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Evaluation of the Phytochemical Properties of *Citrus limon, Citrus sinensis and Citrus aurantifolia*

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ABSTRACT

The phytochemical constituents of the 3 species of Citrus were evaluated using Methanol, Ethanol, ethyl acetate and N-hexane as solvents. Qualitative and quantitative phytochemical analyses were carried out using standard procedures. The high phytochemical contents observed in the plant used in the present study indicate that it is a unique material for medicinal drug screening and research. The results indicated that the extracts (ethanol and methanol) of Citrus (Zest) contains alkaloids, saponins, tannins, glycosides, phenol and carbohydrates. In conclusion, results from this research indicated that *Citrus limon*, *Citrus sinensis* and *Citrus aurantifolia* are rich in phytochemicals and could be used in the treatment and management of different diseases.

Keywords; Phytochemicals, traditional medicine, *Citrus limon*, *Citrus sinensis* and *Citrus aurantifolia*

INTRODUCTION

Natural products such as plants have been integral part of traditional medicine system in ancient times such as in Chinese and Egyptians and the research into antimicrobial and phytochemical screening of compounds from natural sources has always been of great interest for scientist looking for new sources of useful drugs against infection and diseases [1,2,3,4]. The secondary metabolites present in plants have been linked with healing properties of plants, in addition to their active ingredients; plants contain minerals, vitamins, Volatile oil glycosides, alkaloids, bioflavinoids and other phytochemical substances that are important elements [5,6,7,8]. The secondary metabolites present in plants offer synergistic interaction between both known and unknown properties since the medicinal plants have different actions for different purpose [9,10,11]. Several published reports had described the antimicrobial activity of various crude extracts of plants either in single or in combination [12,13]. It has been estimated that about 2.5 million species of higher plants and their therapeutic values are yet to be determined. However, herbal extracts are becoming popular as natural medicine; preservatives and additives [14,15]. According to World Health Organization medicinal plants contain substances in one of its organs such as stem, root, leaves, rhizomes, fruits, flower and seeds that can be used for therapeutic purposes or which are precursor for chemo-pharmaceutical semi-synthesis [16,17,18]. The medicinal plants are employed in the treatment or control of disease condition due to the presence of biochemical components called phytochemical [19,20]. Phytochemicals are considered as bioactive substances of plant origin and are of medical importance. They are otherwise known as secondary metabolites as they are of little important to the plant who manufactured them. These secondary metabolites synthesized naturally in various organs of plants such as leaves, stem, root, flower and seed [21]. Most of the modern drugs used today were originally obtained from plants. Many researches confirmed the herbal extract boost immune system by stimulating the production of white blood cell which fight diseases [22].*Citrus sinensis, Citrus aurantifolia and Citrus limon* belong to Rutaceae, it is a polyembroyonic plant cultivated in several part of the world especially hot subtropical or tropical region such as India, USA, Nigeria, Mexico and Egypt [24]. The plant is shrub in nature and height of about 2 meter tall, evergreen with dense and irregular branches which possess short and stiff spines. The *Citrus aurantifolia* and Citrus sinensis fruits are ovoid berry of about 3-6cm in diameter and sometimes possess apical papilla. When ripe, the fruits turn

yellow from initial blue. The plant is used in traditional medicine for treatment of several diseases such as cold and stomach ailment. It can also be used as an antiseptic, mosquito repellant, antifungal, antibacterial and antiviral agent [8]. The health benefits of *Citrus aurantifolia, Citrus sinensis and Citrus limon* plants are highly associated with the high amount of bioactive constituents it contained such as phenols, flavonoids, carotenoid, vitamins and minerals [19]. Limes contain unique flavonoid compounds that have antioxidant and anti-cancer properties. The flavonoids help to inhibit cell division in many cancer cell lines in addition to its antimicrobial efficacy. The plant also demonstrated bioactive activities for cold, fever, sinusitis, sore throats, asthma and bronchitis [22].

AIMS OF THE STUDY

The aim of this research work was to evaluate of the phytochemical properties of *Citrus limon, Citrus sinensis and Citrus aurantifolia*

MATERIALS AND METHODS

STERILIZATION OF GLASS WARES

All glass wares were sterilized in a hot air oven at 100°c for 1hour and all media prepared were sterilized in an autoclave at 121°C for 15 minutes.

COLLECTION AND IDENTIFICATION OF PLANT MATERIALS

Fresh fruits (Sweet orange, lemon, lime,) were purchased from different Markets, in Enugu State, Nigeria.

PREPARATION OF PLANT MATERIALS

The fruits were rinsed thoroughly with distilled water and were cut into halves. Zests were extracted by "peeling" using a clean knife in an aseptic condition. The Zests were suspended into Methanol, Ethanol, Ethyl Acetate, N- Hexane and Water

PREPARATION OF EXTRACTS

The blended plant (250g) was macerated in 500ml of Methanol, Ethanol, Ethyl Acetate, N- Hexane, and Water in different flat botton flask. The mixture were stirred, covered and allow to stand for 24hours. The mixture was filtered afterwards using sterile Whatman No. 1 filter paper in a Buchner funnel. The filtrate were subjected to concentration using water bath (Chem-index, WB500E, USA) at 30°c for 6h to obtain a concentrate. Thereafter the concentrate was allowed to stand for 24h for proper elimination of the solvent. The extract obtained was stored in refrigerator at 40°c until needed for phytochemical and antimicrobial analysis.

PHYTOCHEMICAL SCREENING

Qualitative and quantitative Phytochemical analyses were carried out using a standard procedures as described by [3,5].

STATISTICAL ANALYSIS

The data were expressed as mean ± SEM of three replicates. The data were subjected to one-way analysis of variance (ANOVA), and differences between means were determined by Duncan's multiple range test using the Statistical Analysis System (SPSS Statistics 17.0, SPSS Inc. Chicago, Illinois, USA) where applicable. $P \leq 0.05$ were regarded as significant.

RESULTS

TABLE 1: Qualitative Phytochemical Constituents Of Lime (*Citrus Aurantifolia*)

QUALITATIVE RESULT_LIME

Parameter		Water	N-hexane	Ethyl Acetate	Methanol	Ethanol
Alkaloids		-	-	-	-	-
Saponins	Emulsion	-	-	-	+	++
	Frothing	-	-	-	+	++
Flavonoids		++	-	++	++	++
Steroids		-	++	-	-	-
Phenoils		-	-	-	-	-
Tanins		++	-	++	++	++
Glycosides		-	-	-	+	+

Key

++	-	Present
+	-	Trace
-	-	Absence

TABLE 2:Qualitative Phytochemical Constituents Of Lemon (*Citrus Limon*)

QUALITATIVE RESULT LEMON

Parameter		Water	N-hexane	Ethyl Acetate	Methanol	Ethanol
Alkaloids		+	-	+	+	+
Saponins	Emulsion	-	-	-	+	+
	Frothing	-	-	+	-	-
Flavonoids		++	-	+	++	++
Steroids		-	-	-	++	++
Phenoils		-	-	-	-	-
Tanins		++	-	++	++	++
Glycosides		-	-	-	++	++

Key

++	-	Present
+	-	Trace
-	-	Absence

TABLE 3:Qualitative Phytochemical Constituents Of sweet oranges (Citrus sinensis)

SWEET ORANGE 1

Parameter		Water	N-hexane	Ethyl Acetate	Methanol	Ethanol
Alkaloids		-	++	+	+	-
Saponins	Emulsion	++	-	-	++	-
	Frothing	++	-	-	-	-
Flavonoids		++	-	++	++	++
Steroids		-	-	-	-	-
Phenoils		++	-	-	-	+
Tanins		++	-	++	++	++
Glycosides		+	-	-	++	++

Key:

++	Present;
+	Trace
-	Absence

TABLE 4:QUALITATIVE RESULT SWEET ORANGE 2

Parameter		Water	N-hexane	Ethyl Acetate	Methanol	Ethanol
Alkaloids		++	-	+	-	+
Saponins	Emulsion	+	+	-	++	++
	Frothing	-	++	+	-	+
Flavonoids		++	-	-	++	++
Steroids		-	-	-	++	-
Phenols		++	-	-	++	++
Tanins		++	-	-	++	++
Glycosides		-	-	-	+	++

Key:

++	Present;
+	Trace
-	Absence

TABLE 5:QUANTITATIVE ANALYSIS PHYTOCHEMICAL CONSTITUENTS OF CITRUS EXTRACT RESULTS (%)

Sample ID	% Alkaloids	% Saponins	% Flavonoid	% Steroids	mgGAE/g Phenoils	% Tanin	% Glycosides
Sweet orange 2	3.06	40.44	9.11	0.56	56.84	0.1357	0.575 1.56
	3.18	34.73	9.01	0.56	55.71	0.1354	0.575 1.56
Sweet orange 1	1.18	22.65	15.25	ND	81.81	0.104	0.373 0.37
	0.65	22.40	15.05	ND	79.56	0.1038	0.373 0.37
Lime orange	3.10	36.03	16.06	0.269	42.05	0.1234	0.447 0.45
	3.11	35.80	16.20	0.271	43.88	0.122	0.447 0.45
Lemon orange	4.68	21.84	7.53	0.438	48.50	0.1067	0.792 0.79
	4.80	15.57	7.55	0.438	49.55	0.1065	0.794 0.79

ND – Not Determined

DISCUSSION

Citrus sinensis, Citrus aurantifolia and Citrus limon belong to Rutaceae, it is a polyembroyonic plant cultivated in several part of the world especially hot subtropical or tropical region such as India, USA, Nigeria, Mexico and Egypt [8]. The *Citrus sinensis*, *Citrus aurantifolia* and *Citrus Limon* are ovoid berry of about 3-6cm in diameter and sometimes possess apical papilla. When ripe, the fruits turn yellow from initial blue. The plant is used in traditional medicine for treatment of several diseases such as cold and stomach ailment. It can also be used as an antiseptic, mosquito repellant, antifungal, antibacterial and antiviral agent. The health benefits of *Citrus aurantifolia, Citrus sinensis and Citrus limon* plants are highly associated with the high amount of bioactive constituents it contained such as phenols, flavonoids, carotenoid, vitamins and minerals [8,15]. Sweet oranges and lemons contain unique flavonoid compounds that have antioxidant and anti-cancer properties. The flavonoids help to inhibit cell division in many cancer cell lines in addition to its antimicrobial efficacy. The plant also demonstrated bioactive activities for cold, fever, sinusitis, sore throats, asthma and bronchitis [8]. Plants can contribute to the advancement of novel chemo-preventive agents as they have been proven essential in forming potentially useful structures. The initial steps to this achievement is performing antibacterial activities in this study, the phytochemical screening indicated that the extracts (Ethanol and methanol) of *Citrus* (Zest) contained *alkaloids, saponins, tannins, glycosides, phenol,* and carbohydrates, the phytochemicals were recorded in all Zest parts. The high phytochemical contents observed in the plant used in the present study indicate that it is a unique material for medicinal drug screening and research. A high quantity of the phytochemical recorded in the zest sample reported the presence of all the phytochemicals [8]. However, flavonoid is present in both zest and seed extracts in the present work. This results in pleasant aroma of citrus found in different geographical location of the plant [11]. Phytochemicals have been extensively studied and their medicinal properties documented. Alkaloids have been reported to possess various pharmacological activities including antihypertensive effects, antiarrhythmic effect, anitimalarial and anticancer activity [7]. Pure isolated alkaloids and their synthetic compounds have been used in medicine as an analgesic, antispasmodic and bactericidal agents. [14], reported the inhibition of pathogenic bacteria by alkaloids. Saponins from fruits and vegetables are

important dietary supplements and are known to exhibit antimicrobial activities and protect plants from microbial pathogens. They could be beneficial in modulating blood lipids, lower cancer risk and improve blood glucose response as well as possess antioxidant activity. Leafy vegetable such as *Citrus* zest and seeds are thus said to possess antimicrobial property attributed with reports on the antimicrobial potentials of *Citrus* obtained from other localities in Nigeria. Saponins which are special class of glycosides, have been reported to have a wide range of pharmacological and medicinal activities, and do not pose serious risk of toxicity in humans, even in large quantities, plants containing saponins are used in wound healing in folkoic medicine because they are good hemagglutinnins. The presence of terpenoids in the zest and seed of *Citrus* support its use in the treatment and management of cancer, ulcers. Plants produce volatile terpenses either to attract specific insect for pollination or otherwise to expel certain pests which consume these plants as food. In addition, terpenoids possess medicine properties such as anticarcinogenic, anti-ulcer, antimicrobial or diuretic activity therefore; Zest of Citrus could be used in ethnomedicine in the management of various ailments due to the presence of these terpenes. Tannin possesses astringent taste and help in healing of wounds and inflamed mucous membrane Tannins are also potent metal ion chelator; proton precipitating agents and biological antioxidants the high tannin content in the seed would make it a suitable choice especially in treatment of wounds bleedings [15].

CONCLUSION

In conclusion, results from this research indicated that *Citrus limon, Citrus sinensis* and *Citrus aurantifolia* are rich in phytochemicals and could be used in the treatment and management of different diseases.

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APPENDIX

Citrus sinensis (Sweet orange)

Citrus limon (Lemon)

Citrus aurantifolia (Lime)