J. Basic. Appl. Sci. Res., 3(5)228-236, 2013
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ISSN 2090-4304
Journal of Basic and Applied Scientific Research
www.textroad.com

Phytochemical Analysis of Some Chemical Metabolites of Colocynth Plant (*Citrullus colocynths l*) and its Activities as Antimicrobial and Antiplasmodial

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ABSTRACT

Medicinal plants are the spine of long-established systems of medication accepted all the way through the world. They are worthless, barely credible and conventional source for the remedial assortment of diseases in the appearance of medicines. The aim of our current study was to evaluate the antibacterial, antifungal and antiplasmodial activities property of *Citrullus colocynthis*. Phytochemical screening of Citrullus colocynthis revealed the presence of glycosides flavonoids, tannin alakloids and saponnin. The primary metabolites such as glycosides- flavonoids- tannin- alakloids and saponnin. The activities of extract metabolites as antimicrobial, antifungal and antipalsmodial have, there been tested. Two cucurbitacin compounds (**E and I**) were found in fruit pulp and two (**B and E**) were found in fruit- rind. The highest inhibition effect against the bacteria studied were exhibited by ethanolic extracts of fruit-pulp and cucurbitacin **E,** where as **B** and **I** cucurbitacin showed moderate effect, cucurbitacin **B** and **I** showed a marked inhibitory effect against fungi. For antiplasmodial effect, the ethanolic extracts of the fruit-pulp at concentration of 500µg/ml, was the most active giving 100% inhibition of the parasite growth. . For antiplasmodial effect, the ethanolic extracts of the fruit-pulp at concentration of 500µg/ml, was the most active giving 100% inhibition of the parasite growth.

KEYWORDS: Citrullus colocynthis, Phytochemical Screening, Antimicrobial, Antifungal Antipalsmodial.

INTRODUCTION

Natural medicines have come from various sources like terrestrial plants, terrestrial microorganism, marine organisms, terrestrial vertebrates and invertebrates (Gill *et al.,* 2011). The plant is recognized in traditional medicine, several medicinal plants are used for the treatment of diabetes mellitus as a purgative, antidiabetic, rheumatism, snakebite, anti-tumor and insecticide (Newman *et al*., 2000). Due to their special importance in safety of communities, the medicinal plants receive attention to research centers. The curative properties of medicinal plants are mainly because it the presence of various complex chemical substances of different composition which occur as secondary metabolites (Karthikeyan *et al*., 2009; Lozoya *et al*, 1989). Properties of medicinal plants are due to the presence of various complex chemical substances from different composition which named secondary metabolites. They are categorized as alkaloids, glycosides, flavonoids, saponins, tannins, carbohydrate and essential oils (Sharafzadeh *et al* 2012). Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc (Gordon *et a*l., 2001).

Ethnobotanic inquiries have recorded the plants used in such a perspective, among which Citrullus colocynthis represent one of the most commonly used species. It is a broad extend annual unsophisticated plant, procumbent herb having diminutive flowers amid of yellow colour. The fruit is extremely bitter (Chaudhary *et al*., 1999). It grows speedy in the grimy soils as well as prevalent in diverse parts of Saudi Arabia. This lodge is used as anticancer agent in numerous drugs and also used as antipyometra in animals (Sudhanshu *et a*l., 2012).Until now, the numbers of chemical methods were used to extract a various parts of Citrullus colocynthis such seeds, pulps, leaves and roots. The presence in the whole plant of three alkaloids and two sterols in a petroleum extract of Citrullus colocynthis fruits have been reported (Afifi *et al*., 1973; Hatam *et al*., 1990). Methanol extract of Citrullus colocynthis fruits were employed to identify the cucurbitacins I, E, L, J, and T(Sonja *et al* 2000). *Other* extraction can be found glycosides as content in cucurbitacin(Nayab *et al*., 2006; Seger *et al*., 2005). The anti-inflammatory, purgative and anti-cancer

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inefficiencies, such as the inhibition of cell adhesion resulting from the cytoskeleton destabilizing in cancer cells exposed to cucurbitacin E (Jian *et al*.,2005)

Herein in this paper, we purpose the antibacterial, antifungal and antiplasmodial activities of Citrullus colocynthis. The phytochemical screening of showed that the glycosides, flavenoids, tannin and sterols have been presented in Citrullus colocynthis. Quantitative and qualitative analysis method were applied to determine the glycosides, flavenoids, tannin, sterols and secondary metabolites insoluble carbohydrates, total soluble sugar and amino acids. The of olocynth oil characterization, and antimicrobial, antifungal and antipalsmodial activities of extract metabolites have, there been estimated.

MATERIAL AND METHODS

Plant material collection

Roots, stems, leaves and fruits (Rind, pulp, seeds) of colocynth plant (*Citrullus colocynth*) were used as basic materials in this study, the plants were collected from wild habitat around Al-Kurma district, after rainy season.

Preparation of plant materials

The collected parts of the colocynth plant were separated for Roots, stems, leaves and fruits (Rind, pulp, seeds) then dried at room temperature and ground to fine size particles by using a motor and pestle and/or a hammer mill size 5 inch. These ready samples will be subjected for the following analysis.

1-Phytochemicals screening of the different parts of *Citrullus colocynthis*

a-Qualitative analysis will carried out for some secondary metabolites

To identify the phytochemicals (Glycosides, flavonoids, chemical tests were carried out by standard methods of analysis described by(Habrone, 1998.) where as cucurbitacins described by (Attard, E. and Scicluna- Spiteri, (2001)),

b-Quantitative analysis will carried out for some secondary metabolites

Determination of total saponin, Determination of total flavonoids will be carried out by method described by(Habrone 1998). Determination of total alkaloids The dried powder of each part of the colocynth plant (50gm) , was subjected to the following process of assay according to Sabri *et al* (1973), where as Determination of total cucurbitacins by extraction method described by(Attard and Scicluna Spiteri, 2001)

c-Isolation of methods for some secondary metabolites in the different parts of *Citrullus colocynthis*

Isolation of cucurbitacins 200 gm of each part of the plant were extracted with 150 ml ethanol (95%), the compounds expected to be cucurbitacins **,** were streaked on TLC then developed with the following different solvent system, benzene : ethyl acetate (85:15 v/v), Tolune : acetone (9:1 v/v), and chloroform : acetone (9:1 v/v), each extracted spot was re-purified by preparative and re-crystallized from cold chloroform – methanol mixture (7:3 v/v). Then TLC to isolate cucurbitacins using three solvent system, benzene: ethyl acetate, (85:15, v/v), toluene : acetone (9:1, v/v) and chloroform: acetone (9:1, v/v). The Rf values were measured for first and second compounds. These values were compared to Rf- values of the authentic standard (cucurbitacin E and cucurbitacin I).The compounds isolated from chloroformic extract of the fruit seeds was fractioned and re-crystallized by TLC spots showed brown colour with vanillic acid/ phosphoric acids. The purified cucurbitacin compounds were further confirmed by UV absorption and MS spectrum.

2- Antibacterial activity, antifungal activity and antiplasmodial activity tests.

Antimicrobial activity test: The petroleum ether, chloroform and ethanol extracts from each part of the colocynth plant was concentrated to dryness at room temperature. A stock solution (500mg/w/v) was prepared from each extract , each stock solution was then tested for anti-microbial activity against six different pathogens microorganism include Bacteria(*staphylococcus aureus, Klebsiella pneumonia, Bacillus cereus*) and Fungi(*Asperigillus niger, Asparigillus flavus, Candida albicans*) . Nystatin and Amoxycilline trihydrate as antibiotic were used for control, the antimicrobial activity test of the petroleum ether,chloroform and ethanol extracts of fruits (pulp, rind, seeds), roots, stems and leaves of *citrullus colocynth* were performed according the procedure of Bauerkirby as described by Prescott *et al*(1993). The inhibition zone around each disc as measured(Berdy,1982)

Antiplasmodial activity: The dried extract of each plant material were separately in RPMI 1640 liquid medium(Invetrogen, UK). The concentration 10,000 and 1000µg/ml were prepared in complete medium (RPMI 164 +10% human serum), on the day of test (Eltahir *et al*, 1999).For parasite cultivation and in-vitro testing, screening tests for parasite cultivation and *in-vitro* testing of different extracts were carried out according to the method recommended by WHO(2001). The highest drug concentration at which no schizonts growth, consider to be end point value for the test

$$\text{Maturation Percentage} = \frac{\text{No. of developed schizonts for test}}{\text{No. of developed schizonts for control}} \times 100$$

Inhibition percentage = 100 – maturation percentage (WHO, 2001)

RESULTS AND DISCUSSION

Powdered of dried parts of the colocynth plant were screened for the detection glycosides, flavonoids, saponins, tannin, alkaloids and curcurbitacins. The results of the quantitative phytochemicals screening Table (1), The different parts of the colocynth plant under investigation under were also analyzed glycosides, flavonoids, saponins, tannin,1 alkaloids and curcurbitacins Table (2). Figure (1 and 2) showed the ultraviolent spectra of E and I of authentic and the first and second compounds isolated from fruit-pulp of colocynth plant, respectively. Figure (3 and 4) showed the ultraviolent spectra of E and B of authentic and the first and second compounds isolated from fruit-rind of colocynth plant. Antimicrobial activities from different parts of *Citrullus colocynth* in addition to the isolated curcurbitacin compounds (E,B and I) are tested against *Staphylococcus aureus, (*gram-positive), *Klebsiella pneumoniae* (gram-negative) and *Bacillus cereus* (gram-positive), results were shown in Table (3). Table (4) showed antibacterial activity of the isolated cucurbitacin compounds. Antifungal activities were carried out for ethanol extracts of the fruit parts, stems,leaves and roots of *Citrullus colocynthis* in addition to the isolated cucurbitacins compound (E, B and I). These materials were tested for their ability to inhibit growth of fungi, *Candida albicans , Asperg*illu*s falvous*, and *Aspergillus niger*. Diameter of the inhibition zones were measured in millimeters, the results were shown in Table (5 and 6). Antiplasmodial activity of the fruit parts of colocynth plant on the number of the developed schizonts shown in Table (7) which is explained the corresponding results in Table (8) which expressed the percentage inhibition of *Plasmodium falciparum* growth after the *in-vitro* tests conducted.

Identification of chemical metabolites of the colocynth plant indicate that there are some variation as far as the type of the secondary metabolite is concerned and the part of the colocynth plant tested. For glycosides, flavonoids, saponins, tannin, alkaloids and curcurbitacins were found in all parts tested at high, moderate and low level, while tannins were also present in all parts tested at trace level. It was reported that alkaloids is active principles found in colocynth roots as reported by Amir and Haby (1960). And have purgative action (Dafni, 1984 and Burkill, 1985).The fruit of C. colocynths contain a mixture of glycosides, which possesses anticancer activity (Claus, 1976). Ahmed (1997, Kumar *et al* 2008)) reported that, phytochemical analysis of the ethanlolic extracts of colocynth plant –parts, revealed the presence of tannins in leaves, unsaturated sterols and tritepenes in roots and saponins in the fruits. Cucurbitacins or the bitter principles of cucurbits, tetracyclic- triterpenoid.

Tannin were determined in all parts of the colocynth plant , the result obtained indicated some variation in their quantities .For instance, the quality level was high in fruit-pulp, low in seeds and moderate in fruit rind (0.61%,0.06% and 0.32% respectively). The other tested part(stem, leaves and roots) contained lower amount of tannin. The highest cucurbitacins concentrations were found in the fruit- pulp. Nadkarmi (1984) also reported that, the active ingredients in the fruit-pulp in colocynth plant. Burkill (1985) confirmed that, the purgative action, which is mainly due to the cucurbitacins which was confined to the fruit-pulp. The bitter principles (cucurbitacins) were identified by spectro The maximum adsorption of the first compound and cucurbitacin E were almost the same at wave length $\lambda_{max}^{\text{chloroform}}$, 248.6 and 247.6 nm,respectively znd also the maximum adsorption of the second compound and the cucurbitacin B were almost the same at wave lengths $\lambda_{max}^{\text{chloroform}}$, 243.0 and 243.0 nm, respectively. The MS fragments of the isolated cucurbitacin E gave the same MS fragment pattern of the anthentic cucurbitacin E sample and with those values reported by (Hatam, 1989, Jian *et a*l 2005).

Table (1) Qualitative analysis of secondary metabolites in the different organs of *Citrullus colocynthis*

Tested secondary metabolite	Tested plant part					
	Pulp	seeds	Fruit rind	Stem	Leaves	Roots
Glycosides	+++	+++	+	++	++	+
Flavnoids	++	++	+	±	±	±
Saponin	+	±	+	-	-	-
Tannins	±	±	±	±	±	±
Alkaloids	+++	+++	++	+	+	++
Curcurbitacins	+++	++	++	++	++	++

The symbol +++, ++, +,±, - indicate a compound present in high, moderate, low, trace and absent level respective

Table (2) Percentage of total tannins, flavonoids, cucurbitacins, saponins, alkaloids and glycosides in the different organs of *Citrullus colocynthis*

Total plant part	Total Tannins %	Total Flavonoids %	Total Cucurbitacins %	Total Saponins %	Total alkaloids %	Total glycosides %
Pulp	0.61	0.71	0.83	0.41	0.06	0.21
Fruit{seeds	0.06	0.62	0.83	0.04	0.08	0.16
Rind	0.32	0.33	0.61	0.03	0.04	0.08
Stems	0.04	0.04	0.54	-	0.02	0.13
Leaves	0.03	0.03	0.52	-	0.01	0.12
Roots	0.02	0.02	0.54	-	0.04	0.05

The mass spectrum of the cucurbitacin E sample showed a molecular ion at m/z (C_{32} H_{43} O_7) the peak at m/z = 496 (9.56%) and 497(2.5%) were due to the loss of CO-$CH_3$and OH groups respectively. The peak at m/z = 400 and 383 were due to the loss of side chain by fission of the double bond between the carbon atoms23 and 24.

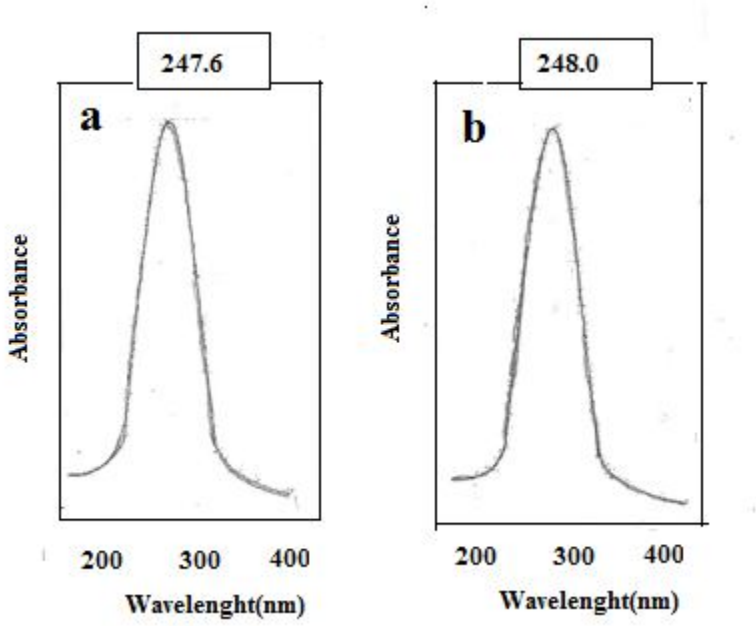

Figure (1) UV spectrum of standard cucurbitacin E (a) and the first compound (b) isolated from the fruit-pulp of *Citrullus colocynth*

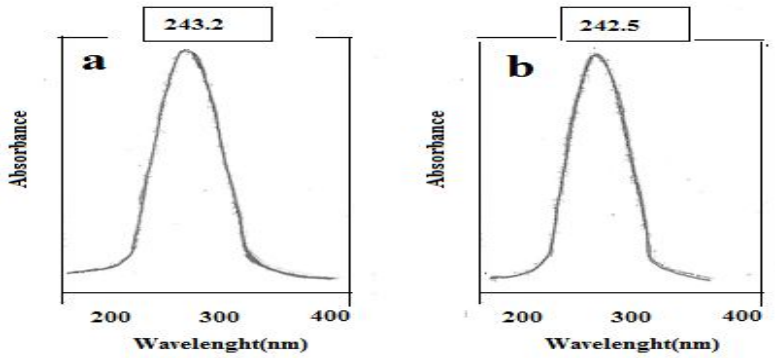

Figure (2) UV spectrum of standard cucurbitacin I (a) and the first compound (b) isolated from the fruit-pulp of *Citrullus colocynth*

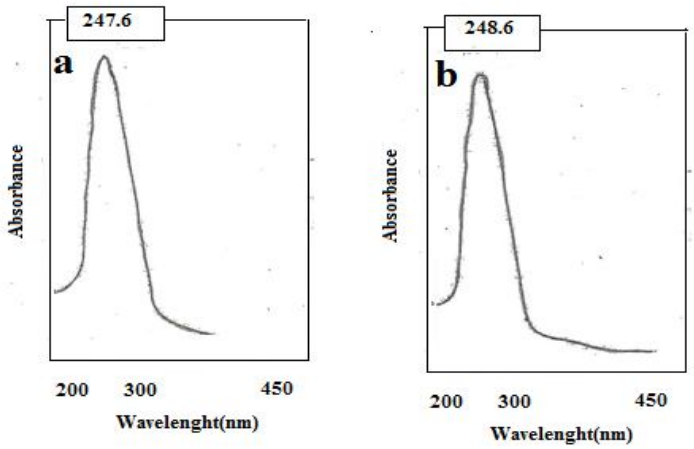

Figure (3) UV spectrum of standard cucurbitacin E (a) and the first compound (b) isolated from the fruit-rind of *Citrullus colocynth*

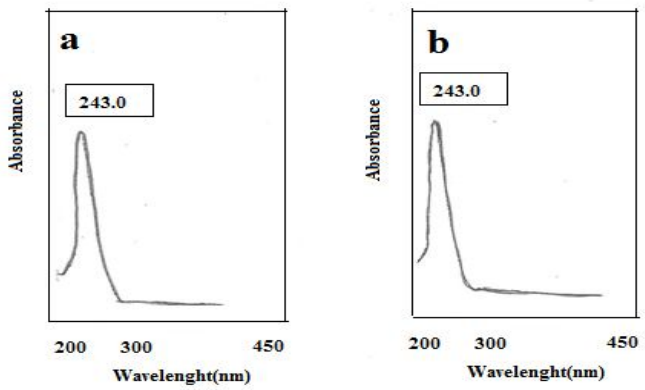

Figure (4) UV spectrum of standard cucurbitacin B (a) and the first compound (b) isolated from the fruit-rind of *Citrullus colocynth*

The mass spectrum of the isolated cucurbitacin B was compared with mass spectrum of the authentic cucurbitacin B sample. The comparison indicated similar mass pattern, which also similar to those reported by (Stuppner and Wangner,1989; hatam,1989).

The mass spectrum of the authentic cucurbitacin B sample showed a molecular ion at m/z = 496 corresponding to the molecular formula (C_{30} H_{43} O_7) Figure (6). The peak at m/z 190,

167,149,140,127,113,95 and83are characteristics of saturated ring A and unsaturated ring B presented in cucurbitacin B. The mass fragments of the isolated cucurbiatcin B on TLC plates gave R_f- values 0.075, 0.023 and 0.026 which is the same MS fragment pattern of the authentic cucurbitacin B compounds. These results were also reported by (Stuppner and wangner,1989; hatam,1989). cucurbitacin B gave a violet colour on TLC with vanillic acid/tification cucurbitacin B phosphoric acid reagent. Comparison of all these (TLC, UV and MS) with authentic samples spectrum leads to the identification of cucurbitacin B (2,26,20 trihydroxy-25 aceto-9 methyl-19 nor lanosta-5,23, diene-3, 11.22trione).

Antimicrobial activities From the result, it was found ethanol extract of the fruit- pulp have higher inhibitory effect against the tested bacteria than the ethanolic extracts of the fruit –rind and the seeds (Memon *et al* 2003, Shahla Najafi, *et al* 2010). On the other hand the ethanolic extract from stems, leaves and roots of colocynth plant have no an inhibitory effects in any one of the studied organism, it was reported that wide range of active secondary metabolites of plants as antimicrobial agents e.g phenolic compounds and their derive atives (Corthout *et al*, 1994), tannins (Sotohy *et al*, 1995; Sato *et al* 1998), flavonoids(Abdel Ghani *et al* 2008), glycosides (Vrkoc, 1975) and alkaloids (Sofoworam, 1979). It was also found that extracts of plants containing curcubitacins were shown to act as bacteriocides and fungicides (Elawad, 1981). Cucurbitacins have strong anti-tumour action, antimicrobial, ant-hepatotoxic, anti-inflammatory, anti-heminthic.

Table (3) Antibacterial activity of the ethanol extracts from different organs of *Citrullus colocynthis*

Ethanol extract of plant used (500mg/ml)	Diameter of inhibition zone in (mm)		
	S. rureus	***K.pneumoniae***	***B. bacillus***
Fruit- pulp	18	9	15
Fruit –rind	14	8	9
Fruit- seeds	13	8	7
Stems	-	-	-
Leaves	-	-	-
Roots	-	-	-
Reference drug (mg/ml)			
Amoxycilline200mg/ml	22	10	18

All values are the mean of three replication
-= No inhibition
Ethanol extract significant at $p \leq 0.001$
Bacterial types**:** significant at $p \leq 0.000$
Significantly different from control at $p \leq 0.001$

Table (4) Antibacterial activity of the isolated cucurbitacin compounds.

Cucurbitacin used (200mg/ml)	Diameter of inhibition zone in (mm)		
	S. rureus	***K.pneumoniae***	***B. bacillus***
Cucurbitacin E	18	9	16
Cucurbitacin B	16	8	10
Cucurbitacin I	16	8	10
Reference drug (mg/ml)			
Amoxycilline100mg/ml	20	10	15

Cucurbitacins type : not significant at $p \leq 0.187$
Bacteria types: significant at $p \leq 0.000$.
Significantly different from control at $p \leq 0.37$

Antifungal activities were carried out for ethanol extracts of the fruit parts, stems, leaves and roots of *Citrullus colocynthis* in addition to the isolated cucurbitacins compound (E, B and I). These materials were tested for their ability to inhibit growth of fungi, *Candida albicans* , *Asperg*illus *falvous*, and *Aspergillus niger*. Ethanol was based as control while antibiotic, Nystatin was used as reference drug. From the results, it was found that the ethanolic extract of the seed were more effective as antifungal agent, specially against *Candida albicans and Asperg*illus *falvou.* While the ethanol extract of stems, leaves and roots of the colocynth plant, had no effect against the tested fungi (*Candida albicans* , *Asperg*illus *falvous*, and *Aspergillus niger)* Mohammed (2005) reported that fungal isolates are more sensitive than bacterial isolates toward the aqueous extract of C. colocynthis fruits based on the effective concentration .The same results were recorded by Aqil and Ahmed (2003). 1'h" inhibitory action of the extract could be attributed to the presence of active compounds in the extract which are water soluble like glycosides and resins which inhibit enzymatic activity in cytoplasmic membrane. The extract inhibited the growth of all tested fungal isolates , this attributed to the presence of the active compounds. Colocynths alkaloids which may be disrupt cylto-plasmic membrane of the organisms through their action on lipids and protein (Anthony, 1976).

Table (5) Antifungall activity of the ethanol extracts from different organs of *Citrullus colocynthis*

Ethanol extract of plant used (500mg/ml)	Diameter of inhibition zone in (mm)		
	C. albicans	*A.falvus*	*A. niger*
Fruit- pulp	15	9	5
Fruit –rind	15	6	5
Fruit- seeds	17	9	6
Stems	-	-	-
Leaves	-	-	-
Roots	-	-	-
Reference drug (µg/ml)			
Nystatin200mg/ml	22	10	18

Cucurbitacins type : not significant at p≤ 0.007
Fungal types: significant at p≤ 0.000.
Significantly different from control at p≤ 0.046

Table (6) Antifungal activity of the isolated cucurbitacins compounds

Cucurbitacin used (200mg/ml)	Diameter of inhibition zone in (mm)		
	C. albicans	*A.falvus*	*A. niger*
Cucurbitacin E	16	9	9
Cucurbitacin B	15	9	9
Cucurbitacin I	15	9	8
Reference drug (mg/ml)			
Amoxycilline100mg/ml	20	10	15

Cucurbitacins type : not significant at p≤ 0.916
Fungal types: significant at p≤ 0.000.
Significantly different from control at p≤ 0.37

Antiplasmodial activity, The most active extract that produce 100% inhibition of the parasite growth at concentration of 500µg/ml, were obtained from the ethanolic extract of the fruit pulp. All extracts of the fruit-parts tested showed ≥ 50% inhibition of the parasites at concentration >50 µg/ml.≥. In all countries where malaria is endemic, plants are used in traditional medicines for treatment of the disease. The ethanolic extract *Cucurbita maxima* of the family Cucurbitaceae, showed a strong antimlarial activity (Amorim, 1991). In Yemen it was reported that *C. colocynthis* and *Tamarindus indica* have antimlaria activity (NISC, 2004). In this study ethanolic extracts of the fruit-pulpproduce 100% inhibition of *Plasmodium falciparum in-vitro* test, while the petroleum ether extracts of the seeds produced only 23.71% inhibition. Thus, the results indicated that colocynth plant display varying antiplasmodial activity against parasite *in-vivo* tests. These results give a good chance and useful guide for further phytochemical analysis to identify more active compounds. The preliminary phytochemical screening of plants under investigation (El-Hadi *et al*, 2010), revealed that none of them had shown the presence of cyanogenic glycosides. The tituents was found two plants which showed high antiplasmodial activity ≤ 50 µg/ml), *Nigella sativa* and *Aristolochia brateolata* showed the presence of sterols, alkaloids and tannins (Ahmed, 2004) .

Table (7) Antiplasmodial activity of solvent extracts from the fruit-parts (pulp-rind-seeds) of Citrullus colocynths on *plasmodium falciparum* in maturation percentage.

Solvent extract	Fruit part used	Number of developed schizonts (maturation %)			
		control	Concentration in µg/ml		
			5	50	500
Petroleum ether extract	Pulp	80(100%)	5(6.25%)	3(3.75%)	2(2.50%)
	Rind	80(100%)	40(50%)	10(12.50%)	5(6.25%)
	Seeds	80(100%)	61(76.29%)	14(17.50%)	10(12.50%)
Chloroformic Extract	Pulp	80(100%)	3(3.75%)	2(2.50%)	1(1.52%)
	Rind	80(100%)	4(5.00%)	3(3.75%)	2(2.50%)
	Seeds	80(100%)	19(23.75%)	8(10.00%)	6(0.05%)
Ethanolic extract	Pulp	80(100%)	2(2.50%)	1(1.1.25%)	0.0(0.0%)
	Rind	80(100%)	3(0.25%)	2(2.50%)	1(1.25%)
	Seeds	80(100%)	4(5.00%)	4(5.00%)	2(2.5%)
Reference drug µg/ml	Control	Concentration in µg/ml			
Chloroquine diphosphate	80(100%)	0.8	1.6	3.2	6.4
		1(1.25%)	0.0(0.0%)	0.0(0.0%)	0.0(0.0%)

Significantly different from control p ≤ 0.001

Table (8) Antiplasmodial activity of solvent extracts from the fruit-parts (pulp-rind-seeds) of Citrullus colocynths on *plasmodium falciparum* in inhibition percentage.

Solvent extract	Fruit part used	Percentage inhibition of *P.flaciarum*			
		control	Concentration in µg/ml		
Petroleum ether extract		0.0	5	50	500
	Pulp	0.0	93.75	96.45	97.50
	Rind	0.0	50.00	87.50	93.75
	Seeds	0.0	23.71	82.50	87.50
Chloroformic extract	Pulp	0.0	96.25	97.50	98.75
	Rind	0.0	95.00	96.25	97.50
	Seeds	0.0	76.25	90.00	99.95
Ethanolic extract	Pulp	0.0	97.50	98.75	100
	Rind	0.0	96.25	97.50	98.75
	Seeds	0.0	99.75	95.00	97.50
Reference drug µg/ml	control	Concentration in µg/ml			
Chloroquine diphosphate	0.0	0.81	1.6	3.0	6.4
		98.75	100	100	100

CONCLUSION

This study conclude that colocynth plant parts were highly effective as antifungal, antibacterial, and antiplasmodial factors. More investigation needed on the location of active ingredients, their physical and chemical properties, their antimicrobial potentialities as well as further research at the molecular level (in - vivo and in-vitro)

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