicated the presence of secondary metabolites in the fractions. The ultraviolet region extends from about 10 to 380nm, but the most useful region in analysis is from 200 to 380nm, called the near-ultraviolet or quartz UV region. This is as a result of chromophores acting as chromatogram and conjugation (where multiple e.g., double and triple bonds are separated by just one single bond each) between the double bonds from oxygen atoms with the single bonds present in the structure. The different colours of the fluorescence rings are due to different atoms present in the compound having different wavelengths. When atoms are excited to a higher energy level, they may fall back to their original position using the same or a different wavelength resulting to emission of different colours [14]. At still higher energies (visible and ultraviolet wavelengths) different levels of electronic transition take place, and rotational and vibrational transitions are superimposed. Thus, indicating that important medicinal compound could be present in the fruit of *Citrus aurantium var.* Dulcis L. pulp fractions.

Table 3 : Physical parameters of *Citrus aurantium var.* Dulcis L. pulp fractions

S/No	Extractives	Weight	Colour	Texture
1	Methanolic	1.3g	Yellow	Viscous
2	Basic	0.3g	Light brown	Solid
3	Hexane(unsaturated)	2.0g	Orange	Oily
4	Acidic	1.2g	Brick red	Solid
5	Hexane(saturated)	40.0g	Red oxide	Oily

Phytochemicals are known to possess antimicrobial properties as reported [13]. This showed that the orange pulps were rich in chemical constituents. These principles have been known for many years to exhibit biological activity, such as effects on the central nervous system, and antibacterial, antiturmour, and anthehelmintic activity [16]. Many alkaloids are known to have effect on the central nervous system and some act as antiparasitic (such as morphine, a pain killer). Quinine was widely used against *Plasmodium falciparum*. In this respect, it is found from the phyto-chemical screening that most plants traditionally used to treat malaria contain alkaloids among other things. Analgesia is another property of many alkaloids containing plants used in traditional medicine. Degenerative disorders, such as gouts and rheumatism, have also been traditionally treated with alkaloid-containing plants. Cochicine compounds are well known in treating gouts [14]. Alkaloids which have anti-inflammatory activity were present in the orange pulp and Saponins which have anti-inflammatory and considered as hemotoxic. Coumarins were present which is precursor for several anticoagulants. Tannins were present which have astringent and detergent properties were also present and can be used against diarrhea [15]. The presence of these compounds in *Citrus aurantium var.* Dulcis L pulpwill be useful in the treatment of diseases associated with the heart, anti-inflammatory action, anticoagulant, diarrhea and dysentery. Steroidal compounds are known to behave like hormones [16] have reported oils, alkaloids and associated with plants to have medicinal value. Others are Tritepenoids, which include: Cardiac Glycosides, Sterols, Saponins and Tritepenes. Mode of action of compounds present in the extracts indicates that the extracts from these pulps have the potential of solving the problem of multi-drug resistance.

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VI. Conclusion

The study is useful for the utilization of natural product waste fruit such as the fruit of *Citrus aurantium var.* Dulcis L. pulpas therapeutic agents especially those that are thrown away been considered not very necessary. These may be more needed for the body wellbeing as it contains very important phytochemicals. Thus, it provides an ethnobotanical data of the medicinal fruits as used by the local practitioners, traditional healers to cure different diseases, and promote a practical use validation and to bring back the extinct knowledge for medicine. Further detailed exploration and collection of ethnobotanical information, chemical studies and screening for medicinal properties which are ignored will also provide less cost effective and reliable source of medicine for the welfare of humanity. However, the observations from the present study need to be further validated with isolations of compounds and pharmaco-chemical studies, in order to confirm their efficacy of such components present in the phytochemicals as a future drug.

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