Investigation of Antimicrobial Activities, Antioxidant Activities And Isolation Of Pure Organic Compounds From *Euphorbia hirta* L.

Yinn Kay Khaing[1], Su Hlaing Hnin[2], Khin Htay Win[3], Thidar Khaing[4], San San Win[5], Htay Htay Shwe[6], Tin Tin Moe[7]

Abstract

In this research work, the whole plant of *Euphorbia hirta* Linn (Kyar-ba-hon) was collected from Meza village, Sagaing Division. Firstly, phytochemical screening of the selected sample was performed with standard procedures. The elemental composition of this plant was examined by using EDXRF method. Furthermore, antimicrobial activities of the sample were tested by Agar-well diffusion method on six tested organisms. The antioxidant activity of ethanol crude extract was determined by using 1-1-Diphenyl-2-picryl hydrazyl (DPPH) radical scavenging assay. In addition compound I and compound II were isolated from the plant of Kyar-ba-hon by using Thin Layer and Column Chromatographic separation methods. The isolated pure organic compound I, yellow crystalline powder (13mg,0. 65%) and pure unknown compound II, (10mg, 0.5%) were obtained. The melting point of pure compound I was measured. The functional groups of these isolated compounds were identified by FT-IR spectral data. From melting point determination, color test and FT-IR spectral data of compound I should be assigned as quercetin.

Keyword : Phytochemical, EDXRF, Antimicrobial activities, Antioxidant activities, FT-IR

Introduction

Natural products produced by living organisms that show biological action have been developed as powerful drugs to combat diseases and to save the lives of millions. Natural products were still a significant source of new drugs especially inthe anticancer therapeutic areas. These facts have led many scientists all over the world to explore useful and fascinating natural products from living things such as plants, animals and microorganisms. (DJ Newman and GM Crag, Prod. 2007) Kyar-ba-hon plant is relatively easy to use: the plant is harvested whole at itsflowering stage, boiled in water for about 15 min and then the decoction is given to the patient as tea. (NM Parcon, AghamMindanaw 2006).The plant *Euphorbia hirta* L. belongs to the family Euphorbiaceae. It is a small annual herb common to tropical countries. It can grow to a height of 40 cm. The plant is commonly called asthma herb. (E.A Soforowa, John Wiley and Sons, 1982).Kyar-ba-hon was distributed throughout the hotter part of Myanmar, often found in waste places along the roadsides.

The stem is slender and often reddish in color, covered with yellowish bristly hairs especially in young parts. The leaves are oppositely arranged lanceolate and are usually greenish or reddish underneath measuring about 5 cm long. The stem and leaves produce white or milky juice when cut. (E.M.Lind and A.C.Tallantire, 1971). The leaves are used to treat cough, asthma, worms and vomiting. It has vermifuge properties and it is also eaten as vegetables. The white latex is used as eye drops to cure conjunctivitis. Paste of leaf is applied externally (twice daily) on the place of scorpion bite. *Euphorbia hirta L.* latex is applied on swellings, piles, and boils. (P.C. Trivedi, K. Maheswari, 2002) Root decoction is also used for snake bites, sores, wounds, boils, and is beneficial for nursing mothers with deficient milk. The entire plant is prescribed as an antidote; it is considered haemostatic, sedative, and soporific. In

[1, 3-7] Dr., Lecturers, Department of Chemistry, University of Mandalay.
[2] M.Sc Student, Department of Chemistry, University of Mandalay.

Australia, the most common use of *Euphorbia hirta* L.is to treat hypertension, asthma, edema, and pectoral complaints. (Yan *et al*., 2011).

In this research, one of Myanmar indigenous medicinal plants, *Euphorbia hirta* L. (Kyar-ba-hon) was selected for chemical investigation. Purecompounds were isolated from the plant of *Euphorbia hirta* L. by using solvent extraction, Thin Layer and Column Chromatographic methods. These isolated compounds were determined by FT IR spectral data.

Material and Methods

Sample collection

The whole plant of *Euphorbia hirta* L. was collected from Meza, Sagaing Division. The plants were avoided from exposure to sunlight to prevent the loss of active components. The sample was cut into small pieces and allowed to air dry. The dry pieces were stored in a well-stoppered bottle and used throughout the experiment.

Preliminary Phytochemical Constituents of *Euphorbia hirta* L.

The phytochemical tests were carried out to detect the presence or absence of organic constituents in the *Euphorbia hirta* L. (Harborne, 1973)

Determination of Elements in *Euphorbia hirta* L. by Energy Dispersive X-ray Fluorescence (EDXRF) Method

The elemental content of the powder of *Euphorbia hirta* L. plant were examined by the energy dispersive X-ray fluorescence (EDXRF) spectrometer at Department of Chemistry, University of Monywa.

Determination of Antimicrobial Activities of *Euphorbia hirta* L. (Kyar-ba-hon)

Antimicrobial Activities of *Euphorbia hirta* L. (Kyar-ba-hon) were performed by agar well diffusion method on six tested organisms, (*Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *E-Coli*) in CRDC (Central Research and Development Centre) Insein, Yangon.

Determination of Antioxidant Activity of the Whole Plant of *Euphorbia hirta* L. by DPPH Radical Scavenging Assay

1-1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging assay was chosen to access the antioxidant activity of ethanol extract of the sample. The antioxidant activity of ethanol extract was determined by DPPH method in Department of Chemistry, University of Mandalay. (Manzocco*et al.,* 1998)

Preparation of Ethanol Crude Extract

Ethanol extract of the whole plant of *Euphorbia hirta* L. was prepared by the following procedure. About 200 g of the dried sample was percolated with 2 L of 95 % ethanol for two months and filtered. Evaporation of the solvent provided ethanol crude extract of the whole plant of *Euphorbia hirta* Linn.

Preparation of Test Sample Solution

0.001 g of the crude extract of the whole plant of *Euphorbia hirta* L. was dissolved in 10 mL of 95 % ethanol under vigorous shaking. After filtration, the filtrate was used as stock solution. Desired concentration (100, 50, 25, 12.5, 6.25 μg/mL) of sample solution were prepared from this stock solution by dilution with appropriate amount of 95 % ethanol.

Preparation of 6μM DPPH Solution

2.436 mg of DPPH was dissolved in 100 mL of 95 % ethanol. This solution was thoroughly mixed at room temperature and it was stored in brown colored flask. This solution kept for no longer than 24 hours.

Preparation of Standard Ascorbic Acid Solution

0.1 mL of ascorbic acid was dissolved in 100 mL of distilled water under shaken and it was used as a standard ascorbic acid solution (stock solution). Desired concentration (50, 25, 12.5, 6.25, 3.125 μg/mL) of ascorbic acid solution were prepared from this stock solution.

Two-Fold Serial Dilutions

A two-fold dilution reduces the concentration of a solution by a factor of two that is reduced the original concentration by one half. A series of two-fold dilutions is described as two-fold serial dilutio

$$\% \text{ inhibition} = \frac{(\text{Absorbance of control-Absorbance of sample})}{\text{Absorbance of control}} \times 100\%$$

This formula is the calculation of percent inhibition of (IC_{50}) value. The half maximal inhibitory concentration (IC_{50}) is a measurement of the effectiveness of a substance in inhibiting a specific biological or biochemical function.

Determination of Half Maximal Inhibitory Concentration

IC_{50} value was calculated by using the linear regressive equation. Ascorbic acid was used as a positive control in the bioassay.

Extraction of Crude Sample

The air dried sample (200 g) were percolated with 95 % ethanol (2000 mL) for two months and then filtered, the filtrate was evaporated at room temperature. Then the ethanol extract was reextracted with ethyl acetate (100 mL). The ethyl acetate extract was evaporated at room temperature. Then, the residue obtained was checked by TLC and chosen the solvent system for Column Chromatography.

Isolation of Pure Compound I and II

The ethyl acetate extract (1.5 g) was chromatographed on silica gel column as eluting was various ratios of n-hexane and ethyl acetate which gave rise to the fractions. Each fraction was checked by TLC and then factions of the same R_f values were combined. Eighteen combined fractions were obtained. The combined fractions IX and XI have found to be main portions. These fractions have shown only one spot on TLC. Combined fraction XI gave compound I, yellow crystalline (0.13 mg) and combined fraction IX gave compound II, pale yellow oily form (0.17 mg). And then compound I and compound II were recrystallized by (3:2 v/v) and (7:3 v/v) in n-hexane and ethyl acetate.

Thin Layer Chromatography of Pure Compounds I and II

According to the Thin Layer Chromatogram, the only one spot on TLC plate were found at fractions IX and XI. The yield percent of the fractions were found to be 0.05% and 0.06% based upon the ethyl acetate crude extract. The R_f values of the compound were 0.60 and 0.3.

Results and Discussion

Botanical Description

Family name - Euphorbiaceae
Botanical name - *Euphorbia hirta* L.
Local name - Kyar - ba- hon
Part used - the whole plant

Figure (1) The Plant of *Euphorbia hirta* L.

Phytochemical Constituents of the Plant of *Euphorbia hirta* L.

Table (1) Results of Phytochemical Tests of *Euphorbia hirta* L.

No	Test	Extract	Reagents	Observation	Remark
1	Alkaloid	1 % HCl	(1) Dragendorff's reagent (2) Wagner's reagent	Pale orange ppt Reddish brown ppt	+
2	Flavonoid	95 % ethanol	Conc: HCl, Mg turning	Pink color solution	+
3	Glycoside	D/w	10 % Lead acetate	White ppt	+
4	Steroid	95 % Ethanol	$CHCl_3$, Acetic anhydride, Conc:H_2SO_4	Green color solution	–
5	Tannin	D/W	2 % NaOH, 10 % $FeCl_3$, Dil H_2SO_4	Pale brown ppt	+
6	Terpene	Pet ether	Acetic anhydride $CHCl_3$	Pink color solution	–
7	Polyphenol	95 % ethanol	1 % $K_3[Fe(CN)_6]$ $FeCl_3$	Greenish blue ppt	+
8	Phenolic	D/W	10 % $FeCl_3$	Brown color solution	+
9	Reducing sugar	D/W	Benedict's solution	Brick ppt	+
10	Saponin	D/W	-	Froth like solution	+

Present = (+) Absence = (–) ppt = precipitate

According to this table, the plant extract consists of alkaloid, flavonoid, glycoside, tannin, polyphenol, phenolic, reducing sugar and saponin, respectively.

Determination of Elemental Contents of the Kyar-ba-hon Plant Sample ssby EDXRF Method

The elemental contents of the Kyar-ba-hon plant were determined and the results were shown in Table (2).

Table (2) The Elemental Composition of Kyar-ba-hon by EDXRF

No	Elements	Symbols	Amount (%)
1	Potassium	K	1.441
2	Calcium	Ca	0.717
3	Silicon	Si	0.640
4	Phosphorus	P	0.374
5	Sulfur	S	0.235
6	Iron	Fe	0.023
7	Titanium	Ti	0.005
8	Manganese	Mn	0.004
9	Zinc	Zn	0.003
10	Strontium	Sr	0.003
11	Copper	Cu	0.002
12	Rubidium	Rb	0.001
13	Bromine	Br	0.001

According to EDXRF result, it can be known that the present of potassium content is highest. The content of calcium, silicon, phosphorus, sulfur and iron are in significant amount. The content of titanium, manganese, zinc, strontium, copper, rubidium and bromine are also small.

Determination of Antimicrobial Activities of the Kyar-ba-hon Plant

The antimicrobial activities of the Kyar-ba-hon plant were performed by Agarwell diffusion method, on six tested organisms as shown in Table (3).

Table (3) Antimicrobial Activities of Kyar-ba-hon

Samples	Solvent	Organisms					
		I	II	III	IV	V	VI
Kyar - ba-hon	n-hexane	–	11 mm (+)	–	11 mm (+)	–	–
	EtOAc	–	14 mm (+)	–	14 mm (+)	14 mm (+)	13 mm (+)
	EtOH	–	11 mm (+)	–	12 mm (+)	11 mm (+)	11 mm (+)

Agar-well – 7 mm Organisms
7 mm ~ 11 mm (+)
12 mm ~ 16 mm (++)
17 mm above (+++)

I = *Bacillussubtilis*
II = *Staphylococcusaureus*
III = *Pseudomonasaeruginosa*
IV = *Bacilluspumilus*
V = *Candidaalbicans*
VI = *E. coli*

According to table (3), the ethyl acetate and ethanol crude extract of Euphorbia hirta L. (Kyar-ba-hon) responds low activities on four tested organisms such as *Staphylococcus aureus, Bacillus pumilus, Candida albicans*and *E-coli.* N-hexane crude extract also responds low activities on two tested organisms such as *Staphylococcus aureus*and *Bacillus pumilus.*

Determination of Antioxidant Activity of the Standard Ascorbic Acid

The result of antioxidant activity using DPPH assay in standard ascorbic acid was shown in Table (4).

Table (4) % Inhibition of Various Concentration of Standard Ascorbic Acid

Sample Concentration (µg/mL)	Mean absorbance	% inhibition	IC_{50} µg/mL
50	0.628	89.936	11.98
25	0.224	64.103	
12.5	0.288	53.84	
6.25	0.341	45.35	
3.125	0.397	36.378	

IC_{50} value was calculated by using linear regressive equation

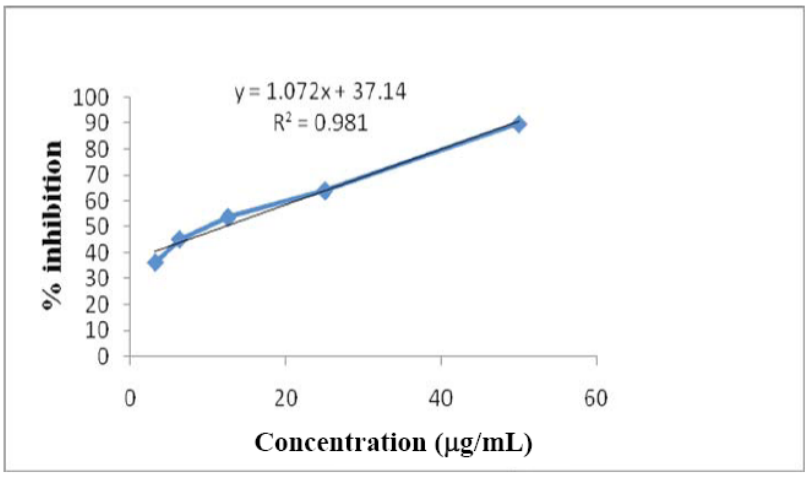


Figure (2) Plot of % Inhibition Vs Concentration of Standard Ascorbic Acid

Determination of Antioxidant Activity of the Plant of Kyar-ba-hon By DPPH Assay

The result of antioxidant activity using DPPH assay of Kyar-ba-hon was shown in Table (5).

Table (5) % Inhibition of Various Concentration of Sample

Concentration µg/mL	Mean absorbance	% inhibition	IC_{50} µg/mL
100	0.031	95.032	6.67
50	0.125	79.968	
25	0.216	65.385	
12.5	0.295	52.724	
6.25	0.363	41.827	

IC_{50} value was calculated by using linear regressive equation

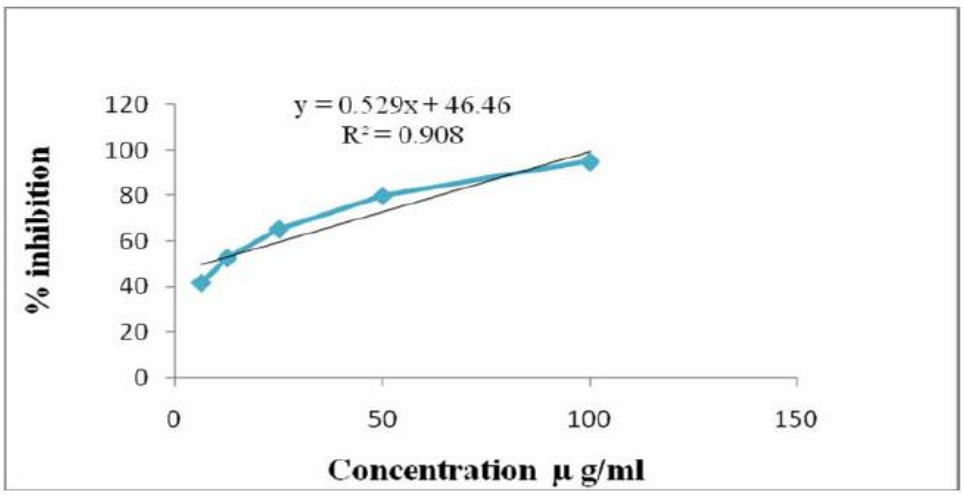


Figure (3) Plot of % Inhibition Vs Concentration of the Plant of *Euphorbia hirta* L.

From the table, the antioxidant activity of the Kyar-ba-hon plant was determined by PPH free radical scavenging assay. In DPPH screening assay the IC_{50} value of the Kyar-ba-honplant was found to be 6.67 μg/mL. It was very much lower than that of standard ascorbic acid (IC_{50}= 11.98 μg/mL). So, the sample extract has higher antioxidant activity than standard ascorbic acid.

Functional Groups Determination of Pure Compound I and Other Pure Compound II

The FT IR spectra of compound I and other unknown compound II were measured at Department of Chemistry, University of Monywa. They were shown in Figure (4) and Figure (5).

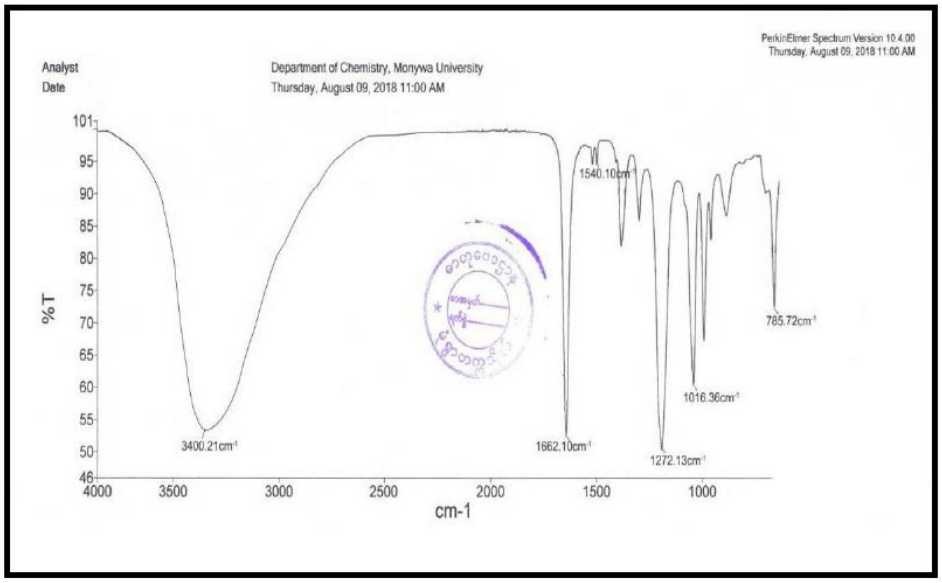
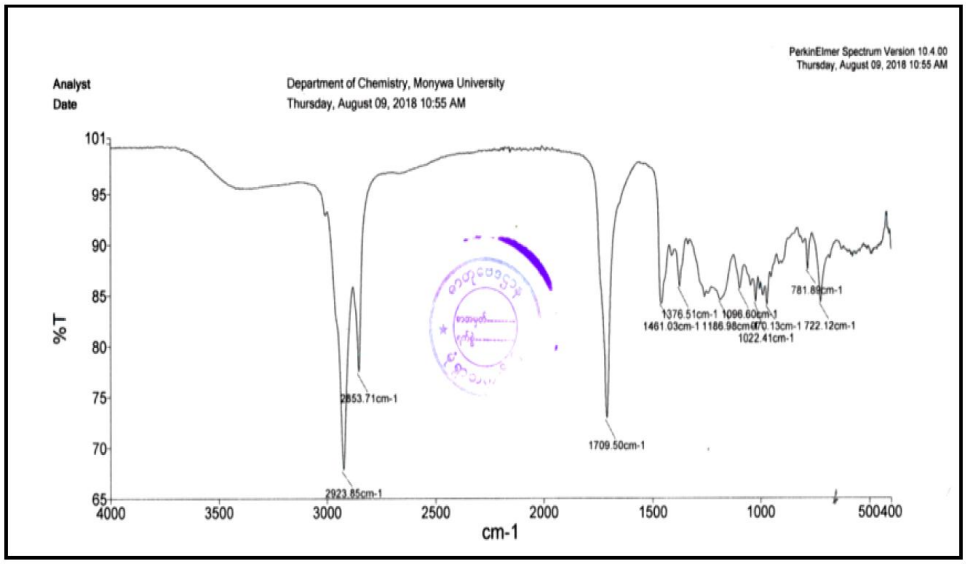


Figure (4) FT IR Spectrum of Compound Figure



(5) FT IR Spectrum of Compound II

Table (6) FT IR Spectral Data of Pure Compound I

No	Absorption wavelength (cm^{-1})	Functional group
1	3400.21	O-H stretching vibration of alcohol group
2	1662.10	C=O stretching vibration of carbonyl group
3	1540.10	C=C ring skeletal stretching vibration of benzene ring
4	1272.13	O-C-C stretching vibration of alcohol group
5	1016.36	C-O-C stretching vibration of ether group
6	785.72	C-H out of plane bending vibration of cis or Z alkenic group

Table (7) FT IR Spectral Data of Pure Compound II

No	Absorption wavelength (cm^{-1})	Functional group
1	2923.85 2853.71	Asymmetric and symmetric C-H stretching vibration of sp^3 hydrocarbon
2	1709.50	C=O stretching vibration of carbonyl group
3	1461.03	C-H in plane bending vibration of allylic hydrocarbon
4	1376.51	C-H in plane bending vibration of gem-dimethyl group
5	1096.60 1022.41	C-O-C stretching vibration of ether group
6	970.13	C-H out of plane bending vibration of trans or E alkenic group
7	781.89 722.12	C-H out of plane bending vibration of cis or Z alkenic group

Conclusion

In this research work, Myanmar Medicinal plant, *Euphorbia hirta* L. (Kyar-ba-hon) was selected for chemical analysis. The phytochemical screening of Kyar-bahon responses positive for alkaloid, flavonoid, glycoside, tannin, polyphenol, phenolic, reducing sugar and saponin respectively. The mineral contents of this plant were examined by EDXRF method. The elements found in the sample are K, Ca, Si, P, S, Fe, Ti, Mn, Zn, Sr, Cu, Rb and Br. Among then, the amount of potassium is the highest. Potassium supplementation may be especially useful in the treatment of high blood pressure. The amount of calcium is the second highest than others. The amount of silicon, phosphorus, sulfur, iron, titanium, manganese, zinc, strontium, copper, rubidium and bromine are found to be 0.001-0.640% respectively. Moreover, antimicrobial activities on the plant of Kyar-ba-hon in three solvents were also determined by Agar-well diffusion method on six tested organisms namely, *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans*and *E.coli species.* The antioxidant activity of the sample was determined in Mandalay University. Percent inhibition of standard ascorbic acid and IC_{50} value of ethanolic extract of plant of Kyar-ba-hon were determined by using DPPH assay. According to results of measuring antioxidant activity, it can be known that IC_{50} value of the ascorbic acid is 11.98µg/ml, but that of selected sample is 6.67µg/ml. Therefore the selected sample has higher antioxidant activity

than standard ascorbic acid. On the other hand, compound (I) and compound (II) were isolated from the plant of *Euphorbia hirta*L. by using Column Chromatographic separation methods. In addition, pure organic compound I (Quercetin) (13mg, 0.65%) and compound II (0.10 mg, 0.005 %) could be isolated by Column Chromatographic methods. The isolated pure organic compound I was assumed to be Quercetin according to determination of flavonoid test, FT IR spectral data and melting point determination.

The prominent functional groups of pure compound II was also determined by FT IR spectroscopic method. According to the FT IR interpretation of this pure compound (II), it consists of sp^3 hydrocarbons, carbonyl groups, allylic hydrocarbon, ether functional group, and cis or Z alkenic group groups respectively.

Acknowledgement

We are greatly indebted to DrThida Win, Rector, University of Mandalay, for her permission to write the research journal. We deeply express my gratitude to Dr Yi YiMyint, Head of Department of Chemistry, University of Mandalay, for her permission and facilities to this research. We are also thankful to DrKhaingKhaingKyu and DrMyatKyaw Thu, Professors, Department of Chemistry for their kind help and strong encouragement to this research.

References

Akomas SC, Ijioma SN and Emelike CU, 2015, "Effect of *Euphorbia hirta*on haematological and biochemical indices in albino rats".*Applied Journal of Hygiene;* 4(1):1-5.

David Harvey, 1956, "Modern Analytical Chemistry", DePauw University, 546-549.

DJ Newman and GM Cragg, Natural products as sources of new drugs over the last 25 years,J.Nat.Prod.2 Abdelmoaty MA, IbrahimMA , Ahmed NS,1.

E.A.Soforowa.Africanherbs.John Wiley and Sons, Chichister.1982,198.

E.M.Lind and A.C.Tallantire.Oxford University Press, Nairobi, 1971,182.

Harborne, J.B, (1973). "Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis". *New York, Chapman and Hall.*

Manzocco.L, Anese M, Nicoli.M.C, 1998, "Antioxidant properties of tea extracts as affected by processing". *Lebens.mittel-Wissenschaft Und- Technologie*31(7-8), 694-698.

Newman DJ and Crag GM , 2007, "Natural products as sources of new drugs over the last 25 years". *J.Nat. Prod.*70, 461-77

OuchiN.Parker JL, LugusJJ.Walsh K, 2011, "Adipokines in inflammation and metabolic disease". *Nat Rev Immunol*; 11:85-97.

Parcon.N.M, 2006, "Preparation of thin layer chromatography profile of extracts from *Euphorbia hirta*Linn".*AghamMindanaw*; 3, 73-7.

Patricia B.Johnson*et al.* 1999, "*Euphorbia hirta*leaf extracts increase urine output and electrolytes in rats", *Journal of Ethno pharmacology*; 65, 63-69.

Ping KY, Darah I, Chen Y, Sreeramanan S and Sasidharan S, 2013, "Acute and subchronic toxicity study of *Euphorbia hirta*L. methanol extract in rats".

Hindawi Publishing Corporation Bio Med Research International, http://dx.doi.org//0.115512013182064

Pollastri S., Tattini M., 2011, "Flavonols: old compounds for old roles". *Ann Bot;*108:1225-1233

Pounikar Y, Jain P, Khurana N, Patil S, Omray LK and Gajbhuye A.,2013, "Medicinal importance of *Euphorbia hirta*Linn". *SchAcad J Pharm;* 2(3):241-246.

"Publishing Corporation Bio Med Research International 2013", http://dx.doi.org//0.115512013182064

Ren W, QianZ, Wang H, Zhu L, Zhang L (2003) "Flavonoids: Promising anticancer agents". *Medicinal Res Rev* 23(4):519-534.

Soforowa.E.A, 1982, "African herbs".*John Wiley and Sons, Chichister*, 198. "The Merck Index", Tenth Edition

Trivedi.P.C, Maheswari.K, 2002, "Ethanobotany and medicinal plant", 5, 270-280. World Health Organization, 15 July 2011, "Fact Sheet: Obesity and Overweight,".http:// www who.int/hpr/gs.fs obesity shtml

Yan SJ, Ye DW, Wang Y (2011),"Ent-KauraneDiterpenoids from *Euphorbia hirta*". Rec.Nat Prod.5 (4):247-251

Yuet Ping K, Darah I, Chen Y,Sreeramanan S, Sasidharan S., 2013, " acute and subchronic toxicity study of *Euphorbia hirta*L. methanol extract in rats". Chin J Nat Med., 5.528-33.

Z and RSR, Jenkins DJA, Diamandis, EP (2002) "Flavonoids and steroid hormonedependent cancers". J Chromatogram B, 777:219-232.