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Phytochemical analysis of a folklore medicinal plant citrullus colocynthis L (bitter apple)

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ABSTRACT

Phytochemical screening of *Citrullus colocynthis* belonging to the family Cucurbitaceae was carried out for finding its medicinal values. A qualitative analysis was performed for the identification of the presence of different composition of elements. The leaf, root, flower (male, female), fruit (pulp, hull, seed) were photochemical analyzed separately and which shows the presence of alkaloids, carbohydrates, and flavonoids. Tannins, gums and mucilages are found to be absent.

Keywords: Citrullus colocynthis, phytochemical analysis, root, leaf.

1. Introduction

The chemical constituent of plants is desirable for the discovery of therapeutic agents and in discovering the actual value of folklore remedies. Medicinal plants contain organic compounds which provide definite physiological action on the human body and these bioactive substances includes tannins, alkaloids, carbohydrates, triterpenoids, steroids and flavonoids ^[1, 2]. These compounds are synthesized by primary or rather secondary metabolism of living organism. Secondary metabolism was chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas ^[3]. A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms [4]. Plant products have been part of phytomedicine since time immemorial. This can be derived from plant leaves, flowers, roots, fruits and seeds ^[5]. Various preparations formed with medicinal plants include decoction, emulsion, apozems, liniments, electroactives and powdered ^[6]. The medicinal plants are equally used in the cosmetic, perfumery and food industries ^[7]. The pharmaceutical industry first extracts the active ingredients before being used in the manufacturing of drugs. Hence, there is the possibility of discovering the evolution of drugs in the medicinal plants ^[8]. Citrullus colocynthis has the traditional use in remedy for cancer, carcinoma, endothelioma, leukemia, tumors of the liver and spleen, even the eye. A decoction of the whole plant, made with juice of fennel is said to help indurations of the liver. Roots may also be used as a purgative against as cites for jaundice, urinary diseases, rheumatism and for snake poison. This plant is available in the southern coastal areas of the Bay of Bengal. Traditional screening methods have been used to study the pharmacological effects of phytochemical compounds. Citrullus colocynthis is commonly known as the colocynth, bitter apple, bitter cucumber. It is a desert viny plant native to the Mediterranean basin and Asia. It resembles a common watermelon vine, but bears, small, hard fruit with a bitter pulp. Citrullus colocynthis is widely used in folk medicine for centuries and as an energy source also. E.g. Oilseed and biofuel. The leaves are diuretic and used in the treatment of jaundice and asthma. The root is useful in inflammation of the breasts, amenorrhea, rheumatism, joint pains and is used externally in ophthalmia and uterine pains. The fruit is pungent, cooling purgative, anthelmintic, antipyretic carminative. It cures, tumors, leucoderma, ulcers, asthma, bronchitis, urinary discharge, enlargement of spleen, tuberculosis glands of the neck, dyspepsia, constipation, anemia's and throat diseases. The fruit pulp is purgative, diuretic, antiepileptic, and is used against gonorrhea. The extracts of fruits, leaves, root and stem were also found to be potentially usable against many gram positive. Some of these extracts also resulted to have an insulin tropic effect.

2. Material and methods

2.1. Sample collection and preparation

Different plant parts of *Citrullus colocynthis* (stem, leaf, fruit, root and flower) were collected during the flowering and fruiting stage from the Karaikal coastal region, Puducherry, India. The collected parts of the plant were washed well in fresh water and shadow dried. The dried leaves were made into powder using mortar and pestle. The leaf powder was kept in a closed conical flask with solvents (1:10 ratio) separately, shake well for 10 min and kept at room temperature for 3 days, after that the extract is filtered. The filtrate was then dried under room temperature. The same procedure was followed for root, seeds, flower and fruits.

2.2. Phytochemical analysis

Phytochemical tests were carried out using different solvent extracts using standardized procedures to identify the constituents as described by Harbone. To assess the activity of selected medicinal plant. Preliminary phytochemical analysis was carried out for the extracts namely water, ethanol, chloroform, ether, acetone, ethyl acetate, butanol and benzene as per the standard method ^[10].

2.2.1. Preliminary phytochemical screening of *Citrullus colocynthis* ^[11, 12, 13]

Phytochemical screening was carried out for all the extracts, as per the standard methods.

I. Detection of alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered.

A. Mayer's test: Filtrates were treated with Mayer's reagent (potassium mercuric iodide).

Formation of a yellow coloured precipitate indicates the presence of alkaloids.

B. Wagner's test: Filtrates were treated with Wagner's reagent (iodine in potassium iodide). The formation of a brown/reddish precipitate indicates the presence of alkaloids.

C. Dragendroff test: Filtrates were treated with Dragendorff's reagent (solution of potassium bismuth iodide). Formation of a red precipitate indicates the presence of alkaloids.

D. Hager's test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Formation of yellow colour indicates the presence of alkaloids.

II. Detection of carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test the presence of carbohydrates.

A. Molisch's test: Filtrates were treated with 2 drops of alcoholic α -Naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.

B. Benedict's test: Filtrates were treated with Benedict's reagent and heated gently.

Orange red precipitate indicates the presence of reducing sugars.

C. Fehling's test: Filtrates were hydrolyzed with dilute HCl, neutralized with alkali and heated with Fehling's A and B

solutions. Formation of a red precipitate indicates the presence of reducing sugars.

III. Test for Tannins

1 g of each powdered sample was separately boiled with 20 ml water for five minutes in a water bath and was filtered while hot and cool filtrate was distilled to 5 ml with distilled water and a few drops (2-3) of 10% ferric chloride were observed for any formation of precipitates and any colour change. A bluish black or brownish green precipitate indicated the presence of tannins.

IV: Detection of flavonoids

A. Alkaline reagent test: Extracts were treated with a few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colorless on the addition of dilute acid, indicates the presence of flavonoids.

B. Lead acetate test: Extracts were treated with a few drops of lead acetate solution. Formation of a yellow colour precipitate indicates the presence of flavonoids.

V. Phyto sterols

A. Salkowski's test: Extract was treated with chloroform and filtrates were treated with a few drops of conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

B. Liebermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrated were treated with a few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. The formation of brown ring at the junction indicates the presence of phytosterols.

VI. Detection of protein and amino acid

A. Xanthoprotein test: the extracts were treated with a few drops of Conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

B. Ninhydrin test: to the extract, 0.25% w/v Ninhydrin reagent was added and boiled for a few minutes. Formation of blue colour indicates the presence of amino acid.

VII. Detection: of phenol

Ferric chloride test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

VIII. Diterpenes

Copper acetate test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of Diterpenes.

IX. Detection of glycosides: Extracts were hydrolyzed with dilute HCl, and then subjected to test for glycosides.

A. Modified Borntrager's test: Extracts were treated with ferric chloride solution and immersed in boiling water for about 5 min. The mixture was cooled and extracted with equal volumes of benzene layer was separated and treated with ammonia solution. Formation of rose pink colour in the ammoniacal layer indicates the presence of anthranol glycosides.

Test for cardiac glycosides: 5 ml of each extract was treated with

2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of concentrated sulphuric acid. A brown ring at the interface indicated the deoxy sugar characteristics of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may be formed.

X. Test for phlobatannins: deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous hydrochloride acid was taken as evidence for the phlobatannins.

Test for combined anthraquinones: 1 g of powered sample of each specimen was boiled with 2 ml of 10% hydrochloride acid for 5 mins. The mixture was filtered while hot and the filtrate was allowed to cool. The cooled filtrate was portioned against the equal volume of chloroform and the chloroform layer was transferred into a clean dry test tube using a clean pipette. Equal volume of 10 % ammonia solution was added into the chloroform layer, shaken and allowed to separate. The separated aqueous layer was observed for any change, delicate rose pink colour showed the presence of an anthraquinone.

Test for free anthraquinones: 5 ml of chloroform was added to 0.5 g of the powder. The resulting mixture was shaken for 5 min. after which it was filtered. The filtrate was then shaken with equal volume of 10% ammonia solution. The presence of a bright pink colour in the aqueous layer indicated the presence of free anthraquinones.

XI. Test for carotenoids. 1 g of each specimen sample was extracted with 10 ml chloroform in a test tube with vigorous shaking. The resulting mixture was filtered and 85% sulphuric acid was added. A blue colour at the interface showed the presence of carotenoids.

XII. Detection of fixed oils and fats

A. Spot test: A small quantity of the extract was pressed between two filter papers. Oil stain on the paper indicated the presence of fixed oil.

Detection of gum and mucilage: Extract was mixed with 10 ml distilled water and 25 ml of alcohol with constant stirring. White or cloudy precipitate indicated the presence of gums and mucilages.

3. Results and discussion

Phytochemical analysis conducted on the *Citrullus colocynthis* plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities ^[14].

Ashes represent the mineral salt or inorganic matter content of the drug. In rigorous conditions, it is a constant and allows us to find falsifications due to other drugs, soils or minerals. The ash value and successive values are tabulated in Table 1.

The powder as such expressed dry brown colour and when it was dissolved in water, no colour change was observed. Various colour changes were observed when treated with different chemical reagents such as FeCl₃, NaOH, H₂O, I₂, HCl, KOH, Ethanol, HNO₃, and H₂SO₄ and are tabulated in Table 2.

Plant material is composed of water, minerals, organic compounds, proteins, lipids and carbohydrates are called primary metabolism, phenol, tannins, flavonoids vitamin C, E are called secondary metabolism. In order to establish the identity, purity, safety and quality of the plant, standardization is an important tool for the herbal drugs. (Table. 3, 4) Plant material is composed of water, minerals, organic compounds, proteins, lipids and carbohydrates are called primary metabolism, phenol, tannins, flavonoids vitamin C, E are called secondary metabolism. In order to establish the identity, purity, safety and quality of the plant, standardization is an important tool for the identity, purity, safety and quality of the plant, standardization is an important tool for the herbal drugs. (Table. 3, 4)

S. No	Parameters	Citrullus colocynthis							
	1. Organoleptic character								
	a) Appearance	Powder							
	b) Colour	Pale green							
	c) Odour	Characteristic odour							
	d)Taste	Bitter							
	2. LOD at 105'C	1.1%							
	3. pH v	value							
	pH of 1 % Aqueous solution	4.56%							
	4. Ash v	values							
	a) Total	13.5%							
	b) Sulphated	10.09%							
	c) Water soluble	5.56%							
	c) Acid soluble	7.08%							
	5. Successive extractive values (%w/w)								
	a) water	28.31%							
	b)Ethanol	25.22%							
	c) Chloroform	3.53%							
	d) Ether	2.51%							
	e) Acetone	5.10%							
	f)Ethyl acetate	3.23%							
	g) Butanal	3.05%							
	h)Benzene	2.34%							

Table 1: Physico-chemical constants of the powder of Citrullus colocynthis

	Reagent	Colour change
1.	Powder as such	Green
2.	Powder + 2% FeCl ₃	No change
3.	Powder + 10% NaOH	Yellow
4.	Powder + 5% KOH	Yellow
5.	Powder + water shaken	Light brown
6.	Powder + Iodine	Yellow
7.	Powder + C_2H_5OH	Light brown
8.	Powder + HNO_3	No change
9.	Powder + H_2SO_4	Light yellow
10.	Powder + HCl	No change

Table 2: Behavior of powder with different chemical reagent

Fig 3: Qualitative phytochemical screening of *Citrullus colocynthis*

S.	Teata	Store	Loorog	Fl	Flower		Fruit			Constituent	
No	Tests	Stem	Leaves	Male	Female	Hull	pulp	Seed	Koot	Constituent	
1. Alkaloids											
	a) Mayer's test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve		
	b) Wagner's test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	Procence of	
	c) Hager' test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	alkaloids	
	d) Dragendorff's test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	arkatolus	
2. Car	bohydrates										
	a) Molisch's test			+ve	+ve		+ve	+ve	+ve	Presence of	
	b) Fehling's test	-ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	carbohydrate	
	c) Benedict's test	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve		
3.	Flavonoids test										
	a) NaOH	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	Presence of flavonoids	
	b) lead acetate	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve		
4.	Saponins										
	a) Foam test	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	Presence of	
	b) Froth test	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	saponin	
5.	Proteins										
	a) Biurett's test	+ve	+ve	-ve	-ve	+ve	-ve	+ve	-ve	Presence of	
	b) Ninhydrin's test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	protein	
6.	Phytosterols/Terpenoid	ls									
	a) Lieberman Burchard's test	-ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	Presence of sterols and	
	b) Salkowski's test	+ve	+ve	+ve	-ve	-ve	-ve	-ve	+ve	terpenoids	
7.	Tannins and phenol								1		
	a) Ferric chloride test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve		
	b) Lead acetate test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve		
	c) Alkaline reagent	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	Absence of	
	d) Mg/HCl reduction test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	Tannin	
8.	Glycosides										
	a) Borntrager's test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	Presence of	
	b) Legal's test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	athranol glycosides	
9.	Fixed oils										
	a) Spot test	-ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	Presence of	
	b) Saponification test	-ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	fixed oils	
10.	Gums and Mucilage										
		-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	Absence of gums ad mucilage	

S. No	Tests	Water	Ethanol	chloroform	ether	acetone	Ethyl acetate	buta nal	Benzene
1. Alka	1. Alkaloids								
	a) Mayer's test	+++	+++	+++	+++	+++	+++	+++	+++
	b) Wagner's test	+++	+++	++	+++	+++	++	++	+++
	c) Hager' test	+++	+++	+++	+++	++	+++	++	++
	d) Dragendorff's test	+++	+++	-	++	++	-	++	+
2. Carl	bohydrates								
	a) Molisch's test	++	++	-	++	++	++	+++	++
	b) Fehling's test	+	+	-	+	+	-	+	+
	c) Benedict's test	++	++	-	+	++	++	+++	++
3.	Flavonoids test	+++	+++	+++	+++	+++	+++	+++	+++
4. Sape	onins								
	a) Foam test	++	++	++	++	++	-	-	++
	b) Froth test	++	++	++	++	++	-	-	++
5. Prot	eins								
	a) Biurett's test	++	++	+++	+++	+++	+++	+++	+++
	b) Ninhydrin's test	-	-	-	-	-	-	-	-
6. Phyt	tosterols/Terpenoids								
	a) Lieberman Burchar's test	+++	+++	-	+++	+++	+++	-	+++
	b) Salkowski's test	-	-	+++	-	-	-	+++	-
7. Tan	nins and phenols								
	a) Ferric chloride test	-	-	-	-	-	-	-	-
	b) Lead acetate test	-	-	-	-	-	-	-	-
	c) Alkaline reagent	-		-	-	-	-	-	-
	d) Mg/HCl reduction test	-	-	-	-	-	-	-	-
8. Glycosides									
	a) Borntrager's test	+++	+++	+++	+++	+++	+++	+++	+++
	b) Legal's test	-	-	-	-	-	-	-	-
9. Fixed oils									
	a) Spot test	-	-	-	-	-	-	-	-
	b) Saponification test	-	-	-	-	-	-	-	-
10.	Gums and Mucilage								
		-	-	-	-	-	-	-	-

Table 4: Qualitative phytochemical screening of leaves of Citrullus colocynthis

The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolism ^[15]. They possess biological properties such as anti-apoptosis, anti-aging, anti carcinogen, anti inflammation, anti atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities ^[16]. Several studies reported that the plant parts are rich in phenolic compounds ^[17, 18]. Natural antioxidants mainly come from plants in the form of phenolic compounds such as flav phenolic acid, tocopherols etc. ^[19]. Tannins binds to proline rich protein and interfere with protein synthesis.

Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against a wide array of microorganism *in vitro*. It was reported that the activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall (20). They are also an effective antioxidant and show strong anticancer activities ^[21, 22, 23].

I. Alkaloids

Several workers have reported the analgesic ^[24, 25], antispasmodic preparation of insecticides, various drugs and the synthesis of steroid hormones ^[31]. Alkaloids have been associated with

and antibacterial ^[26, 23] properties of alkaloids. Alkaloids were strongly present in *Citrullus colocynthis*. The presence of alkaloids in most of these samples supported the reports of various authors ^[27]. Alkaloids are known to play some metabolic roles and control development in living systems ^[27]. The compound has a protective role in animal and it is used in medicine, especially the steroidal alkaloids which constituents most of the valuable drugs. Additionally, plant extract showing active trypanocidal activity was found to contain alkaloids, flavonoids, phenolic and or terpenes ^[28].

II. Saponin

The plant extracts were also revealed to contain saponins, which are known to produce inhibitory effect on inflammation ^[29]. Saponins are glycoside of both triterpenes and sterols and are used as expectorant and emulsifying agent ^[27, 30]. Hence saponin as sugar derivatives may be steroids or triterpenoids. The occurrence of steroidal saponins from numerous studies showed their importance and interest in pharmacy due to relationship with such compound as sex hormones mostly in the development of female contraceptive pills. Additionally, saponin is equally used in medicine and pharmaceutical industries because of its foaming ability with the production of frothy effect. Saponin is used in the medicinal uses for centuries and one of their common biological properties is their cytotoxicity ^[32].

Saponine has the property of precipitating ad coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness ^[33, 23]. Steroids have been reported to have anti-bacterial properties ^[34].

III. Flavonoids

These flavonoids display a remarkable array of biochemical and pharmacological actions, viz. Anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral and anti carcinogenic, activities ^[35]. These compounds appear to play vital roles in defense against pathogens and predators and contribute to physiological functions such as seed maturation and dormancy ^[36]. They are synthesizes from phenyl propanoids and acetate derived precursors. Flavonoids are important for human being due to their antioxidative and radical scavenging effects, as well as their potential estrogenic and anticancer activities ^[37]. The compound to be useful in disease resistance.

Table 5: Active conten	s of various Extracts:
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Water	Ethanol	Chloroform	Ether	acetone	Et. acetate	Butanol	Benzene
Alkaloids	Alkaloids	Alkaloida	Alkaloida	Alkaloids	Alkaloida	Alkaloida	Alkaloids
Carbohydrate	Carbohydrate	Flavonoida	Flavonoida	Carbohydrate	Carbohydrata	Corbobudroto	Carbohydrate
Flavonoids	Flavonoids	Flavoiloius	Flavoiloius	Flavonoids			Flavonoids
Saponin	Saponin	Saponin	Tormonoida	Saponin	Tarranaida	Tarmonoida	Saponin
Terpenoids	Terpenoids	Dratain	Drotoin	Terpenoids	Dratain	Dratain	Terpenoids
Protein	Protein	Anthropol	Arthropol	Protein	Anthropol	Anthropol	Protein
Anthranol	Anthranol	Anthranol	Anthranol	Anthranol	Anthranoi	Anthranoi	Anthranol
glycosides	glycosides	grycosides	grycosides	glycosides	grycosides	grycosides	glycosides

Table 6: Mechanism of action of some phytochemicals.

phytochemicals	Activity	Mechanism of action					
		Complex with cell walls, binds to adhesions					
		Inhibits release of autacoids and prostaglandins.					
Flavonoids	Antimicrobial	Inhibits contractions caused by spasm gens. Stimulates normalization of the deranged water transport across the mucosal					
1 lavonoidis	Antidiarroeal						
		cells.					
		Inhibits GI release of acetylcholine.					
Terpenoids and	Antimicrobial	Membrane disruption.					
essential oils	Antidiarrheal	Inhibits release of autacoids and prostaglandins.					
	Antimicrobial Antidiarrheal Anthelmintic	Intercalates into cell wall and DNA of parasites.					
		Inhibits release of autacoids and prostaglandins.					
A 111-1-1-		Possess anti-oxidizing effects. Thus reduces nitrate generation which is useful for					
Alkaloids		protein synthesis. Suppresses transfer of sucrose from stomach to small intestine.					
		Diminishing the support of glucose to the helminthes. Acts on CNS, causing					
		paralysis					
Lectins and							
polypeptides	Antiviral	Blocks viral fusion or adsorption, forms disulfide bridges.					
Glycosides	Antidiarrheal	Inhibits release of autacoids and prostaglandins.					
	Anticancer	Possesses membrane permeabilizing properties.					
Saponins	Anthelmintic	Leads to vacuolization and disintegration of teguments.					
	Antidiarrheal	Inhibits histamine release in vitro					
Steroids	Antidiarrheal	Enhance intestinal absorption of Na ⁺ and water.					

IV. Sterols

The presence of sterols in samples helps in the lowering of plasma cholesterol & LDB cholesterol. Hence its inclusion in the ruminant diet will assist in reducing drastically the morbidity & mortally caused by cardiovascular disease. Another point of concern is that plant sterol could act as a natural preventive dietary product ^[38]. Glycosides are known to lower the blood pressure, according to many reports ^[39]. The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and these plants are providing to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

However, all these chemicals were not extractable in one solvent? Alkaloids, phenolic, phenolic flavonoids and glycosides were present in chloroform extract; tannins, steroids and saponins were found in pet. ether extract; phenols, flavonoids and glycosides were present in methanolic extract; alkaloids phenolic, flavonoids and saponin were found in aqueous extract while acetone extract showed the presence of only steroids.

The availability of specific phytochemicals in plant gives it specific medicinal properties. Therefore the presence of above phytochemicals in *Citrullus colocynthis* can be correlated with its medicinal potential. Similar reports on the phytochemical composition of various medicinal plants were made earlier by many workers ^[40]. However, it is very essential to isolate the bioactive fractions from these major groups so that it can be used further in designing specific drugs.

The phytochemical screening revealed the presence of protein, carbohydrate, reducing sugar, flavonoids, saponins and alkaloids. The presence of these secondary metabolites suggests that the plant might be much of medicinal importance. The physical-chemical evolution of drugs is an important parameter in detecting adulteration or improper handling of drugs. It can serve as a valuable source of information and provide appropriate standards to establish the quality of this plant material in future study or application. (Table 5, 6)

4. Conclusion

The studied medicinal plant part has been potent bioactive compounds which could be used for therapeutic purpose and/or as precursors for the synthesis of useful drugs. It suggested that the decoctions, emulsion, apozenes or liquid extract or liniment or powders and others prepared from this medicinal plant may be very rich in nutrient composition and chemical substances, which may be of great importance to pharmaceutical companies. The phytochemical study of *Citrullus colocynthis* gives valuable information about the chemicals present in the plant. The behaviors of leaf powder upon treatment with different chemical reagents was also analyzed. The various qualitative chemical tests showed the presence of diterpenoids, saponin, sterols, flavonoids, carbohydrate and alkaloids. Aromatic acid, gums and mucilage and tannin were totally absent in the leaf, root, and seed of this plant.

5. References

- 1. Edogo HO, Okwu DE, Mbaebie BO. Phytochemicals constituents of some Nigerian medicinal plants. Afr J Biotechnol 2005; 4(7):685-688.
- 2. Mann J. Secondary metabolism. Oxford University press, London, 1978, 154.
- 3. Vasu K, Goud JV, Suryam A, Singara CMA. Biomolecular and phytochemical analysis of three aquatic angiosperons. Afr J Microbiol Res 2009; 3(8):418-421.
- Cowan MM. Plant products as antimicrobial agents. Clin Microbial Rev 1999; 564-582.
- 5. Criagg GM, Dravid JN. Natural product drug discovery in the next millennium. J Pharm Biol 2001; 39:8-17.
- Hilow MA, Abdi SB, Jeremiah E, Abdulahi HY. Ethnoveterinary medicine. Workshop on ethnoveterinary medicine in Kenya, Edn by Mathias E, Mundy P and Wilanyama JB, 2002.
- 7. Heinrich M. Ethnophamazieund Ethanobatanik. Eine Einfuhrung. Stuttgart (Germany) 2001.
- 8. Trease GE, Vans WC. Pharmacology. Edn 11, Braillier Tiridel and Macmilan publishers, 1983.
- Neube NS, Afolayan AJ, Okoh. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. African journal of bio technology 2009; 12c:1797-1806.
- Das K, Tiwari RKS, Shrivastava DK. Techniques for evolution of medicinal plant products as antimicrobial agent: current methods and future trends. Journal of medicinal plants research 2010; 42:104-111.
- Roopashree TS, Dang R, Rani SRH, Narendra C. Antibacterial activity of anti-psoriatic herbs: *Cassia tora*, *Momordica charantia* and *Calendula officinalis*. International Journal of Applied Research in Natural products 2008: 1(3):20-28.
- Obasi NL, Egbuonu ACC, Ukoha OP, Ejikeme PM. Comparative phytochemical and antimicrobial screening of some solvent extracts of *Samanea saman* pods. African Journal of pure and applied chemistry 2010; 4(9):206-212.
- 13. Audu SA, Mohammed I, Kaita HA. Phytochemical screening of the leaves of *Lophira lanceolata* (Ochanaceae). Life science Journal 2007; 4(4):75-79.
- 14. Sofowra A. Medicinal plants and traditional medicine in

Africa Spectrum Books Ltd., Ibadan, Nigeria, 1993, 191-289.

- 15. Singh R, Singh SK, Arorab S. Evaluation of antioxidant potential of ethyl acetate extract/fractions of *Acacia auriculiformis* A. Conn Fod Chem Toxicol 2007; 45:1216-1223.
- 16. Han X, Shen T, Lou H. Dietary polyphenols and their biological significance. Int J Mol Sic 2007; 950-988.
- Brown JE, Pice-Evans CA. Luteolin rich artichoke extract protects low density lipoprotein from oxidation *in vitro*. Free Radical Res 1998; 29:247-255.
- 18. Krings U, Berger RG. Antioxidant activity of roasted foods. Food chem 2001; 72:223-229.
- Ali SS, Kasoju N, Luthra A, Singh A, Sharanabasava H, Sahuand A, Bora U. Indian medicinal herbs as source of antioxidants. Food Res Int 2008; 41:1-15.
- 20. Marjorie C. Plant products as antimicrobial agent. Clinical microbial Rev 1996; 12:564-582.
- 21. Salah N, Miller NJ, Pagange G, Tijburg L, Bolwell GP, Rice E *et al.* Polyphenolic flavonoids as Scavenger of aqueous phase radicals as chai breaking antioxidant. Arc Bio chem Broph 1995; 2:339-346.
- Del-Rio A, Obudululio BG, Casfilles J, Main FG, Ortuno A. Uses and properties of citrus flavonoids. J Agric Food Chem 1997; 45:4505-4515.
- Okwu DE. Phytochemicals and vitamin content of indigenous species of southeastern Nigeria. J Sustain Agric Environ 2004; 6(1):30-37.
- 24. Antherden LM. Textbook of pharmaceutical chemistry. Edn 8, Oxford University Press, London, 1969, 813-814.
- 25. Harbone JB. Phytochemical methods. Chapman and Hall. London 1973, 113.
- 26. Stray F. The natural guide to medicinal herbs and plants. Tiger Books international, London, 1998, 12-16.
- 27. Edeoga HO, Omobuna G, Uche LC. Chemical composition of *Hyptis suaveolens* and *Ocimum gratissimum* hybrids from Nigeria. Afri J Biotechnol 2006; 5(910):892-895.
- 28. Le-Grand A. Anti-infection, phyto therapy of free Savannah. Senegal (West Africa) (III). J Ethanopharmacol 1989; 25:315-388.
- 29. Just MJ, Recio MC, Giner RM, Cucller MU, Manez S, Billia AR. Antiinflammatory activity of unusual lupine saponins from *Bupleurum fruticescens* 1998; 64:404-407.
- 30. Akundu MN. An investigation of anti-abortive properties of the leaves of *Ocimum gratissimum* M.Sc Thesis, university of Nigeria, Nsukka, Nigeria, 1984.
- 31. Okwu DE. The potential of *Ocimum gratissimum*, *Pengularia extensa* and *Tetrapleura tetraptera* as species and flavoring agents. Nig Agric J 2003; 34:143-148.
- Nobori T, Miurak K, Wu DJ, Takaboyashik LA, Carson DA. Detection of cyclin dependent, Kinase-4 inhibition gene in multiple human cancers. Nature 1994; 46:753-756.
- Sodipo OA, Akiniyi JA, Ogunbomosu JU. Studies on certain on certain characteristics of extracts of bark of *Pausinystalia macroceras* (K Schemp) Picrre Exbeille. Global J Pure Appl Sci 2000; 6: 83-87.
- Raquel FE. Bacterial liquid composition and antimicrobial efficacy of cationic steroid compounds. Biochemica Et Biophysica acte 2007; 2500-2509.
- 35. Middleton E, Andkandaswami C. The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer, in the flavonoids,

advances in research science (Wd.) Horborn, I.R., Chapman and hall, London, 1993, 619-645.

- 36. Winkel-shirley B. Biosynthesis of flavonoids and effects of stress. Curr Opin Plant boil 2002; 5:218-223.
- 37. Springob K, Saito K. Metabolic engineering of plant secondary metabolism promising approach to the production of pharmaceuticals. Sci Cul 2002; 68:76-85.
- Piirunon VM, David GL, Miettinen T, Toivo J, Lamp AM. Plant sterols, Biosynthesis function and their importance of human nutrition. J Sci Food Agric 2000; 87:939-966.
- 39. Nyarko AA, Addy ME. Elects of aqueous extract of an *Adenia Cissampeloides* on blood pressure and serum analyte of hypertensive patients. Phytotherapy Res 1990; 41(1):25-28.
- Chopra RN, Nayar SL, Chopra IC. Glossary of Indian medicinal plants. Vo1. Council of Scientific and industrial Res, New Delhi, 1956.