Original Research Article

Anti-oxidant and Anti-inflammatory activity of *Moringa oleifera* ~~(~~Flowers~~)~~

ABSTRACT

~~Objectives~~Aims: The ~~aim of the present~~ study ~~was~~ isolated ~~the~~ a new compound from ~~ethyl acetate extract of~~ *Moringa oleifera [flowers – not typeset in italics]* and identifi~~edy~~ied its ~~the~~ anti-oxidant and anti- inflammatory activities.

Study design: Mention the design of the study here.
Place and Duration of Study: Sample: Department of Medicine (Medical Unit IV) and Department of Radiology, Services Institute of Medical Sciences (SIMS), Services Hospital Lahore, between June 2009 and July 2010.
Methodology~~Methods~~: ~~Flavonoids, Biflavonoids are the major constituent of ethyl acetate extract of Moringa Oleifera flowers. The e~~Extraction and fractionation was carried out from solvents of ethanol, benzene, petroleum ether, diethyl ether and ethyl acetate. ~~–~~The a~~A~~nti-inflammatory activity of the sample was determined by HRBC membrane stabilization and Albumin denaturation methods. Anti-oxidant activity of the sample was determined by DPPH assay and ABTS method.

Results: Flavonoids, Biflavonoids are the major constituents of an ethyl acetate extract of the flowers. The results of the study, suggest that the sample isolated from the ethyl acetate fraction possesses anti-oxidant activity and anti-inflammatory activity. However, these effects need to be confirmed using [in vivo – typeset in italics] models and clinical trials before ~~for~~ its ~~effective~~ utilization as a therapeutic agent~~s~~.

Conclusion:

Keywords: *Moringa oleifera*, Antioxidant activity, Anti-inflammatory activity, HRBC method, ABTS assay. – arrange keywords alphabetically

1. INTRODUCTION

Paragraph 1: Oxidative stress

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Paragraph 2: Inflammation and associated pain

Paragraph 3: *Moringa oleifera*

~~The phytochemicals are extracted from all parts of the plant body, but the concentration of these components varies from part to part. Normally, parts known to contain the highest concentration of the principles are preferred to therapeutic purposes and it can either be the leaves, stems, barks, roots, bulks, rhizomes, woods, flowers, fruits or the seeds. The literature review revealed the antimicrobial, analgesic, antifertility and antibacterial, anti-inflammatory, anti-oxidant, purgative and hepatic protective activities of the plant body [1].~~ (Above information too general to be of value – get straight to the point!)

Expand this paragraph by defining oxidative stress. Indicate causes of oxidative stress. As oxidative stress plays a central role in liver pathologies and their progression, the use of antioxidants has been proposed as therapeutic agents, as well as drug coadjuvants, to counteract liver damage [2 – renumber all citations].

Expand this paragraph by defining Inflammation and inflammatory pain. Indicate causes of Inflammation and inflammatory pain. Pain and inflammatory responses in the peripheral and central nervous systems play~~take part in~~ key roles in the growth and persistence of many pathological pain states [3]. A variety of natural compounds are able to alleviate pain targeting inflammation by reducing the synthesis of inflammatory mediators, or modulating inflammatory and nociceptive pathways [4].

Moringa oleifera [Lam.- not typeset in italics], a member of the Moringaceae family ~~also known as Drumstick or Horseradish tree~~, is cultivated in home-gardens in ~~rown to~~ the sub-Himalayan regions of India[indicate source of this information], Pakistan[indicate source of this information], Bangladesh and Afghanistan[indicate source of this information]. Due to its~~the~~ importance as a ~~uses of *M. oleifera* in~~ traditional medicine, many investigations have previously reported on pharmacological properties such as antifertility[5], anti-inflammatory[6], antispasmodic[7], and diuretic activities [~~5-~~7]. However, ~~The~~ it is known that phytochemical~~s are extracted from all parts of the plant body, but the~~ concentration ~~of these components~~ varies from part to part. To the best of our knowledge there have not been any phytochemical studies focusing on the flowers of *M. oleifera*. Thus this study set out to investigate the anti-oxidant and anti- inflammatory activities of this plant part as a possible clinical therapeutic agent.

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2. MATERIALS AND METHODS

2.1 Collection of fFlowers

Fresh flowers of *M.oringa oleifera* were collected from Karaikudi, Sivagangai (Dt), Tamil Nadu, India, during the month of April 2017 and taxonomically identified by Dr. S. John Britto, Director, The Rrapinat Herbarium and Centre for Molecular Systematics (Authentication No. AR003 dated: 05/04/2017) at the. St. Joseph's College (Campus), Tiruchirappalli, Tamil Nadu, India.

2.2 Extraction and fractionation

The 3 kg of fFreshly collected flowers (3 kg) of *Moringa oleifera* collected were extracted with 90% ethanol. The combined alcoholic extract was concentrated [in vacuo – typeset in italics] and the aqueous extract was successively fractionated with petroleum ether (60-80^{0}C) (6x250 ml), Peroxide free diethyl ether (4x250 ml) and ethyl acetate (8x250 ml). Petroleum ether fraction and diethyl ether fraction did not give in any isolable compounds. Ethyl acetate fraction on concentration yielded a dry powder. The dried compound was dissolved in DMSO and were used for further studies..

2.3. In vitro antioxidant activity

2.3.1 DPPH Assay mMethod

The DPPH free radical is reduced to a corresponding hydrazine, when it reacts with hydrogen donors. The DPPH radical is purple in colour and upon reaction with hydrogen donor changes to yellow colour. It is a decolouration assay, which is evaluated by the addition of the antioxidant to a DPPH solution in ethanol or methanol and the decrease in absorbance was measured at 490 nm [8].

Reagents:

A. **Preparation of 2,2-Diphenyl 1-picryl hydrazyl solution (DPPH, 100 µM)**:
(Cannot start sentence with a numerical!) Twenty two milligrams 22mg of DPPH was accurately weighed and dissolved in 100 ml of methanol. From this stock solution, 18 ml was taken and diluted to 100 ml using methanol to obtain 100 µM DPPH solution.

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B. Preparation of test solutions:

(Cannot start sentence with a numerical!) Twenty one milligrams~~21 mg~~ -of ethyl acetate fraction compound was dissolved in distilled DMSO to get a solution of 21_mg/ml concentration. This solution was serially diluted to prepare lower concentrations.

C. Preparation of standard solutions:

(Cannot start sentence with a numerical!) 10_mg of ascorbic acid was accurately weighed and dissolved in 1_ml of Dimethyl sulfoxide (DMSO) to obtain 10_mg/ml concentrations. These solutions were serially diluted with DMSO to prepare lower concentrations.

D. Procedure:

The antioxidant activity -was carried out in a 96 well micro-titre plate. To 200_µl of DPPH solution, 10_µl of each of the test sample or the standard solution was added separately in wells of the micro-titre plate. The final concentration of the test and standard solutions used were 1000, 500, 125 and 31.25 µg/ml. The plates were incubated at 37^0C for 30 minutes and the absorbance of each solution was measured at 490 nm, using a micro plate reader.

ABTS radical scavenging activity

ABTS radical scavenging activity was performed as described by Re *et al.* (1999) with a little modification. 7.0 mM ABTS in 14.7 mM ammonium peroxo-disulphate was prepared in 5.0 ml distilled water. The mixture was allowed to stand at room temperature for 24 hours. The resulting blue green ABTS radical solution was further diluted such that its absorbance is 0.70 ± 0.020 at 734 nm. Various concentrations of the sample solution (in ethanol) (20.0 µl) were added to 980.0 µL of ABTS radical solution and the mixture was incubated in darkness for 10 minutes. The decrease in absorbance was read at 734 nm. A test tube containing 20.0 µL of ethanol processed as described above was served as the control tube. Different concentrations of ascorbic acid were used as reference compound.

2.4 Anti- inflammatory activity

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4.1 The human red blood cell (HRBC) membrane stabilization method

The anti-inflammatory studies (Gopalkrishnan *et al*., 2009; Sakat *et al*., 2010 – these references must be numbered! Use Vancouver system throughout paper) was adopted with few modifications. The blood was collected from human volunteer who had not intake any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.5 % citric acid and 0.42 % NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10 % suspension was prepared. Various concentrations of test drug were prepared in mg/ml using distilled water and to each concentration, 1 ml of phosphate buffer, 2 ml hypo saline and 0.5 ml of HRBC suspension were added and incubated at 37^0C for 30 min then centrifuged at 3,000 rpm for 20 minutes. The hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac (100 Jg/ml) was act as a reference standard and its control was prepared by omitting the test drug. The experiments were performed in triplicates and mean values of the three were considered. The percentage (%) of HRBC membrane stabilization was calculated. [9,10]

Percentage of Protection (%) =
(100- OD of drug treated sample/OD of Control) X 100

4.2 Albumin denaturation method

The method as prescribed (Sakat *et al*., 2010 - – this reference must be numbered! Use Vancouver system throughout paper) was followed with some modifications. The reaction mixture was consisting of test sample and 1% solution of bovine albumin fraction. pH of the reaction mixture was adjusted using small amount of HCl. The mixtures were incubated at 37°C for 20 minutes and then heated to 51°C for 20 minutes. After cooling the samples the turbidity was measured spectrophotometrically at 660 nm. Diclofenac sodium was used as a standard drug. The experiment was performed in triplicates and the mean value of the three was considered. Percentage inhibition of protein denaturation was calculated. [11,12]

3. RESULTS AND DISCUSSION

It is indicated by the journal guidelines that the discussion about the tables and figures should appear in the text before the appearance of the respective tables and figures. No tables or figures should be given without discussion or reference inside the text.

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151 Anti-oxidant activity:

152 The compound isolated from the ethyl acetate fractions of *Moringa oleifera* flowers exhibited
153 significant anti-oxidant activity when compared with DPPH assay. It is evidenced from the data
154 presented in Table -1. The result showed the percentage of cytotoxicity for 1000 µg/ml as 70.54,
155 for 500 µg/ml as 63.01, for 125 µg/ml 46.57, and for 31.25 µg/ml 29.45 – do not repeat data
156 exactly in text format and then again in table format. DISCUSSION NEEDED It is evident from
157 the data presented in Table 2 that the sample possesses ABTS assay activity. The result showed
158 the percentage of cytotoxicity for 1000 µg/ml as 76.34, for 500 µg/ml as 68.19, for 125 µg/ml as
159 49.56, and for 31.25 µg/ml as 36.78.

160 **Table No. 1.: Anti-oxidant– activity of the compound –isolated from the ethyl acetate**
161 **fraction of flowers of *Moringa oleifera* by DPPH assay.**
162 **Moringa oleifera by DPPH assay Journal guidelines: Tables should be explanatory enough**
163 **to be understandable without any text reference. Double spacing should be maintained**
164 **throughout the table, including table headings and footnotes. Table headings should be**
165 **placed above the table.**

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S. No	Concentration (µg/ml)	% CTC_{50} Cytotoxicity (µg/ml)	IC_{50} (µg/ml)
1	1000	70.54	369.4
2	500	63.01	
3	125	46.57	
4	31.25	29.45	

170 *Table No. 1: Anti-oxidant activity of the compound isolated from the ethyl acetate fraction of flowers of*
171 *Moringa oleifera by DPPH assay*

172

173 Report outstanding/exceptional results of Figure 1 and discuss.

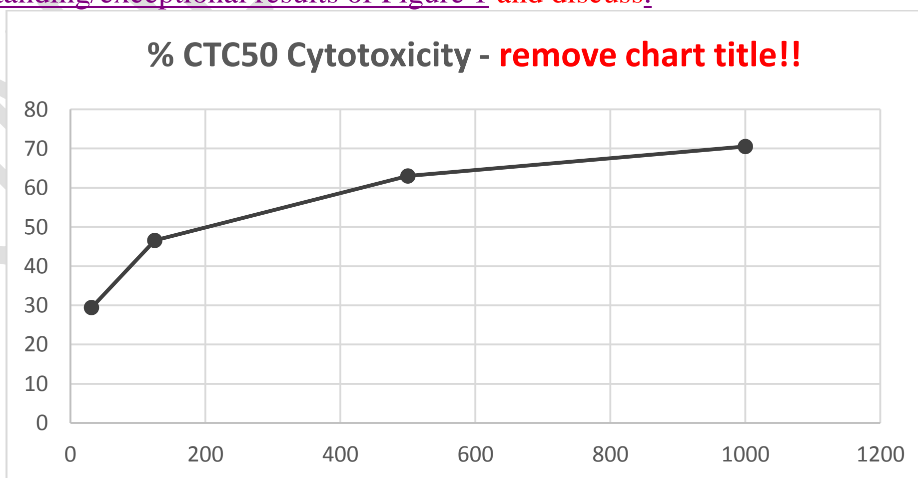

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Figure ~~Graph No.~~1. ~~:~~ ~~Graphical representation of~~ A~~a~~nti-oxidant activity– of the compound isolated from the ethyl acetate fraction of flowers of *Moringa oleifera* by DPPH assay.

According to journal guidelines: Each figure should have a caption. The caption should be concise and typed separately, **not on the figure area**. Figures should be self-explanatory. Information presented in the figure should not be repeated in the table. All symbols and abbreviations used in the illustrations should be defined clearly. Figure legends should be given below the figures.

~~**3.2 ABTS radical scavenging activity:**~~

~~ABTS radical scavenging activity was performed as described by Re *et al.* (1999) with a little modification. 7.0 mM ABTS in 14.7 mM ammonium peroxo-disulphate was prepared in 5.0 ml distilled water. The mixture was allowed to stand at room temperature for 24 hours. The resulting blue green ABTS radical solution was further diluted such that its absorbance is 0.70 ± 0.020 at 734 nm. Various concentrations of the sample solution (in ethanol) (20.0 µl) were added to 980.0 µL of ABTS radical solution and the mixture was incubated in darkness for 10 minutes. The decrease in absorbance was read at 734 nm. A test tube containing 20.0 µL of ethanol processed as described above was served as the control tube. Different concentrations of ascorbic acid were used as reference compound.~~

It is evident from the data presented in Table 2 that the sample possesses ABTS assay activity. The result showed the percentage of cytotoxicity for 1000 µg/ml as 76.34, for 500 µg/ml as 68.19, for 125 µg/ml as 49.56, and for 31.25 µg/ml as 36.78.

Table ~~No.~~ 2. ~~:~~ Anti-oxidant activity of the compound isolated from the ethyl acetate fraction of flowers of Moringa oleifera by ABTS assay.

S. No	Concentration (µg/ml)	% CTC_{50} Cytotoxicity(µg/ml)	IC_{50} (µg/ml)
1	1000	76.34	211.9
2	500	68.19	
3	125	49.56	
4	31.25	36.78	

~~***Table No. 2 : Anti-oxidant activity of the compound isolated from the ethyl acetate fraction of flowers of Moringa oleifera by ABTS assay.***~~

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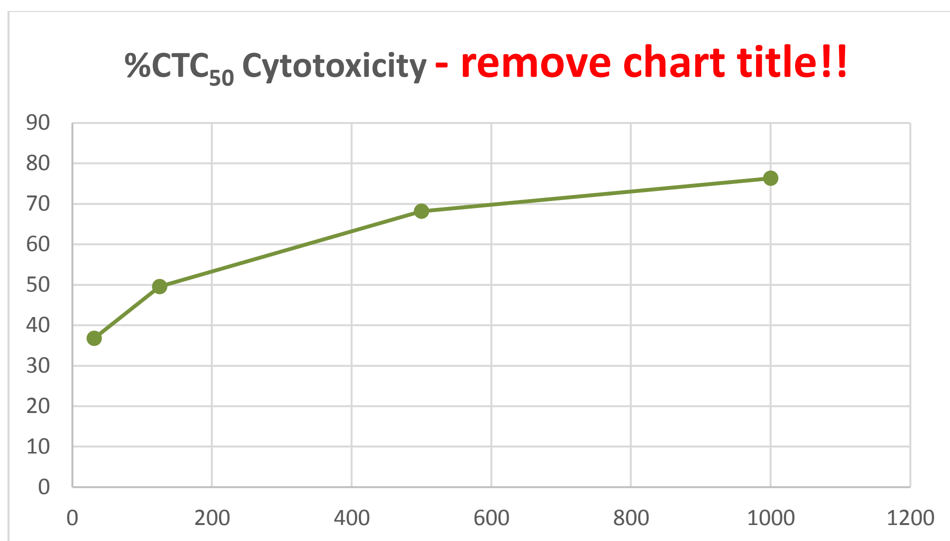

201 ***Graph No.2: Graphical representation of radical scavenging activity of the compound isolated from the ethyl***
202 ***acetate fraction of flowers of Moringa oleifera by ABTS assay.***

203 **4. ~~ANTI- INFLAMMATORY ACTIVITY~~**

204 **~~4.1 The human red blood cell (HRBC) membrane stabilization method~~**

205

206 ~~The anti-inflammatory studies (Gopalkrishnan *et al*., 2009; Sakat *et al*., 2010) was adopted with~~
207 ~~few modifications. The blood was collected from human volunteer who had not intake any~~
208 ~~NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution~~
209 ~~(2 % dextrose, 0.8 % sodium citrate, 0.5 % citric acid and 0.42 % NaCl) and centrifuged at 3,000~~
210 ~~rpm. The packed cells were washed with isosaline and a 10 % suspension was prepared. Various~~
211 ~~concentrations of test drug were prepared in mg/ml using distilled water and to each~~
212 ~~concentration, 1 ml of phosphate buffer, 2 ml hypo saline and 0.5 ml of HRBC suspension were~~
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214 ~~hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm.~~
215 ~~Diclofenac (100 Jg/ml) was act as a reference standard and its control was prepared by omitting~~
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217 ~~considered. The percentage (%) of HRBC membrane stabilization was calculated.~~ [9,10]

218 **~~Percentage of Protection (%) =~~**

219 **~~(100- OD of drug treated sample/OD of Control) X 100~~**

220

221 **~~4.2 Albumin denaturation method~~**

The method as prescribed (Sakat *et al*., 2010) was followed with some modifications. The reaction mixture was consisting of test sample and 1% solution of bovine albumin fraction. pH of the reaction mixture was adjusted using small amount of HCl. The mixtures were incubated at 37°C for 20 minutes and then heated to 51°C for 20 minutes. After cooling the samples the turbidity was measured spectrophotometrically at 660 nm. Diclofenac sodium was used as a standard drug. The experiment was performed in triplicates and the mean value of the three was considered. Percentage inhibition of protein denaturation was calculated.[11,12]

Percentage of inhibition (%) =
(OD of Control- OD of Sample/ OD of Control) X 100

The compound isolated from the ethyl acetate fractions of *Moringa oleifera* flowers exhibited significant anti-inflammatory activity of the human red blood cell (HRBC) membrane stabilization and the results are presented in Table 3. –The result showed the percentage of inhibition in membrane stabilization for 100 µg/ml as 29.96 ± 0.41, for 200 µg/ml as 39.48 ± 0.59, -for 400 µg/ml as 48.09 ± 0.61, for 600 µg/ml as 58.93 ± 1.40, and -for 800 µg/ml as 62.36 ± 1.86. The inhibition of Albumin denaturation activity exhibited by the compound are given in Table 4. The results showed the percentage of inhibition in membrane stabilization for 100 µg/ml as 29.62 ± 0.58, for 200 µg/ml 39.18 ± 0.86, for 400 µg/ml as 59.64 ± 0.94, -for 600 µg/ml as 68.17 ± 1.27, and for 800 µg/ml as 72.96 ± 1.49. The anti-inflammatory effect of the compound isolated from ethyl acetate fraction (test sample) of *Moringa oleifera* may be due to presence of active constituent flavonoids.

Table 3: The human red blood cell (HRBC) membrane Stabilization activity of the compound isolated from the ethyl acetate fraction of flowers of *Moringa oleifera*.

S. No	Concentration (µg/ml)	% of Inhibition Membrane Stabilization Mean ± S.E.M(S-I)
1	100	29.96 ± 0.41
2	200	39.48 ± 0.59
3	400	48.09 ± 0.61
4	600	58.93 ± 1.40
5	800	62.36 ± 1.86

Table 3: The human red blood cell (HRBC) membrane Stabilization activity of the compound isolated from the ethyl acetate fraction of flowers of Moringa oleifera

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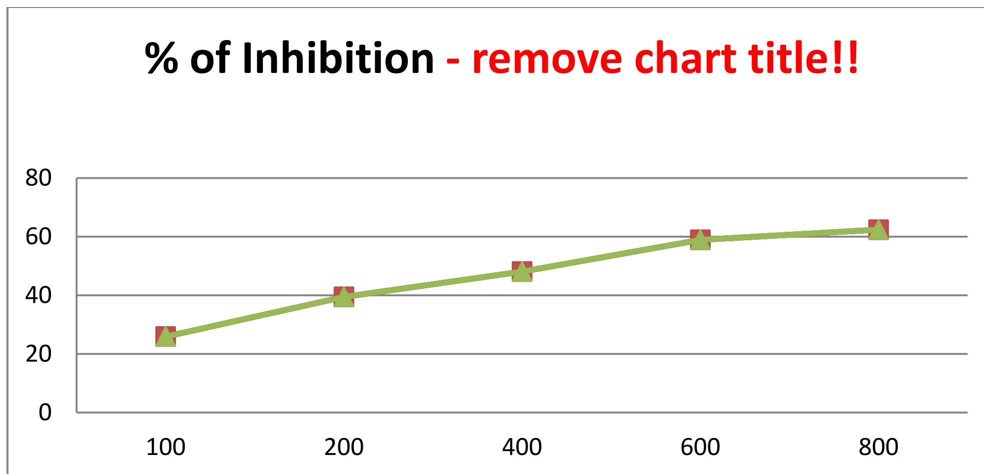

254 ***Figure ~~Graph~~ 3: Graphical representation of human red blood cell (HRBC) membrane Stabilization activity of***
255 ***the compound isolated from the ethyl acetate fraction of flowers of Moringa oleifera***
256

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257 Report and discuss outstanding/exceptional results of Table 4 1.
258 Table 4: The Inhibition of Albumin Denaturation activity of the compound isolated from the
259 ethyl acetate fraction of flowers of *Moringa oleifera*.
260

S. No	Concentration (µg/ml)	% of Inhibition Membrane Stabilization Mean ± S.E.M(S-I)
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2	200	39.18 ± 0.86
3	400	59.64 ± 0.94
4	600	68.17 ± 1.27
5	800	72.96 ± 1.49

261

262 ***Table 4: The Inhibition of Albumin Denaturation activity of the compound isolated from the ethyl acetate***
263 ***fraction of flowers of Moringa oleifera***
264 Report and discuss results of Figure 4.
265
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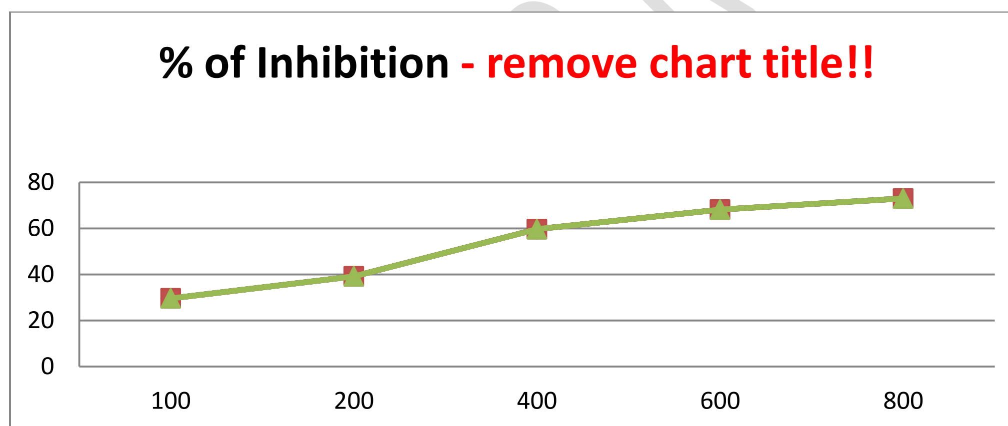

267
268
269
270 FigureGraph 4.: Graphical representation of Inhibition of Albumin Denaturation activity of the
271 compound isolated from the ethyl acetate fraction of flowers of *Moringa oleifera*.
272

273 **5. RESULTS AND DISCUSSION**

274 **5.1 Anti-oxidant activity:**

275 The compound isolated from the ethyl acetate fractions of *Moringa oleifera* flowers
276 exhibited significant anti-oxidant activity when compared with DPPH assay. It is evidenced from
277 the data presented in Table-1. The result showed the percentage of cytotoxicity for 1000 µg/ml

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278 as 70.54, for 500 µg/ml as 63.01, for 125 µg/ml 46.57, and for 31.25 µg/ml 29.45. It is evident
279 from the data presented in Table 2 that the sample possesses ABTS assay activity. The result
280 showed the percentage of cytotoxicity for 1000 µg/ml as 76.34, for 500 µg/ml as 68.19, for 125
281 µg/ml as 49.56, and for 31.25 µg/ml as 36.78.

282 **5.2 Anti-inflammatory activity:**

283 The compound isolated from the ethyl acetate fractions of *Moringa oleifera* flowers
284 exhibited significant anti-inflammatory activity of the human red blood cell (HRBC) membrane
285 stabilization and the results are presented in Table 3. The result showed the percentage of
286 inhibition in membrane stabilization for 100 µg/ml as 29.96 ± 0.41, for 200 µg/ml as 39.48 ±
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289 Table 4. The results showed the percentage of inhibition in membrane stabilization for 100
290 µg/ml as 29.62 ± 0.58, for 200 µg/ml 39.18 ± 0.86, for 400 µg/ml as 59.64 ± 0.94, for 600 µg/ml
291 as 68.17 ± 1.27, and for 800 µg/ml as 72.96 ± 1.49. The anti-inflammatory effect of the
292 compound isolated from ethyl acetate fraction (test sample) of *Moringa oleifera* may be due to
293 presence of active constituent flavonoids. The results strongly suggest anti-inflammatory effects
294 and anti-oxidant effects of the test sample by percentage of inhibitions, which are given in the
295 Table 1,2,3,4.

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299 **6. CONCLUSION (Short Communication papers do not have conclusion section as a rule)**

300 In the present study, both DPPH assay and ABTS have showed a highest potential
301 antioxidant activity and also the human red blood cell (HRBC) membrane stabilization activity
302 of the test sample. It could be concluded that the compound isolated from the ethyl acetate
303 fraction of flowers of *Moringa oleifera* is of phytopharmaceutical importance.

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COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

AUTHORS' CONTRIBUTIONS

Authors may use the following wording for this section: " 'Author A' designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. 'Author B' and 'Author C' managed the analyses of the study. 'Author C' managed the literature searches…… All authors read and approved the final manuscript."

~~7.~~ REFERENCES – REFER TO JOURNAL GUIDELINES ON HOW REFERENCES SHOULD BE LISTED.

1. Solomon S, Muruganantham N and Senthamilselvi M M. Anti-oxidant and anti-inflammatory activity of *Thespesia populnea* (flowers). Pharmacophore. 2015; 6(1): 53-59
2. Mukazayire MJ, Allaeys V, Duez P, Calderon PB, Stevigny C, and Bigendako, MJ. ~~"~~Evaluation of the hepatotoxic and hepatoprotective effect of Rwandese herbal drugs on *in vivo* (guinea pigs barbiturate-induced sleeping time) and *in vitro* (rat precision-cut liver slices, PCLS) models.~~,"~~ Experimental and Toxicologic Pathology. 2010; 62(3):289–299.
3. Watkins LR, Milligan ED, Maier SF, Glial proinflammatory cytokines mediate exaggerated pain states: implications for clinical pain.~~,~~ Advances in Experimental Medicine and Biology. 2003; 521: 1–21.
4. Smejkal K, Selected natural phenolic compounds - potential treatment for peripheral neuropathy? Ceska Slovenska Farmacie. 2014; 63:55–70.

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333 5. Fahey JW. Moringa oleifera: Aa review of the medical evidence for its nutritional,
334 therapeutic, and prophylactic properties. Part 1. Tree for Life Journal. 2005; INDICATE
335 VOLUME OF JOURNAL 1:5.
336 6. Shukla S, Mathur R, Prakash A O. Antifertility profile of the aqueous extract of
337 Moringa oleifera roots. Journal of Ethnopharmacology. 1988; 22:51–62.
338 7. Caceres A, Saravia A, Rizzo S, Zabala L, De Leon E, Nave F. Pharmacologie properties
339 of Moringa oleifera. 2: Screening for antispasmodic, anti-inflammatory and diuretic
340 activity. Journal of Ethnopharmacology. 1992; 36:233–237.
341 8. Ilavarsan R and Mallika M et al.ADD ALL AUTHORS -(2005), "INDICATE TITLE OF
342 PAPER. African Journal of Traditional, Complementary and Alternative MMedicines ".
343 2005; 2(1):70-85.
344 9. Mizushima Y and Kobayashi M. Interaction of anti-inflammatory drugs with serum
345 proteins especially with some biologically active proteins. Journal of Pharmacy
346 and Pharmacology.1968; 20:169-173.
347 10. Sakat S, Juvekar AR, Gambhire MN. In vitro antioxidant and inflammatory activity of
348 methanol extract of Oxalis corniculata Linn. International Journal of Pharma and
349 Pharmacological Sciences. 2010; 146-155.
350 11. Brown JH and Mackey HK. Inhibition of heat-induced denaturation of serum proteins by
351 mixtures of nonsteroidal anti-inflammatory agents and aminoacids. Proceedings of the
352 Society for Experimental Biology and Medicine, 1968; 128:225-8.
353 12. Grant NH, Alburn HE and Kryzanaukas C. Stabilization of serum albumin by
354 anti-inflammatory drugs. Biochemical Pharmacology. 1970; 19:715-22.

355 Prescribed journal Reference style

356 References must be listed at the end of the manuscript and numbered in the order that they
357 appear in the text. Every reference referred in the text must also present in the reference list
358 and vice versa. In the text, citations should be indicated by the reference number in brackets
359 [3].

360 Only published or accepted manuscripts should be included in the reference list. Articles
361 submitted for publication, unpublished findings and personal communications should not be
362 included in the reference list but may be mentioned in the text (e.g., T Nelson, Purdue

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363 University, USA, Unpublished results or personal communication). Avoid citing a "personal
364 communication" unless it provides essential information not available from a public source,
365 in which case the name of the person and date of communication should be cited in
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367 accuracy from the source of a personal communication. An unpublished result which has
368 been accepted for publication in any journal should be cited as "in press". Journal name
369 abbreviations should be those found in the NCBI databases (Link:
370 http://www.ncbi.nlm.nih.gov/nlmcatalog/journals).

371 Note: All SDI journals will follow this new reference style, effective from 09 Oct, 2012.

372

373 All references should follow the following style:

374 Reference to a journal:

375

376 For Published paper:

377 1. Hilly M, Adams ML, Nelson SC. A study of digit fusion in the mouse embryo. Clin Exp
378 Allergy. 2002;32(4):489-98.

379

380 Note: List the first six authors followed by et al.

381 Note: Use of DOI number for the full-text article is encouraged. (if available).

382 Note: Authors are also encouraged to add other database's unique identifier (like PUBMED
383 ID).

384

385 For Accepted, unpublished papers.

386 Same as above, but "In press" appears instead of the page numbers.

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1. Saha M, Adams ML, Nelson SC. Review of digit fusion in the mouse embryo. J Embryol Exp Morphol. 2009;49(3): (In press).

Note: List the first six authors followed by et al.

Note: Use of DOI number is encouraged (if available).

Note: Authors are also encouraged to add other database's unique identifier (like PUBMED ID).

For Articles not in English

Forneau E, Bovet D. Recherches sur l'action sympathicolytique d'un nouveau dérivé du dioxane. Arch Int Pharmacodyn. 1933;46:178-91. French.

Reference to a book:

Personal author(s)

Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology. 5th ed. Edinburgh: Churchill Livingstone; 2003.

Editor(s) or compiler(s) as authors

Beers MH, Porter RS, Jones TV, Kaplan JL, Berkwits M, editors. The Merck manual of diagnosis and therapy. 18th ed. Whitehouse Station (NJ): Merck Research Laboratories; 2006.

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Authored chapter in edited publication

Glennon RA, Dukat M. Serotonin receptors and drugs affecting serotonergic neurotransmission. In: Williams DA, Lemke TL, editors. Foye's principles of medicinal chemistry. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2002.

Reference to Web-resource or Electronic articles.

Hugo JT, Mondal SC. Parallels between tissue repair and embryo morphogenesis: a conceptual framework. Global Health. 2006;16:4. Accessed 29 March 2012. Available: http://www.globalizationandhealth.com/content/1/1/14.

Anonymous. Parallels between tissue repair and embryo morphogenesis: a conceptual framework. Global Health. 2006;16:4. Accessed 29 March 2012. Available: http://www.globalizationandhealth.com/content/1/1/14.

Reference to Organization as author

Diabetes Prevention Program Research Group. A study of digit fusion in the mouse embryo. J Embryol Exp Morphol. 2009;49(2):259–276.

Nomenclature and Units

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433 Internationally accepted rules and the international system of units (SI) should be used. If
434 other units are mentioned, please give their equivalent in SI.
435
436 For biological nomenclature, the conventions of the International Code of Botanical
437 Nomenclature, the International Code of Nomenclature of Bacteria, and theInternational
438 Code of Zoological Nomenclature should be followed.
439
440 Scientific names of all biological creatures (crops, plants, insects, birds, mammals, etc.)
441 should be mentioned in parentheses at first use of their English term.
442
443
444 Chemical nomenclature, as laid down in the International Union of Pure and Applied
445 Chemistry and the official recommendations of the IUPAC-IUB Combined Commission on
446 Biochemical Nomenclature should be followed. All biocides and other organic compounds
447 must be identified by their Geneva names when first used in the text. Active ingredients of all
448 formulations should be likewise identified.
449
450 Math formulae
451 All equations referred to in the text should be numbered serially at the right-hand side in
452 parentheses. Meaning of all symbols should be given immediately after the equation at first
453 use. Instead of root signs, fractional powers should be used.
454 Subscripts and superscripts should be presented clearly. Variables should be presented in
455 italics. Greek letters and non-Roman symbols should be described in the margin at their first
456 use.
457

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To avoid any misunderstanding zero (0) and the letter O, and one (1) and the letter l should be clearly differentiated.

For simple fractions use of the solidus (/) instead of a horizontal line is recommended.

Levels of statistical significance such as: *P <0.05, **P <0.01 and ***P <0.001 do not require any further explanation.

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