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Total phenolic, flavonoid contents and *in-vitro* anti-inflammation evaluation of ethanol extracts of *Hibiscus sabdariffa* calyx, *Malus domestica* and their 1:1 extracts blend on protein denaturation

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Abstract: *Hibiscus sabdarifa* and *Malus domestica* are well known and widely used herbs, which contains several interesting bioactive constituents and possesses health promoting properties. The aim of this study is to determine the total phenolic and flavonoid contents, evaluate and compare the anti-inflammatory effects of ethanol extracts of the two extracts and a 1:1 blend of the extracts against the denaturation of proteins *in vitro.* The respective extracts were analyzed for their contents of polyphenols and flavonoids. The test extracts and reference drug (Ibuprofen) of varying concentrations were also incubated with egg albumin under controlled experimental conditions and subjected to determination of absorbance to assess the anti-inflammatory property. The results obtained exhibited a concentration-dependent inhibition of protein denaturation by both extracts, the 1:1 blend as well as the reference drug. The EC_{50} for extracts as well as those for the blend and the reference drug were determined by the dose-response curve using Graphpad Prism 5.0 software.
Keywords: *Hibiscus sabdariffa; Malus domestica; flavonoids; ibuprofen; anti-inflammation; protein denaturation*

I. Introduction

From ancient past, man has used plants or fruits in the treatment and prevention of many ailments [1]. According to Eni *et al*.[2] a plant becomes medicinal only when its biological activities have been enthno-botanically reported or scientifically established. Much of the medicinal uses of plant or fruits seem to have been developed through observation of wild animals and by trial and error. The world health organization [3] estimated that 80% of the world population uses medicinal plant for the treatment of disease and in African countries, this rate is much higher. It has been estimated that up to 90% of the population in developing countries rely on the use of medicinal plants to help meet their primary health care need [4].

At least 25% of drugs used in modern pharmacopoeia are derived from plant while many are synthetic analogue built based on prototype compounds isolated from plants [5]. Over 50% of all modern clinical drugs are of natural products based pharmaceutical industries [6]. Natural products have contributed significantly towards the development of modern medicine. Recently traditional medicine worldwide is being re-evaluated by extensive research on different plant species and their active therapeutic principles [7,8]. The rich wealth of plant kingdom can represent a novel source of newer compounds with significant biological activities such as anticancer, anti-pneumonia, anti-diarrhea, anti-dysentery, antiviral, antimicrobial include anti-inflammatory activities. The major merits

of herbal medicine seem to be their perceived efficacy, low incidence of serious adverse effects and more affordable treatment [9].

Inflammation is a bodily response to injury, infection or destruction characterized by heat, redness, pain, swelling and disturbed functions. Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. It is the body response to inactivate or destroy the invading organisms, to remove the irritants and set the stage for tissue repair. It is triggered by the release of chemical mediators from injured tissue and migrating cells [10]. Anti-inflammatory refers to the property of a substance or treatment that reduces inflammation. Anti-inflammatory drugs make up about half of analgesics, remedying pain by reducing inflammation as opposed to opioids, which affect the central nervous system [7,8,11,12].

The numbers of animals required for the isolation of an anti-inflammatory prototype from complex natural product extracts using the activity-directed isolation pathway is alarming. It is for the above reason that the chick carrageenan response assay for the discovery of molecules with anti-inflammatory nociception properties was proposed [13,7,8].

The most commonly used drug for management of inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs) and steroids which have several adverse effects especially gastric irritation leading to formation of gastric ulcers [14,7,8]. Grant *et al.*[15] have reported that one of the features of several non-steroidal anti-inflammatory drugs for example indomethacin, ibufenac, flufenamic acid and salicylic acid is their ability to stabilize (prevent denaturation) heat treated bovine serum albumin (BSA) at pathological pH (pH 6.2 – 6.5).

Some plants, vegetables and fruits are reported to be rich sources of antioxidants, such as vitamin A, vitamin C, Vitamin E, carotenoids, polyphenolic compounds, flavonoids and hydrolysable tannins, which can significantly destroy the free radicals, reactive oxygen species responsible for degenerative diseases [16,17] or prevent free radical damage, thus reducing the risk of chronic diseases. These plants include *Ocimum basilicum* (basil), *Prunella vulgaris* (self-heal) *Cinnamomum cassia, Ginkgo biloba L., Camellia sinensis Lin., Aloe vera, Malus domestica, hibiscus sabdariffa* calyx and quite a number of others. This study is carried out to investigate and compare the possible anti- inflammatory effects of methanol extracts of *Malus domestica* and *Hibiscus sabdariffa* calyx against the denaturation of protein in vitro. *Malus domestica* is a fruit of the genus *Malus* (about 25 species) belonging to the family *Rosaceae*, the most widely cultivated tree fruit which is well known the world over for its delicious and nutritious fruits. The skin of ripe apples is generally red, yellow, green or pink, although many bi- or tri-colored varieties may be found [18].The skin may also be wholly or partly russet i.e. rough and brown and may be covered in a protective layer of epicuticular wax [19].The fruits are usually eaten fresh and raw except for the seeds, making the nutritional values fully available for the body [20-23]. Evaluation of the antimicrobial, antimutagenic, anticarcinogenic, antioxidant, antidiabetic/osteoporosis properties and related qualities of apple fruits and juices have been reported [22]. Clinical observations have shown that apple consumption is associated with reduction in risk of cancer [24-26]. Antioxidant activity of apple components is known to inhibit cancer cell proliferation, decrease lipid oxidation and lower cholesterol [1, 20-22]. Apples are rich source of photochemical that have been reported to reduce risk of cardiovascular diseases, asthma, dysentery, heart problem, diabetes, cataracts, Alzheimer's disease/cognitive decline and pulmonary functions[1,21].

Hibiscus Sabdariffa (Roselle or Sour Tea) is a tea where the usually dark colored flowers are used to brew. It appears to inhibit carbohydrate absorption to a degree and appears to be effective in reducing blood pressure. *Hibiscus sabdariffa* calyx is commonly called Roselle in English, Yakuwa in Hausa. However, the fresh calyx commonly called soboroto in Nigeria. It belongs to the family *Malvaceae. Hibiscus sabdariffa* is an herbaceous and perennial plant cultivated in the tropical and subtropical regions. It has limited traditional medicinal use, and tends to be a treatment for high blood pressure, gastrointestinal disorders, diaphoresis, and anuria [27]. The red flowers have sometimes been used traditionally for their pigmentation as a dye, which may have a role in histological studies [28]. *Hibiscus sabdariffa* extract protected human erythrocytes against lipid peroxidation [29]. The vegetable is widely grown and commonly used as port herb or soup in the northern part of Nigeria. In many countries of the world, it is eaten raw in salads, cooked and used as flavouring in cakes and making jellies, jams, soups, sauces, pickles and puddings. It is also used as colouring to herb teas and can be roasted as coffee substitute. Herbal drink made with roselle, scent leaf, garlic and ginger could be a home-made cure for diarrhoea and pneumonia. In recent times it has been commercialised

because of its touted medicinal potentials. Zobo is the hot water extract of *Hibiscus Sabdariffa* calyx and has been shown to possess medicinal/pharmaceutical properties including anti-diabetic, anti-hypertensive and anti-inflammatory properties [30]. A study [31] published in 2007 compared *Hibiscus sabdariffa L*. to the drug lisinopril on people with hypertension. *Hibiscus* "decreased blood pressure (BP) from 146.48/97.77 to 129.89/85.96 mmHg, reaching an absolute reduction of 17.14/11.97 mmHg (11.58/12.21%, $p < 0.05$)". Blood pressure "reductions and therapeutic effectiveness were lower than those obtained with lisinopril ($p < 0.05$)". The authors [31] and others [32-34] concluded that *hibiscus* "exerted important antihypertensive effectiveness with a wide margin of tolerability and safety, while it also significantly reduced plasma ACE activity and demonstrated a tendency to reduce serum sodium (Na) concentrations without modifying potassium (K) levels". They attributed the blood pressure reducing effect of *hibiscus* to its diuretic effect and its ability to inhibit the angiotensin-converting enzyme through the presence of anthocyanins [34,35].

II. **Materials and Methods**

II.1 Methods

The method used for assessing the anti-denaturation effects of natural products with anti-inflammatory properties is simple and inexpensive as has been reported by several authors [7,8.15,36, 37]

II.2 Chemicals and drugs

The standard reference drug, Ibuprofen Emzor brand was purchased from Beautiful Pharmaceutical Drug store, opposite North-Gate, Ahmadu Bello University, Samaru-Zaria. Other reagents (hydrochloric acid, sodium dihydrogenphosphate, disodiumhydrogenphosphate, sodium chloride, glycerol) used were of analytical grades from BDH, M&B, Sigma or, Fluka.

II.3 Plant materials

The *Malus domestica* fruit and *Hibiscus sabdariffa* calyx *were* obtained from Samaru market, Zaria, Kaduna state of Nigeria, and were identified at the herbarium, Department of Biological Sciences in Faculty of Sciences, Ahmadu Bello University Zaria, Kaduna state of Nigeria.

II.4 Preparation of extracts

The *Malus domestica* fruit material was washed and chopped into bits and air-dried for 14 days. The *Hibiscus sabdariffa* calyces were purchased dried and used as. The crude samples were separately ground in a laboratory grinder until a smooth powder was obtained in order to ensure high surface area for increased diffusion. About 440g of each plant powdered sample were packed in a Soxhlet extractor and extracted with 1.5liters of methanol for one week. This cycle was repeated for 7 days. After extraction, the solvent was removed, typically by means of a rotary evaporator, yielding the crude extracts. And the non-soluble portion of the extracted plants remains in the thimble and were discarded. The extracts were dried on water bath at 70^{o}C for 72hours. The dry extracts of *Malus domestica* fruit and *Hibiscus sabdariffa* calyx were kept in a vacuum desiccator until when ready for use.

II.5 Estimation of total phenolic compounds and flavonoids

The total phenolic contents of the two extracts were determined spectrophotometrically by applying the Folin-Ciocalteu assay with gallic acid as standard [8, 38]; while a slight modification of the method of Chang *et al.*[8, 39] was used for estimation of flavonoids contents of the test extracts with rutin as standard.

II.6 In-vitro anti-inflammatory activity bioassay

The screening for anti-inflammatory activity was carried out according to a modification of the *in vitro* protein denaturation bioassay methods described by Jagtap *et al.*[40], Sakat *et al.*[41] and Shallangwa *et al.*[8] Anti-inflammatory bioassay *in vitro* consist of reaction mixture (5mL) which are 0.2mL of egg albumin (from fresh hen's egg), 2.8mL of phosphate buffered saline (PBS, pH 6.4) and 2mL of varying concentrations of apple extract (of final concentrations 50, 100, 200, 400, 800, 1600 µg/mL, respectively). Similar composition was made where no drug or test sample was added but only doubly distilled water to serve as control. Then the mixtures were incubated at (37±2ºC) in Corsair Heating & Catering Limited incubator for 15 min. Denaturation was induced by keeping the reaction mixtures at 60±2ºC in water bath for 10 min. After cooling, the turbidity was measured at 660 nm (UV-Visible U2800 Spectrophotometer, Hiatachi Ltd). Percentage of inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and the average taken. Similar procedures were applied to *Hibiscus sabdariffa* calyx extract too. The Ibuprofen tablet at the final concentrations of 50, 100, 200, 400, 800, 1600µg/mL, respectively were also used as reference drug and treated similarly for determination of absorbance. Each experiment was done in triplicate and the average taken. The percentage inhibition of denaturation was calculated by using following formula:

$$\%\ Inhibition = 100x\left(V_t / V_c - 1\right)$$

Where,
V_t= mean absorbance of test sample.
V_c=mean absorbance of control.

II.7 Statistical analysis

All the pharmacological test data are expressed as the Mean±Standard Error of the Mean (SEM). The 50% Inhibitory Concentrations (IC_{50}) expressed as 50% Effective Concentrations (EC_{50}) were determined after logarithmic transformation of the concentration-response curve using GraphPad Prism 5.0 software. The Efficiency Index (EI) was expressed as EC_{50}/E_{max}.

III. Results and Discussion

III.1 Total phenolic compounds and flavonoids

The overall extraction yield of *Malus domestica* fruit and *Hibiscus sabdariffa* calyx were 42.05% and 39.82% dry weight materials. The study also revealed that the extracts were rich in phenolic and flavonoids. This result is in agreement with previous studies done by Jelodarian *et al* [42]; Yang *et al* [43]. Total polyphenols of the extract of *Malus domestica* fruit and *Hibiscus sabdariffa* calyx were estimated by the Folin-Ciocalteu method as 8.4±4.1µg GAE/mg and 21.87±3.5µg GAE/mg, respectively, dry extract weight. The total flavonoid content of the plant methanolic extracts were estimated and presented in Table1.

Table 1*: Extraction yield, total polyphenols and total flavonoids contents of extracts from Malus domestica and Hibiscus sabdariffa calyx*

parameter	M. domestica	H. sabdariffa
Total yield (%W/W)	42.05	39.82
Total polyphenols contents (µg GAE/mg dry extract weight)	8.4±4.1	21.87±3.5
Total flavonoids contents (µg RE/mg dry extract weight)	4.8±3.2	12.6±2.2

GAE: Gallic acid equivalents and RE: Rutin equivalents

It has been reported widely that phenolic and flavonoids content show significant antioxidant action on human health [7,42-45]. The polyphenolic compounds like flavonoids, phenolic acids and tannins are known to have an ideal chemical structure for effective free radical-scavenging activities that have shown to be more effective as antioxidants *in vitro* than vitamins E and C. This fact, has been established by many researches [46,47]. Furthermore, a number of previous studies have demonstrated a strong correlation between phenolic content, antioxidant properties and anti-inflammatory properties [7,8,48-50].

III.2. *In vitro* anti-inflammatory activity bioassay

In the present study, the evaluation of anti-inflammatory effects was undertaken using the effect of *Malus domestica* and *Hibiscus sabdariffa* calyx extracts on protein denaturation. Denaturation of proteins is well documented and is caused by inflammation process, mostly in conditions like arthritis [40,49,50]. Therefore, using agents that can prevent protein denaturation would be worthwhile for anti-inflammatory drug development. Several anti-inflammatory drugs have shown dose dependent ability to inhibit heat induced protein denaturation [8]. Protection or inhibitory effect against protein denaturation, which is the main mechanism of action of NSAIDS, plays an important role in the anti-inflammatory activity of NSAIDS [7]. The use of *in vitro* methods to study anti-inflammatory activities has its own advantages compared to using animals in experimental pharmacological research, because it addresses the ethical issues and the lack of rationale for the use of live animals when other suitable methods could be employed [7,8].

The ability of *Malus domestica* and *Hibiscus sabdariffa* calyx extracts to inhibit protein denaturation may contribute to their anti-inflammatory properties. In the present investigation, the *in vitro* anti-inflammatory effect of *Malus domestica* and *Hibiscus sabdariffa* calyx extracts, including a 1:1 blend of their extracts, were evaluated against denaturation of egg albumin. The results are as presented in Table 2.

***Table 2**: Anti-inflammatory data on M. domestica, H. sabdariffa, extracts blend of M. domestica and H. sabdariffa (1:1) and, Ibuprofen*

Concentration (µg/mL)	*M. domestica* (% inhibition)	*H. sabdariffa* (% inhibition)	1:1 blend of *M. domestica and H. sabdariffa*) (% inhibition)	Ibuprofen (% inhibition)
Control	--	--	--	--
1600	551.9±7.1	549.2±15.7	534.2±3.3	278.2±13.4
800	523.1±20.4	529.6±13.1	462.8±3.3	151.4±8.4
400	326.4±14.6	463.3±7.3	339.9±11.7	122.6±3.2
200	285.6±6.5	422.0±7.9	336.1±9.3	118.5±13.6
100	270.4±9.8	421.2±5.2	288.9±2.7	97.2±5.6
50	205.4±7.6	407.3±13.6	285.1±4.9	78.8±6.2

(Values are expressed as SEM of 3 readings)

The study showed a concentration-dependent inhibition of protein (egg albumin) denaturation by *Malus domestica, Hibiscus sabdariffa* calyx and the I:I blend of the two extracts, within the concentration ranges of 50.0 to 1600.0µg/mL. The reference drug, Ibuprofen, also exhibited concentration-dependent inhibition of protein denaturation. Table 2 showed that at low concentrations(50-400µg/mL), the *Hibiscus sabdariffa* calyx was more effective than the 1:1 blend extract, *Malus domestica* and Ibuprofen, in that order. At the moderate concentration of 800µg/mL there was slight change in the trend of percentage inhibition of denaturation; *Hibiscus sabdariffa* calyx extract (529.6±13.1) did just a little better than the *Malus domestic* extract (523.1±20.4), and the two extract were both separately better than the 1:1 blend (462.8±3.3) with Ibuprofen (151.4±8.4) being the least effect in preventing denaturation. But at higher concentration (1600µg/mL) the *Malus domestica* extract (551.9±7.1) showed that it is more effective than the *Hibiscus sabdariffa* calyx

extract (549.2±15.7) in inhibiting denaturation of protein, followed by the 1:1 blend of extracts (534.2±3.3) and Ibuprofen (278.2±13.4), in that order, respectively.

The extract concentration for 50% inhibition expressed as effective concentration (EC_{50}) was determined by the dose-response curve using GraphPad Prism 5.0 software and values are as presented in Table 3.

Table 3: *EC_{50} values for M. domestica, H. sabdariffa, extracts blend and Ibuprofen*

	M. domestica	*H. sabdariffa*	Extracts blend 1:1 (*M. domestica* / *H. sabdariffa*)	Ibuprofen
IC_{50} (µg/mL	510±3.1	481.1± 2.2	692.6±4.1	571.3±2.1

(Values are expressed as SEM of 3 readings)

This is defined as the concentration sufficient to obtain 50% of a maximum inhibition of protein denaturation. In this study the EC_{50} values showed that the *Hibiscus sabdariffa* calyx extract is more effective than the *Malus domestica*, followed by the reference drug, Ibuprofen; with the 1:1 blend extract being the least effective. This was almost the same trend of efficiency observed at the moderate concentration of 800µg/mL (Table 2), only that the reference was a little better than the blend.

The increments in the absorbance of the test samples with respect to the control indicated that there was stabilization of protein. That is, there was inhibition of protein (egg albumin) denaturation, which is also a measure of the anti-inflammatory effect of test extracts, blend and the reference drug, ibuprofen [7,8,15,36].

IV. Conclusion

It has been reported that one of the features of several non-steroidal anti-inflammatory drugs is their ability to prevent denaturation of protein. From the results of this study, it can be concluded that *Malus domestica* and *Hibiscus sabdariffa* calyx as well as their blend possessed marked anti-inflammatory effects as they can limit the denaturation of protein process *in vitro*. This can contribute to the validation of the anti-inflammatory activity of these plants and may provide some evidence for their folk uses separately and in combinations. These results also provide the motivation for further planning of clinical nutrition and anti-inflammatory research studies on bioactive compounds of the plants and development of anti-inflammatory products.

V. References

[1]. Chan A., Shea T., Dietary supplementation with apple juice decreases endogenous amyloid-beta levels in Murine brain. *J. Alzheimer's Dis.,* 16 (2009)176-171.

[2]. Eni A.O., Oluwawemitan I. A. and U.S. Oranusi U.S., Microbial quality of fruits and vegetables sold in Sango Ota, Nigeria. *Afr. J. food Sci.,* 4:5(2010) 291-296.

[3]. World Health Organization (WHO), Traditional medicine; growing needs and potentials WHO policy perspectives medicines, (2002).

[4]. Segismundo A.B., Florendoand P.E., Pablico A.R.P, *In-Vitro* Antifungal Activity and Phytochemical Screening of *Gouania javanica Miq*. Leaves. *UNP Research Journal*, Vol. XVII (2008) 1-10.

[5]. Food and agriculture organization (FAO), Now-wood news; an information Bulletin on Non-wood forest products (2000) p. 25.

[6]. Singh M.K., Khare G., Iyer S.K,, Sharwan G. and Tripathi D.K., Clerodendrum serratum: A clinical approach, J.App.Pharm.Sci., 02:02 (2012) 11-15.

[7]. Shallangwa G.A., Hamidu A., Abba H., Dallatu Y. A. and D.T. Bilham D.T., *In-vitro* Evaluation of Aqueous Extracts of *Citrus sinensis*, Aloe vera and Their 1:1 Extracts Blend on Protein Denaturation during Acute Inflammation. *Journal of Biologically Active Products from Nature*, 3:5-6 (2013) 295-302.

[8]. Shallangwa G. A., Musa H. and Nyaga G.T, 2015. *In-Vitro* Evaluation of Ethanolic Extracts of *Zingiber officinale*, *Sygzium aromaticum* and their 1:1 Extracts Blend on Protein Denaturation During Acute Inflammation. *JPRC,* 1:1 (2015)1– 8.

[9]. Iwu M.M., Duncan A.R. and Okunji C.O., 1999. New Antimicrobia of plants Origin. In: Janick, J, (ed). Perspective in new crops and new uses- ASHS press, Alexandra., V.A, (1999)546-582.

[10]. Chandra S., Dey and P., Bhattacharya S., 2012. *Mikania scandens* flower extract Preliminary *in vitro* assessment of anti-inflammatory property of. J*APER*, 1(2012) 25-31.

[11]. Williams L.A.D., Hibbert S.L, Porter R.B.R., Bailey-Shaw Y.A. and Green C.E., Jamaican plants with *in vitro* anti-oxidant activity. Research Signpost. In: *Biologically active natural products for the 21st Century*. Williams L. A. D (ed.) (2006) pp.1–12.

[12]. Gan R.Y., Kuang L., Xu X.R., Zhang Y.A., Xia E.Q., Song F.L. and Li H.B., Screening of Natural Antioxidants from Traditional Chinese Medicinal Plants Associated with Treatment of Rheumatic Disease. Molecules, 15 (2010) 5988- 5997.

[13]. Roach J.T. and Sufka K.J. Characterization of the chick carrageenan response. *Brain Research,* 994 (2003) 216–225.

[14]. Elujoba A.A., The role of pharmacognosy in phytotherapy, the challenges of our time. *Nig. J. Nat. Prod.,* 2(1997) 34-36.

[15]. Grant N.H., Alburn H.E. and Kryzanauska C., Stabilization of serum albumin by anti-inflammatory drugs*. Biochem. Pharmacol.,* 19(1970) 715–22.

[16]. Fresquet F., Pourageaud F., Leblais Y., Brandes R.P., Savineau J. P., Marthan R. and Muller B., Role of reactive oxygen species and gp91 phox in endothelial dysfunction of pulmonary arteries induced by chronic hypoxia. *Br. J. Pharmacol.,* 48:5(2006)714-723.

[17]. Shallangwa G.A., Jibrin G.P., Musa H., Hamidu A., Dallatu Y. A., Abba H. and Moyosore A.A., *In vitro* evaluation of Aloe vera and *Camellia sinensis* aqueous extracts effect on protein denaturation during acute inflammation. *Biointerface Res. Appl.Chem.,* 3:3(2013): 566-572.

[18]. Janick J., Cummins J.N., Brown S.K. and Hemmat M., Apples, In: Jules Janick and James N. Moore. Fruit Breeding, Volume I: Tree and Tropical Fruits. John Wiley & Sons, Inc. (1996) p. 9.

[19]. Nelson L.S., Shih R.D., Balick M.J., Handbook of poisonous and injurious plants. Springer. (2007)211–212.

[20]. Avci A., Atli T., Eruder I, Varl M., Devrim E., Turgay S. and Durak J., 2007. Effect of apple consumption on plasma and erythrocyte antioxidant parameters in elderly subjects. *Exp. Aging Res.,* 33(2007) 429-437.

[21]. Boyer J. and Liu R. H., Apple phytochemicals and their health benefits*. Nutrition Journal.* 5 (2004)1–15.

[22]. Gerhauser C., Cancer chemo preventive potential of apples, apple juice and apple components. *Planta Med*., 74(2008)1608-1624.

[23]. Liu R.H., Health Benefit of fruit and vegetables are from additive and synergistic combinations of phytochermicals. *Am. J. Clin. Nutr.,* 78 (2003) 5175-5205.

[24]. Gallus S., Talamini R., Giacosa A., Montella M., Ramazzotti V., Franceschi S., Negni C. and La-Vecchia E., 2005. Does an apple a day keep the Oncologist away? *Ann. Oncol.* 16 (2005)1841-1844.

[25]. Michels K.B., Giovannucci E, Chan A.T., Singhania R., Fuchs C.S. and Willett W.C., Fruits and vegetable consumption and colorectal adenomas in the Nurses' health study. *Cancer Res.,* 66(2006) 3942-3953.

[26]. Theodoratou, E., J. Kyle, R. Cetnarskyj, S.M. Farrington, A. Tenesa, R. Barnetson, M. Porteous, M. Dunlop and H. Campbell, 2007.Dietary flavonoids and the risk of colorectal cancer.Cancer Epidemiol. Biomarkers Prev., 16: 684-693.

[27]. Seujange Y., Leelahavanichkul A., Yisarakun W., Khawsuk W.,, Meepool A., Phamonleatmongkol P., Saechau W., Onlamul W., Tantiwarattanatikul P.,, Oonsook W., Eiam-Ong S.and Eiam-Ong S., *Hibiscus sabdariffa Linnaeus* aqueous extracts attenuate the progression of renal injury in 5/6 nephrectomy rats. *Ren Fail.* 35:1(2013)118-25. Doi: 10.3109/0886022X.2012.741468. Epub 2012 Nov 19.

[28]. Bassey R.B., Bakare A.A. Peter A.I., Oremosu A.A. and Osinubi, A.A., Factors influencing extract of *Hibiscus sabdariffa* staining of rat testes. *Biotech Histochem.,* (2012) 403-407. DOI:10.3109/10520295.2012.679365

[29]. Suboh S.M., Bilto Y.Y. and Aburjai T.A. Protective effects of selected medicinal plants against protein degradation, lipid peroxidation and deformability loss of oxidatively stressed human erythrocytes. *Phytother. Res.,* 18(2004) 280-28

[30]. Osueke J.C. and Ehirim F.N., Chemical, Nutritional and Sensory analysis of zobo drink (*H. sabdariffa)* and selected soft drinks. *Journal of Agriculture and Food Science*. :1(2004)33–37.

[31]. Hopkins A.L., Lamm M.G., FunkJ. and Ritenbaugh C., *Hibiscus sabdariffa L.* in the treatment of hypertension and hyperlipidemia: a comprehensive review of animal and human studies, (2013) Doi: 10.1016/j.fitote.2013.01.003 PMCID: PMC3593772 NIHMSID: NIHMS444438

[32]. Sindi H.A., Marshall L. J. and Morgan M.R.A., Comparative chemical and biochemical analysis of extracts of *Hibiscus sabdariffa.*Food Chem., 164(2014) 23–29.

[33]. Ismail A., Ikram E.H.K. and Nazri H.S.M., Roselle (*Hibiscus sabdariffa L*.)Seeds – Nutritional Composition, Protein Quality and Health Benefits. *Food,* 2:1(2008)1-16.

[34]. Herrera-Arellano A., Miranda-Sánchez J., Avila-Castro P., Herrera-Alvarez S., Jiménez-Ferrer J.E., Zamilpa A., Román-Ramos R., Ponce-Monter H., Tortoriello J., Clinical effects produced by a standardized herbal medicinal product of *Hibiscus sabdariffa* on patients with hypertension. A randomized, double-blind, lisinopril-controlled clinical trial*. Planta Med.,* 73:1(2007) 6–12. doi:10.1055/s-2006-957065. PMID 17315307

[35]. Formagio A.S.N., Ramos D.D., Vieira M.C., Ramalho S.R., Silva M.M., Zárate N.A.H., Foglio M.A. and Carvalho J.E. 2015. Phenolic compounds of *Hibiscus sabdariffa* and influence of organic residues on its antioxidant and antitumor properties. *Braz. J. Biol.,* 75:1(2015) 69-76.

[36]. Williams L.A.D., O'Connar A., Latore L., Dennis O., Ringer S., Whittaker J.A., Conrad J., Vogler B., Rosner H. and Kraus W., 2008. The *in vitro* anti-denaturation effects induced by natural products and non-steroidal compounds in heat treated (immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals, in the early stages of the drug discovery process. *West Indian Medical Journal*, 57:4(2008) 327-331.

[37]. Zakrzewski K. and Goch H., 1968.Human serum albumin.Tyrosyl residues and strongly binding sites*. Biochemistry*, 7(1968)1835–42.

[38]. Proestos C., BoziarisI S., Nychas G.J.E. and Komaitis M.,, Analysis of flavonoids and phenolic acids in Greek aromatic plants: Investigation of their antioxidant capacity and antimicrobial activity. Food Chem., 95 (2006) 664-671.

[39]. Chang C., Yang M. and Wen H., Estimation of total flavonoids content in *propolis* by two complementary colorimetric methods. *J Food Drug Anal*, 10:3 (2002) 178-182.

[40]. Jagtap V.A., Agasimundin Y.S., Jayachandran E. and Sathe B.S., *In-Vitro* Anti - Inflammatory Activity of 2- Amino- 3- (Substituted Benzylidinecarbohydrazide)- 4,5,6,7- Tetrahydrobenzothiophenes. *J Pharm Res.,* 4:2(2011) 378- 379.

[41]. Sakat S., Juvekar A.R. and Gambhire M.N., *In vitro*antioxidant and anti-inflammatory activity of methanol extract of*Oxalis corniculataLinn*. *Int. J Pharm Pharm Sci*., 2:1(2010.)146- 155.

42. Jelodarian S., Ebrahimabadi A., Khalighi A. and Batooli H., Evaluation of antioxidant activity of *Malus domestica* fruit extract from Kashan area. *Afr. J. Agric. Res.,* 10:20 (2015) 2136- 2140.

[43]. Yang L., Gou Y., Zhao T, Zhao J, Li F, Zhang B. and X. Wu X., Antioxidant capacity of extracts from calyx fruits of roselle (*Hibiscus sabdariffa* L.). *Afr. J. Biotechnol.,* 11:17(2012) 4063-4068

[44]. Hollman P.C.H. and Katan M.B., 1999. Dietary flavonoids: Intake, health effects and bioavailability. *Food Chem. Toxicol.,*37(1999) 937-942.

[45]. Halliwell B., Rafter J and Jenner A., Health promotion by flavonoids, tocopherols, tocotrienols, and other phenols: direct or indirect effects? Antioxidant or not? *Am. J. Clin. Nutr.*, 81(2005) 268S-276S.

[46]. Apak R., Guclu K., Ozyurek M., and S.E. Karademir S.E.,. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *J. Agric. Food Chem.*, 52 (2004) 7970-7981.

[47]. Kim D.O. and Lee C.Y., Comprehensive study on vitamin C equivalent antioxidant capacity (VCEAC) of various polyphenolics in scavenging a free radical and its structural relationship. Crit. Rev. *Food Sci. Nutr.,* 44 (2004) 253-273.

[48]. Wojdylo A., Oszmianski J .and Czemerys R., 2007. Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chem.,* 105 (2007) 940-949.

[49]. Chandra S., Chatterjee P., Dey P. and Bhattacharya S., 2012. Evaluation of *in vitro* anti-inflammatory activity of coffee against the denaturation of protein. *Asian Pac J Trop Biomed., (*2012*)*S I 78-S 180.

[50]. Zhen J., VillaniT.S., Guo Y. Qi Y., Chin K., Pan M. H., Ho C.T., Simon J.E. and Wu Q., Phytochemistry, antioxidant capacity, total phenolic content and anti-inflammatory activity of *Hibiscus sabdariffa* leaves. *Food Chem.,* 190 (2016) 673–680.

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