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Determination of antioxidant and anti-inflammatory activities, as well as *in vitro* cytotoxic activities of extracts of *Anastatica hierochuntica* (Kaff Maryam) against HeLa cell lines

Faten Abou-Elella¹, Eman A. Hanafy¹ and Yahaya Gavamukulya^{2*}

¹Department of Biochemistry, Faculty of Agriculture, Cairo University, 12613 Giza, Egypt.

²Department of Biochemistry and Molecular Biology, Faculty of Health Sciences, Busitema University, Mbale Regional Referral Hospital, 1460 Mbale, Uganda.

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Anastatica hierochuntica L. is distributed throughout the Arabian Peninsula, and North Africa. It is locally called "Kaff Maryam". All parts of the plant are used in folk medicine. This study aimed to investigate possible antioxidant activity of various extracts of Kaff Maryam, anti-inflammatory activities, as well as *in vitro* cytotoxic activities. Five extract types were used, namely, the whole ethanolic extracts, ethyl acetate, petroleum ether, water, and butanol extracts. The explored items included determination of total phenolics using the Folin-Ciocalteu assay and total flavonoids using Muller's method, anti-oxidant activity using three assays (2, 2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt (ABTS), reducing power, and 2, 2-diphenyl-1-picrylhydrazyl (DPPH)), membrane stabilization test for anti-inflammatory studies, as well as *in vitro* cytotoxic activities against HeLa cell lines using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. The results obtained indicated high flavonoid and phenolic contents in all the five extracts types. All extracts registered high antioxidant activity using three assays, but generally, the ABTS assay recorded the highest antioxidant activity as compared to the reducing power and DPPH methods. The membrane stabilization test showed that extracts of Kaff Maryam had good anti-inflammatory activity. Extracts of Kaff Maryam also registered very good cytotoxic activities against HeLa cell lines. Kaff Maryam extracts have good antioxidant, anti-inflammatory, and cytotoxic activities and the results of this study provide the basis for further investigation of Kaff Maryam for potential identification of novel bioactive compounds with therapeutic properties.

Key words: *Anastatica hierochuntica*, Kaff Maryam, antioxidant, anti-inflammatory, HeLa cell lines, cytotoxicity.

INTRODUCTION

There is a global increase in the use of medicinal plants for health reasons. In developing countries, herbal drugs and traditional remedies are relatively more popular, because of cultural acceptability and belief that being

natural, they are safe and non-toxic. Although, there are some new approaches to drug discovery, such as combinatorial chemistry and computer based molecular modeling design, none of them can replace the

importance of natural products in drug discovery and development (Gavamukulya et al., 2015; Heinrich and Bremner, 2006; Thirumal et al., 2012). Many synthetic drugs cause severe side effects that are not acceptable except as treatments of last resort for terminal diseases, such as cancer and the metabolites discovered in medicinal plants may avoid the side effect of synthetic drugs (Valko et al., 2007), because ideally, toxicity of such drugs is yet not well understood (Barnes et al., 2007; Salah et al., 2011). The medicinal values of these plants lie in their phytochemical components which produce definite physiological actions on the human body. The most important of these components are alkaloids, tannins, flavonoid and phenolic compounds (Nakashima et al., 2010).

Anastatica is a monotypic genus with the type species *Anastatica hierochuntica*. The genus is a member of the family Brassicaceae (formerly called Cruciferae), tumbleweed and a resurrection plant (Ihsanullah, 2012; Noura et al., 2011). *Anastatica* is found in arid areas in the Middle East and Sahara Desert, including parts of North Africa and regions of Iran, Egypt, Palestine, Israel, Iraq, Jordan, and Pakistan (Ahmad et al., 2006; Masayuki et al., 2003). *A. hierochuntica* L. locally called 'Kaff Maryam', is a well-known desert zone medicinal plant. Novel melanogenesis inhibitor flavonoids with antioxidant potential were isolated from it (Law et al., 2009). The plant is widely consumed as a tea beverage. It is powdered, mixed with honey and taken for the treatment of many conditions, in particular as a remedy for difficult childbirth and uterine hemorrhage (El-Ghazali et al., 2010; Khalifa and Ahmad, 1980). In addition, it is used to treat asthma, gastrointestinal disorders, depression, high blood pressure, indigestion, headache, cold, fever, malaria, epilepsy, fatigue, diabetes, heart disease, and infertility (Batanouny, 1999; Eman et al., 2011).

Antioxidants are a group of substances that are useful for fighting cancer and other processes that potentially lead to diseases, such as atherosclerosis, Alzheimer, Parkinson, diabetes, and heart disease (Aboul-Enein et al., 2012; Valko et al., 2007). Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), which are effective in their role as antioxidants, are commercially available and currently used in industrial processes. However, since suspected actions as promoters of carcinogenesis and other side effects have been reported, their use in food, cosmetic, and pharmaceutical products has been decreasing (Politeo et al., 2007). Thus, there has been an upsurge of interest in naturally-occurring antioxidants from vegetables, fruits, leaves, oilseeds, cereal crops, tree barks, roots, spices, and herbs (Rababah et al., 2004).

Inflammation is a very important aspect in membrane

injury. The mechanism of inflammation injury is attributed in part to release reactive oxygen species (ROS) from activated neutrophil and macrophages. This over release leads to tissue injury by damaging the macromolecule and lipid peroxidation of membranes. ROS propagate inflammation by stimulating the release of the cytokines, such as tumor necrosis factor, inter-leukine 1, which stimulate recruitment of additional neutrophil and macrophages. Thus, free radicals are important mediators that provoke or sustain inflammatory processes and consequently their neutralization by antioxidants and radical scavengers can attenuate inflammation (Lavanya et al., 2010).

The present study aimed to provide a comprehensive analysis of the total phenolic and total flavonoids contents, antioxidant, anti-inflammatory, and cytotoxic activities of different fractions of *A. hierochuntica* L.

MATERIALS AND METHODS

Preparation of *A. hierochuntica* extracts

The samples of *A. hierochuntica* were collected in the month of September 2014. They were identified by Dr. Narmein Shanan of the Ornamental Department at the Faculty of Agriculture in Cairo University. A voucher specimen was deposited in the Faculty Herbarium under the voucher number 14562 for future reference. The dried *A. hierochuntica* (115 g) was ground into powder and extracted with 500 ml of 70% ethanol by plant tissue homogenization method with modifications as previously described (Gavamukulya et al., 2014). The extraction was repeated twice and the total extracts were filtered, and the obtained extracts were concentrated using rotary evaporator to dryness. The crude extract was partitioned with 3×100 ml petroleum ether, and then successively partitioned with 3×100 ml of ethyl acetate, 3×100 ml of n-butanol, and 3×100 ml of water, respectively. The resulting four extracts were evaporated to dryness in vacuum.

Measurement of total phenolic content

The total phenolics content of the five different ethanolic extracts was determined with the Folin-Ciocalteu reagent (Singleton et al., 1999). Ferruleic standards were prepared (0 to 500 µg). To 50 µl of each sample (three replicates), 2.5 ml diluted Folin-Ciocalteu's reagent (1/100) and 2 ml of Na₂CO₃ (7.5%, w/v) were added and incubated at 45°C for 15 min. The absorbance of all samples was measured at 765 nm using a UV-VIS spectrophotometer (GAT UV-9100). A standard curve was plotted using different concentrations of Ferruleic and the results were expressed as Ferruleic equivalent.

Measurement of total flavonoid content

The total flavonoids content of the four different ethanolic extracts was evaluated (Christ and Muller, 1960). Rutin standards were prepared (10 to 100 µM). A standard curve was plotted using different concentrations of rutin and the results were expressed as

*Corresponding author. E-mail: gavayahya@yahoo.com. Tel: +256 775 869 783.



Figure 1. Kaff Maryam plant (BintBatman, 2013).

rutin.

Anti-oxidant activity using the reducing power assay

The reducing power of all samples was determined as described by Dorman et al. (2003). 100 μ l were added to 1 ml of distilled water and mixed with phosphate buffer (2.5 ml, 0.2 Mol/L, pH 6.6) and potassium ferricyanide [$K_3 Fe (CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. 2.5 ml of trichloroacetic acid (TCA, 10%) was added to the mixture, which was then centrifuged at 3000 rpm (MSE Mistral 2000, UK) for 10 min. The reaction was initiated by the addition of 200 μ l $FeCl_3$ (0.1%) and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The value is expressed as gallic acid equivalent (GAE)(Figure 1).

Measurement of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging activity

DPPH radical scavenging activity of tested extracts was evaluated as described by Blois (1958). The reaction mixture contained 50 μ l of test samples (or 80% MeOH as a blank) and 5 ml of a 0.04% (w/v) solution of DPPH in methanol. Different standard antioxidants BHT and BHA were used as a positive control. Discoloration was measured at 517 nm after incubation for 30 min. Measurements were performed in triplicate.

2, 2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt (ABTS) assay

The antioxidant activity of the samples was also measured by ABTS assay according to the method of Re et al. (1999). $ABTS^{+}$ was produced by reacting 7 mmol/L ABTS aqueous solution with 2.45 mmol/L potassium persulfate in darkness for 12 to 16 h at room temperature. Prior to assay, this solution was diluted in aqueous and equilibrated at 30°C. Sample of 0.2 ml was mixed with 3.0 ml of diluted ABTS cation radical solution. The absorbance at 734 nm was measured using spectrophotometer. The percentage inhibition was calculated of the control absorbance at 734 nm.

Anti-inflammatory activity using human red blood cell (HRBC) membrane stabilization assay

HRBC membrane stabilization assay was performed (Gandhidasan et al., 1991). The assay system consisted of 100 μ g/ml of the test methanol extract, 1 ml phosphate buffer (0.15 M, pH 7.4), 2 ml of hyposaline (0.36%), and 0.5 ml of HRBC suspension. Standard was prepared by using Diclofenac sodium (50 mg/ml). Control consisted of distilled water instead of hyposaline. The assay systems were incubated at 37°C for 30 min and centrifuged at 3000 rpm for 20 min. The hemoglobin (Hb) content of the supernatant solution was estimated spectrophotometrically at 560 nm. The percentage stabilization of HRBC membrane was calculated by using the formula: (Absorbance control-absorbance sample) / (absorbance control) \times 100.

Cytotoxic activity using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay

The culture medium was prepared using RPMI 1640 media with 1.2 g/L sodium carbonate and L-glutamine (Gibco, Grand Island, USA), 10% inactivated fetal bovine serum (Gibco), and 100 units/ml penicillin and 100 mg/ml streptomycin were added. The cytotoxic activity of the different 4 fractions on leukemia cell line (HeLa cell line) was determined by the MTT assay (Denizot and Lang, 1986). Cells at exponentially growing phase were used. Five thousand cells per well (100 μ l) were plated in 96-well plates in the presence of various concentrations of the extracts (10, 20, and 30 μ g/ml) for 24 h at 37°C in 5% CO_2 incubator. Fytoside was used as the positive control. The activity of mitochondrial succinic dehydrogenase was measured by incubation for 4 h in the presence of 0.5 mg/ml of MTT. Absorbance reflects the viable cell number and was measured at 570 nm. % Cell death was calculated using the following formulas (Thirumal et al., 2012). % Cell death = (Control OD – Sample OD) / Control OD \times 100.

Statistical analysis

Quantitative and graphical data was analyzed using Microsoft Excel Package. The results of each series of experiments (performed in

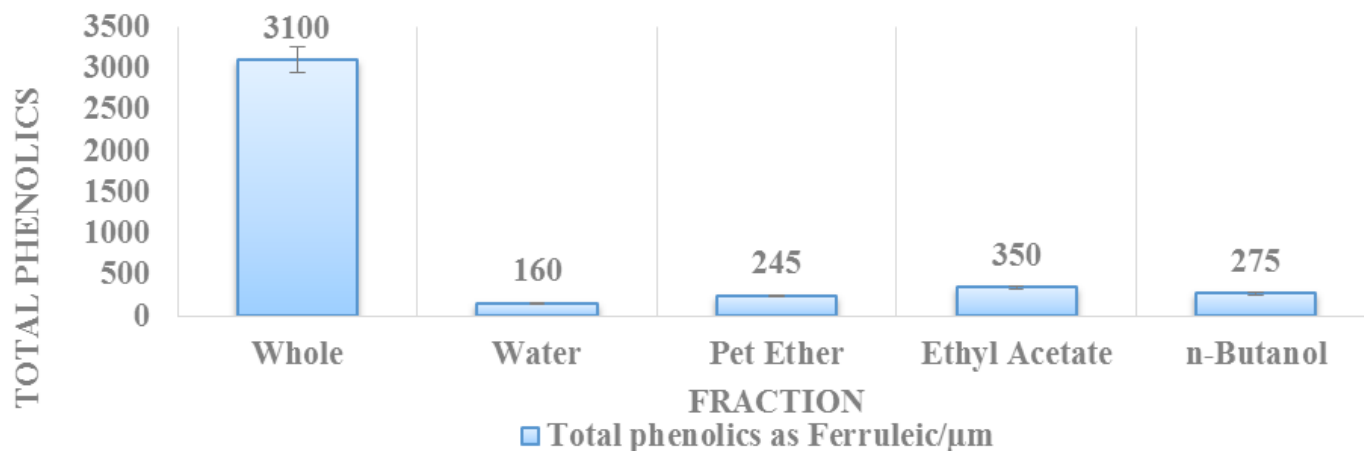


Figure 2. The total phenolic content of the different extracts of Kaff Mariam (Standard curve equation: $y=1\text{E-}04x$; $R^2=0.9357$).

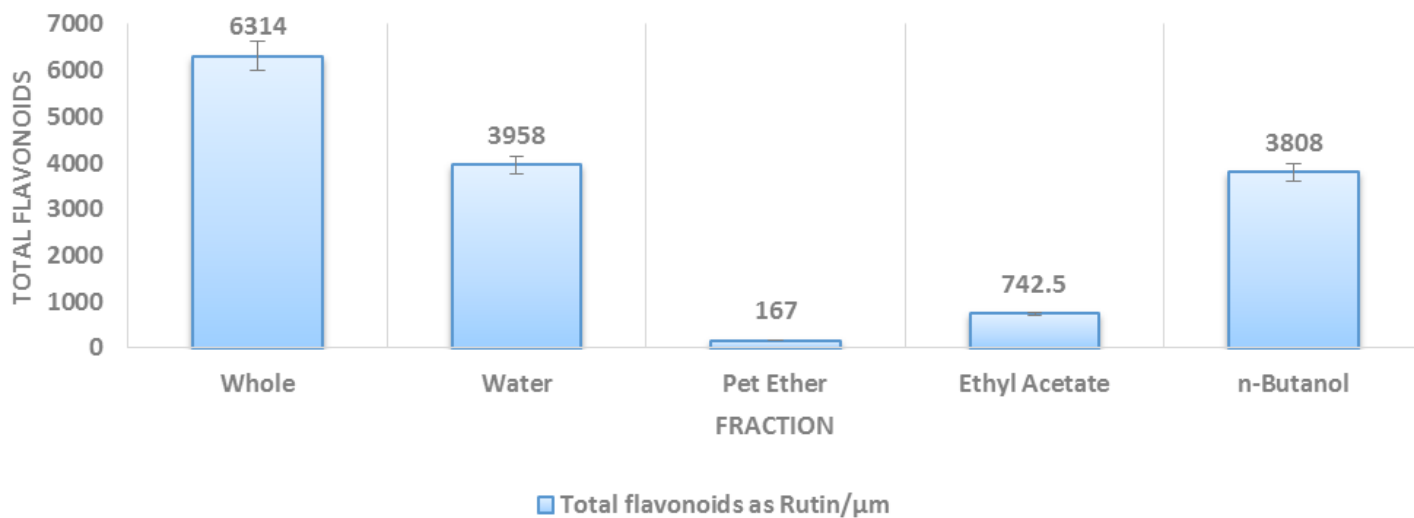


Figure 3. Total flavonoids of different fractions of Kaff Mariam (Standard curve equation: $y=0.0016x$; $R^2=0.9762$).

triplicates) were expressed as the mean \pm standard deviation. Graphs of percentage activity against logarithm of concentration were generated for the different parameters (Reducing power, DPPH, ABTS, anti-inflammatory, and cytotoxic activity), and the IC_{50} values estimated therein. P-values less than 0.05 were considered statistically significant.

RESULTS

Determination of total phenolics

It was noted that the highest content was registered in the whole extract at 3100 ferruleic acid equivalents (FAE), while the lowest was in the water extract at 160 FAE. Phenols play a crucial role in the antioxidant activity of plant extracts and an increase in the content is

a direct indication of the expected activity (Figure 2).

Determination of total flavonoids

A standard curve for rutin was generated for the determination of total flavonoid content. Figure 3 shows the total flavonoid content of the different extracts of Kaff Mariam. The highest content was registered in the whole extract at 6314 μm as rutin, while the lowest was in the pet ether extract at 167 μm as rutin. As shown in Figure 2, the whole, water and n-butanol extracts had relatively higher flavonoid content of more than 3000 μm as rutin, while that of ethyl acetate and pet ether had slightly lower content. The role of flavonoids in health has been well covered in various literatures, and thus this recorded high

Table 1. Reducing power of different fractions of Kaff Mariam.

Sample	% Activity			IC ₅₀	
	Concentration (µg/ml)	100	200		400
Whole extract		73.32±0.06	100±0.12	100±0.31	23.63
Pet ether		0	0	0	0
Ethyl acetate		16.77±0.02	29.09±0.07	47.33±0.12	472.1
Butanol		6.29±0.02	8.12±0.003	19.55±0.009	11401.9
Water		0	5.08±0.004	23.53±0.05	2168.99
BHA		100±0.14	100±0.05	100±0.08	N/A
BHT		100±0.20	100±0.09	100±0.06	N/A

Standard curve equation: $y=0.0039x$; $R^2=0.7283$

Table 2. DPPH activity of different fractions of Kaff Mariam.

Sample	% Activity			IC ₅₀	
	Concentration (µg/ml)	100	200		400
Control		0	0	0	0
Whole extract		35.34±0.003	64.88±0.06	72.41±0.06	150.85
Pet ether		0	0	14.84±0.004	13448.6
Ethyl acetate		0	32.11±0.02	68.73±0.04	278.3
Butanol		0	12.89±0.02	52.70±0.03	419.18
Water		0	12.17±0.02	49.20±0.008	459.72
BHA		59.61±0.14	58.55±0.18	65.01±0.18	11.71
BHT		75.29±0.04	75.3±0.09	75.37±0.07	6x10 ⁻¹⁸⁹

content projects higher antioxidant, scavenging, and cytotoxic activity in the corresponding fractions.

Determination of antioxidant activity

Determination of reducing power

Gallic acid was used as a standard for the determination of reducing power of the extracts. As shown in Table 1, the reducing power of the different extracts was determined across the ranges of concentrations (100, 200, and 400 µg/ml). Generally, with exception of pet ether, which did not register any reducing power activity by this method, all the remaining extracts registered some good activity. There was a general increase in reducing power activity with increase in concentration of the extracts. From the different extracts activity, the whole extract had the highest IC₅₀ at 23.63, since the lower the IC₅₀, the higher the reducing power and eventually the antioxidant activity. Ethyl acetate followed in activity, while the remaining fractions had graded lower activity, water and butanol, respectively. Standard antioxidants BHA and BHT were used to compare the effectiveness of the method and as shown in the Table 1, registered the highest activity.

DPPH radical scavenging activity

Table 2 shows the antioxidant activity of the different extracts using the DPPH radical scavenging assay. The control registered no activity at all, as expected, and the standard antioxidants BHA and BHT showed very good antioxidant activity across the range of concentration as shown, resulting in very low IC₅₀ values and resultantly very high antioxidant activity. All the extracts showed good activity which increased with increase in concentration of the extracts, giving on average IC₅₀ values of 150 to 460, with exception of pet ether extract that only showed activity at 400 µg/ml, and a staggering IC₅₀ of 13448.6. The highest antioxidant activity was registered by the whole extract with an IC₅₀ of 150.85.

ABTS anti-oxidant activity

The antioxidant activity of the extracts was also determined using the ABTS assay. Generally, the best activity was registered using this method as indicated in Table 3. With exception of the pet ether extract which showed the lowest activity (IC₅₀ 1085), the rest of the extracts had very good activity which increased with increasing concentration. The whole extract had the

Table 3. ABTS activity of different fractions of Kaff Mariam.

Sample	% Activity			IC ₅₀	
	Concentration (µg/ml)	100	200		400
Control		0	0	0	
Whole extract		91.47±0.006	91.68±0.001	93.18±0.02	3x10 ⁻¹³
Pet ether		36.25±0.006	34.75±0.002	45.42±0.13	1085
Ethyl acetate		65.78±0.01	70.36±0.02	92.53±0.02	51.388
Butanol		78.14±0.03	80.28±0.03	98.51±0.004	17.69
Water		74.31±0.05	85.29±0.008	96.16±0.009	21.37
BHA		99.25±0.002	99.57±0.001	100±0.18	3x10 ⁻³⁸
BHT		68.23±0.01	79.85±0.008	99.36±0.002	47.09

Table 4. *In vitro* anti-inflammatory activity of tested ethanolic extracts of Kaff Maryam by membrane stabilization test.

Plant extracts (100 µg/ml)	% Anti-inflammatory activity
Whole	57±14.73
Water	48±10
Pet ether	16.67±0.58
Ethyl acetate	7±3.46
Butanol	28±0
Sodium diclofenac	71.33±2.31

highest activity registering an IC₅₀ value of 3x10⁻¹³, far higher than that of one of the positive controls, BHT which was recorded at 47.09. Similarly, the water and butanol extracts had IC₅₀ values higher than that of BHT.

Figure 4 is a general comparison of the antioxidant activity of the five extracts using the different assays. In all the methods used, the whole extract recorded the highest activity with the lowest IC₅₀ values, while the pet ether extract recorded the lowest activity in all the assays. A general summary indicates that the best method of assay was the ABTS method which generated the highest activities, followed by the DPPH assay, and least on the list was the reducing power assay. All methods show high antioxidant activity of the plant, Kaff Mariam.

Anti-inflammatory activity by HRBC membrane stabilization test

Table 4 summarized the HRBC membrane stabilization effect of the whole ethanolic extract and the different extraction fractions. Accordingly, this is one of the primary reports on the *in vitro* membrane stabilization potential of Kaff Maryam extracts. In this study, the maximum anti-inflammatory activity was found in the whole ethanolic extracts of Kaff Maryam at a concentration of 100 µg/ml (57±14.73), whereas the standard drug diclofenac showed 71.33±2.31%

protection. The other four ethanolic extracts have weak anti-inflammatory activity, with water extracts having a slightly higher one at 48±10%, while the ethyl acetate fraction registered the lowest activity at 7±3.46%.

Cytotoxic activity against HeLa cell line

The cytotoxic activity of the extracts of Kaff Mariam against HeLa cell lines was determined using the MTT assay. With exception of the negative control which showed no cytotoxic effect, all the extracts had an increase activity with increasing concentration. Fytoside, the positive control had the highest activity with an IC₅₀ of 0.00002, and it is anticipated as this is usually in a purified form. The level of increasing activity was registered in a graded level with ethyl acetate fraction having the highest activity, followed by water and pet ether, then the whole extract and finally the butanol fraction registered the lowest activity. The results, as shown in Table 5 indicate a generally high cytotoxic activity in the Kaff Mariam extracts.

DISCUSSION

It is well-known that plant phenolic compounds are highly effective free radical scavengers and antioxidants. Polyphenolic compounds have inhibitory effects on

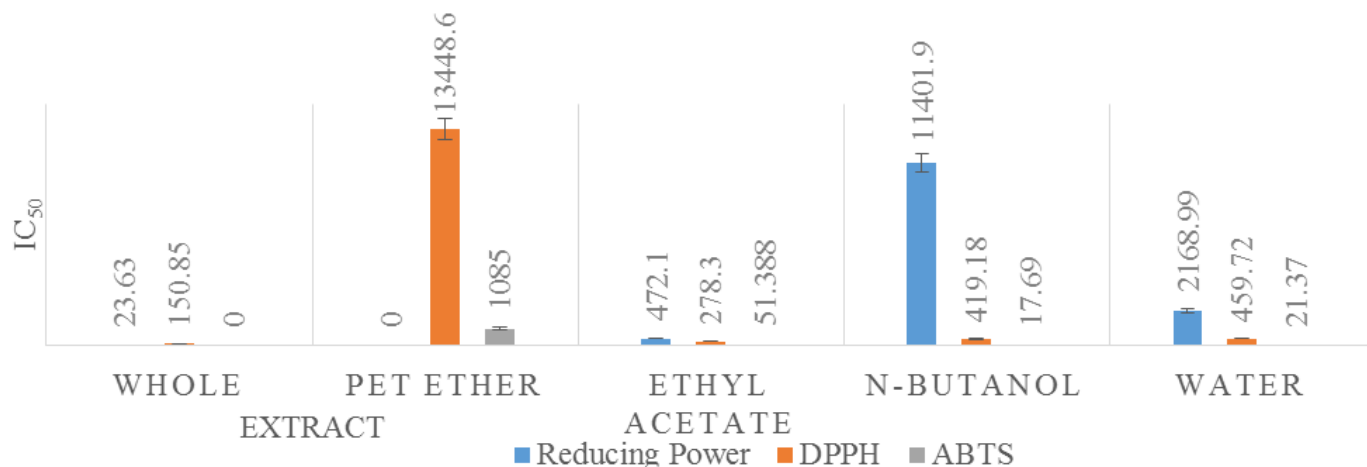


Figure 4. Comparison of IC₅₀ values of the different anti-oxidant assays.

Table 5. Cytotoxic activity of the different ethanolic extracts of Kaff Mariam against HeLa cell lines.

Sample	% Activity (Cell death)			
	10 µg/ml	20 µg/ml	30 µg/ml	IC ₅₀
Negative control	0	0	0	0
Whole extract	60.67±1.16	64±1.17	68±1	2.02
Pet ether	77.67±3.06	81.67±1.53	84.67±1.53	0.12
Ethyl acetate	81.67±2.31	82.67±3.51	86.33±1.16	0.0036
Butanol	55.67±12.2	83.67±0.58	85±1.73	7.54
Water	78.67±1.16	82.33±1.53	85.67±0.58	0.12
Fytoside	77.75±0.5	78±3.65	80.25±1.26	0.00002

mutagenesis and carcinogenesis in humans, when up to 1.0 g daily is ingested from a diet rich in fruits and vegetables (Tanaka et al., 1998). The total phenolic content of Kaff Maryam was high and the extracts exhibited potent antioxidant activity. It has also been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The action mechanisms of flavonoids are through the scavenging or chelating process (Kessler et al., 2003). Compounds, such as flavonoids, which contain hydroxyl functional groups, are responsible for the antioxidant effect in the plants (Das and Pereira, 1990). Carotenoids may act as a singlet oxygen quencher and can transfer one electron to the radicals, giving rise to a stable carotenoid radical cation regenerating the original molecule (Mortensen and Skkibsted, 1997). All these factors taken together may influence the performance of the plant with respect to phytochemical content. The total phenolics and total flavonoids of the Kaff Maryam extracts indicate high content within the range of solvents used. The highest content registered by the whole extract could be as a result of the ability of the ethanolic extract

to extract more of the bioactive components, as compared to the rest of the solvents.

Ferric-Ferrous transition to measure the antioxidant activity of Kaff Maryam extracts using the reducing power assay was investigated. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Meir et al., 1995). The antioxidant activities of putative antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical scavenging (Diplock, 1997). The reducing power of Kaff Maryam showed significant activity across the different ranges of extraction solvent fractions used. However, the activity was low compared to that of the remaining two assays, DPPH and ABTS as subsequently discussed in details. Generally, high reducing power registered indicates a very high antioxidant potential of the plant under study. It exhibited a dose dependent reducing activity within the applied concentrations.

The proton radical scavenging action is known as an

important mechanism of antioxidants. The model of scavenging the stable DPPH radical is a widely used method to evaluate antioxidant activity in a relatively short time compared with other methods. The effect of antioxidants on DPPH radical scavenging was thought to result from their hydrogen donating ability (Shimada et al., 1992). DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares et al., 1997). The decrease in absorbance of the DPPH radical caused by antioxidants, because of the reaction between antioxidant molecules and the radical, progresses, which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a discoloration from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate the anti-oxidative activity of natural antioxidants. Phenolic compounds of Kaff Maryam extracts are probably involved in their antiradical activity (Hsu et al., 2006). Although, the activity is relatively lower than that of BHT and BHA, the extracts may be viable source of bioactive compounds with better activities after fractionation. The results obtained using this method indicate a relatively high antioxidant activity of the plant. The differences in activity arise from the ranges of extraction solvents used. However, in general, all the extracts showed high activity as measured by the DPPH method, an indication of their high antioxidant activity.

The ABTS antioxidant assay can be used to determine the total antioxidant capacity of biological fluids, cells, and tissue. It can also be used to assay the antioxidant activity of naturally occurring or synthetic compounds for use as dietary supplements, topical protection, and therapeutics. The assay measures ABTS⁺ radical cation formation induced by metmyoglobin and hydrogen peroxide. This method was used to assay for the antioxidant activity of the different fractions of Kaff Maryam. A strong dose dependent activity was registered by all the extracts, with the highest activity being shown by the whole extract of ethanol. In general, the ABTS assay generated the highest antioxidant activity as compared to the remaining the assays of reducing power and DPPH assay. The ABTS method could have generated the highest activity due to its various advantages as compared to the former methods of reducing power and DPPH assays (Shalaby and Shanab, 2013).

Results of anti-inflammatory activity by HRBC membrane stabilization demonstrated that ethanolic extracts of Kaff Maryam inhibit heat-induced hemolysis of erythrocytes. This indicates that this extract possess biological membrane stabilization properties preventing stress-induced destruction of the plasma membrane. Stabilization of lysosomal membrane is important in limiting the anti-inflammatory response by preventing the release of lysosomal constituents of activated neutrophils such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon

extracellular release (Murugasan et al., 1981). The erythrocyte membrane is analogous to lysosomal membrane 66 and its stabilization implies that the extract may as well stabilize lysosomal membranes.

The *in vitro* cytotoxic activity of extracts of Kaff Maryam against HeLa cell lines was determined using the MTT assay. With exception of the negative control, which showed no effect on the HeLa cell lines, the rest of the extracts showed very high cytotoxic activity, a good step towards the authentication of the use of this plant in therapeutics include cancer management. All extracts showed high cytotoxic activity as compared to their corresponding low antioxidant activity, such as that of the pet ether fraction. This trend of where extracts which show high cytotoxic activity have antioxidant activity, but not vice versa is not a new phenomenon as it has been earlier on demonstrated (Aboul-Enein et al., 2012; Gavamukulya et al., 2014).

An integrated part of cancer cell development is the resistance to programmed cell death (apoptosis) and therefore re-establishment of apoptosis in cancer cells is a target mechanism for anticancer agents (Joshi et al., 1999). Some plant derived products are known to selectively induce apoptosis in cancer cells, which represent the ideal property for successful anticancer agents (Joshi et al., 1999; Wamidh, 2011). The current study showed the highly effective action of the ethanolic extracts of Kaff Maryam and can be used in the management and treatment of cancer and other common diseases. This is in line with a study which showed that any extract had anticancer and cytotoxic activity if it had an IC₅₀ value less than 1000 µg/ml after 24 h contact time, and that the smaller the IC₅₀ value of a test compound, the more toxic the compound (Hirano et al., 1995). The results obtained in this study all have IC₅₀ values far lower than 100, indicating the strong cytotoxic activity of Kaff Maryam.

The present findings exhibited a relation between antioxidant, cytotoxic and anti-inflammatory activities of ethanolic extracts of Kaff Maryam. In this regard, the antioxidant compounds play an important role in the later stages of cancer development. There is increasing evidence that oxidative processes promote carcinogenesis, although the mechanisms are not well understood. The antioxidants may be able to cause the regression of premalignant lesions and inhibit their development into cancer. Several herbs and spices in addition to plenty of other medicinal plants are reportedly exhibiting antioxidant activity (Abou-Elella and Ahmed, 2015; Rajapakse and Kim, 2011; Souza et al., 2011). Majority of the active antioxidant compounds are flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, and isocatechins. In addition, vitamins C and E, β-carotene, and - tocopherol present in natural foods, are known to possess anticancer potential (Abou-Elella and Ahmed, 2015; Genovese et al., 2012). Thus, potential antioxidant, anti-

inflammatory, and anticancer properties of plant extracts (or isolated products of plant origin) can possibly be explored for developing the anticancer drugs.

Conclusion

The objective of this study was to compare the antioxidant activity of extracts of Kaff Maryam using various ethanolic extract fractions, determining anti-inflammatory activities, as well as investigating the *in vitro* cytotoxic activities of the same extracts on HeLa cell lines. Very good antioxidant activity was recorded across the three assays used, as well as relatively high total phenolics and total flavonoids. The extracts were also found to have good anti-inflammatory activity, and high cytotoxic activity against HeLa cancer cell lines. The identification of novel bioactive compounds with cytotoxic properties, and the elucidation of the mechanisms by which the cytotoxic properties derived from the natural products work are of immense importance. The results of this study provide the basis for further investigation of Kaff Maryam for potential identification of novel bioactive compounds with therapeutic and anti-cancer properties.

Conflict of interests

The authors have not declared any conflict of interest exists.

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