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Ethnomedicinal and antioxidant potentials of some wild fruits of Shinyanga region in Tanzania

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Abstract

Wild fruits are potentially natural foods, healthy and economically important in our communities. Lack of data on the nutritive and medicinal values of the wild fruits leave them neglected in our communities. Investigation on medicinal use and antioxidant potential of wild fruits in the drylands area of Shinyanga region was conducted in 2022 at Iselamagazi ward (33° 8'14.52"E to 33° 8'26.97"E and 3°32'28.44"S to 3°32'40.38"S) in Shinyanga rural District. Wild fruits were collected using standard methods from the purposely selected areas while ethnomedicinal information was collected using a questionnaire method. Extraction of plant material was done by solvent extraction method using ethanol 99.8% v/v as a solvent. Extract of the wild fruits were evaluated calorimetrically using the spectrophotometer UV-Visible model 6305 Jenway, UK. Ethnomedicinal information from the questionnaire were also collated. Antioxidant properties were obtained using 2, 2-diphenyl-1picrylhydrazyl DPPH) method. Results indicate that the wild fruits are potential natural antioxidants with total phenolic content (gallic acid equivalence) ranging from 19.513-175.983 mg GAE/g and total flavonoid content (*Ruta graveolens*) ranging from 9.171-176.99 mg RUE/g. *Adansonia digitata* showed the highest antioxidant capacity (175.983mg GAE/g) and *Trema orientalis* showed the lowest antioxidant capacity (17.898mg GAE/g). Scavenging ability values of the fruits ranged from 0.368-44.77 taurine (TAU) in total phenolic and 0.233-5.68 TAU in total flavonoid content. More than 20 diseases were reported to be treated by the investigated fruits based on ethnomedicinal information of the investigated community. Propagation and fruits utilization sensitization of the wild fruits in our communities will improve health and livelihood but also conserve our environments.

Keywords: Antioxidants, anti-radical, ethnomedicinal, wild fruits, scavenging capacity

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INTRODUCTION

Antioxidants are important in living cell and food we eat as they can act as scavengers and convert produced harmful free radical into the form which is not reactive after donating hydrogen atoms (Yeung *et al*, 2018). When radicals are produced without antioxidants to capture them, it can result in body stress that in most cases are interpreted as body disorder resulting in many chronic diseases such as cancer, diabetes, heart and nervous system disorder (Catana *et al.*, 2018). The most occurring natural antioxidants have been investigated and most are found in plant parts like leaves, root and fruits (Battino *et al* 2018). So the food we eat contains antioxidant in many forms and their functions in quenching free radicals differ from one antioxidant to another (Yeung *et al.*, 2017). To mention few, Superoxide Dismutase (SOD) antioxidant are involved in quenching of superoxide radical (O2) by converting it to hydrogen peroxide and water, Glutathione peroxidase naturally occurring in human body as selenium or Manganese containing Enzyme which catalyses the reduction of H_2O_2 and lipid hydro peroxide generated during lipid peroxidation to water using reduced glutathione as substrate (Yeung *et al*, 2017). Other antioxidants include Vitamins, C, E, A carotenoids which are grouped as line two defence antioxidants, for example Beta – carotene (vitamin A) scavenge directly with O-OH were as glutathione scavenge may radicals O2 OH but also detoxify included oxidizing air pollutants such as Ozone, No_2, free radicals from cigarette smoke. Vitamin E scavenge peroxyl radical produced as intermediate molecule during lipid peroxidation there by protecting cell membrane. Flavonoids are group of phenolic compound present in plant they serve as lipid peroxidation and lipoxygenases inhibitor (Moosavi *et al*, 2018).

The knowledge of the community on medicinal use, local names and habitants of the fruit in the given area is the one which determine the utility and conservation of species in the given community (Njau, 2005). The Wild fruit are normally considered as neglected food items due to the lack of data on nutritive value compared to domesticated and cultivated fruits (Moosavi *et al*, 2018). This study therefore was conducted to raise knowledge on the medicinal use scavenging capacity of antioxidants on harmful compounds in human body. This will enable increase of utility of the fruits by the community but also conservation involvement by the community will increase.

MATERIALS AND METHODS

Collection of plant materials and Antioxidant activity determination

Wild fruits were collected from eight plant species of Shinyanga dry region of Tanzania and were analysed for potential Antioxidant capacity, antiradical activity and information on their medical use and local names were collected from the community members. The main criteria of selecting Shinyanga as a sampling area is because they have dry and semi-desert environment (Njau, 2005) that threaten the livelihood, economy and health of the people in Shinyanga since most of the regions have no natural tree except for few areas including Iselamagazi ward (33° 8'14.52"E to 33° 8'26.97"E and 3°32'28.44"S to 3°32'40.38"S) which was the only ward selected for sampling of the fruits and ethnomedicinal information with the main criteria being availability of the wild fruits.

The Antioxidant capacity of the wild fruits collected were evaluated calorimetrically using the spectrophotometer UV-Visible model 6305 Jenway UK. The principle behind this procedure is that the organic radical DPPH react with the Antioxidant in methanolic solution and the reduction of the red colour of DPPH is monitored by measuring the absorbance. In detail the antioxidant in air dried ground sample were extracted by using ethanol.

Preparation and extraction of fruit materials

In the laboratory, each morphological part of the plant was handled separately. The plant materials were cleaned of debris using running tap water. The leaves were first sorted from branches and shade dried. The pods were chopped into small pieces before sun dried first to reduce the moisture content then shade dried. The dried plant materials were then grounded into powder using laboratory mill and stored in airtight bags in a cool dry room until used in the extraction. Solvent extraction was carried out according to the method described by Parekh and Chanda (2006) with modifications. 1000 grams of the powdered plant material was soaked in ethanol (99.8% v/v) in a conical flask, covered with aluminium foil and left on a bench for 72 hours in a dark place at room temperature with frequent shaking. The mixture will then be filtered using Whatmann No. 1 filter paper and the filtrate will be concentrated on water bath at 50°C using rotary evaporator until all the solvent is

recovered. The obtained crude extracts will be stored at 4°C in airtight bottles until use.

Thirty grams of each sample were mixed with 150ml of 95% ethanol and shaken for 48 hrs after which filtration was performed by using Whatman number 4 filter paper and centrifuged for 15min at 1500g. After filtration the ethanol was evaporated under vacuum by using rotary evaporator at 50 mmHg at a temperature of 40$^{\circ}C$. The residue was retained for antioxidant assay as described below. The extract yield was determined gravimetrically by weighing the residue (extract) and % yield calculated from equation % yield= $\frac{W1X100}{W2}$ Where as

W_1=weight of extract (residue after evaporation), W_2=weight of air-dry ground plant sample.

Determination of free radical scavenging using DPPH or (2,2-diphenyl-1picrylhydrazyl)

Free radical scavenging activity was determined by free radical method as described by Brand-Williams *et al*., (1995) with some modification. In each of four beakers 5 ml of 1 mg/ml metabolic extract sample was poured in 100 ml beakers followed by serial dilution to make four concentrations of 1, 0.1, 0.01, and 0.001 mg/ml. The absorbance of each concentration of the extract was read at 515 nm as an absorbance test. Blank solution of methanolic free radical was prepared by mixing 5 ml methanol with 100 µl DPPH and treated in dark for 30 min in vials. The absorbance for the control was read at 515 nm giving a value of 0.888. Five millilitres of each concentration of the sample extract to be measured was poured in 4 separate vials and 100 µl of DPPH methanolic free radical solution was then added. The mixture was allowed to react and absorbance was read at 515 nm at 0 min to every minute till the next 15 min. The degree of decline in the intensity of purple colour of DPPH solution was detected. The decrease in absorbance of the DPPH radical at 515 nm was determined continuously at every 15 min until the reaction reached the plateau. This was achieved within 60 min when the whole solutions changed into yellow. The extent of discolouration of a solution when mixed with a methanolic extracts indicates the reduction of DPPH free radical by probable antioxidant leading in a loss of absorbance and defines the radical scavenging efficiency of the added extracts by hydrogen donation (Enujiugha, 2010).The reaction medium of each concentration was calculated with DPPH free radicals at 515 nm using the equation: %DPPH RSA = (Absorbance control – Absorbance sample/Absorbance control) × 100 (Rane *et al*., 2019).

The results in percentage of DPPH free radicals' reduction were expressed by means of EC50, which corresponds to the minimal antioxidant concentration effective for the DPPH radical to be reduced by 50%. Based on the EC50 the result was expressed in terms of mg dry matter of sample/standard equivalent per gram of DPPH radicals in the reaction medium. The parameter EC50 is the weight in mg of the sample required to decrease one gram of the initial DPPH radical concentration by 50% (Siddhuraju & Becker, 2007).

3. Determination of Total Phenolic (TP)

The TP was determined by Folin-Ciocalteu (FC) assay as per Onwuka *et al*., (2012) using gallic acid standard with minor modification. The assay was carried out by pipetting 200µl of the 1 mg/ml sample into three 8 ml vials followed by addition of 1 ml of FC reagent and then 0.8 µl of 35% of sodium carbonate. The mixture was mixed on vortex for 20s and placed in dark for 30 minutes. In four 100 ml beaker each contained 200 µl of gallic acid where water was added for different dilution. The absorbance of the mixture and successive diluted gallic acid read absorbance at 765 nm at room temperature. A calibration curve was created using standard gallic acid solutions each time an analysis run. The level of TP in the extract was calculated from the calibration curve using factorial equation below at every experiment. Three replicates of total phenolic were recorded for comparison. Results were expressed in mg of the gallic acid equivalent to per gram of sample. It means,

TP (mg GAE/g) = A_{765}/0.702) × 80.

Determination of Total Flavonoid (TF)

Total flavonoid was determined by modifying Aluminium chloride calorimetric method as described in Pękal-Pyrzynska (2014). One ml of 1 mg/ml sample was poured in a vial followed by addition of 1 ml of 2% weight per volume of Aluminium chloride in methanol containing 5% of acetic acid. The mixture was placed in dark for 30 minutes. The sample mixture and 1 ml of 10 mg/ml rutin standard was read at the absorbance of 425 nm. Total

flavonoids were calculated using equation below by comparing a standard curve of rutin to each sample`s absorbance. Three replicates of flavonoid results were recorded for comparison. Total flavonoid content was expressed as mg of standard equivalent per g of dry weight. It means, TF (mg RUE/g) = (A_{425}÷0.47) ×1000.

Collection of Ethnomedicinal information

The interview method by questionnaire was used to collect information from purposely selected ward (Iselamagazi) of Shinyanga. The ward was selected based on two criteria: One is availability of wild fruits and second is that, the rest of the region is dry and semi-desert threatening the livelihood, economy and health of the communities in the region. Fruits were collected using standard method and transported to the University of Dar es Salaam Botany Department for identification and laboratory work. The calorimetric results of antioxidant capacity, antiradical activity and ethnomedicinal information were presented on tables for interpretation and discussion.

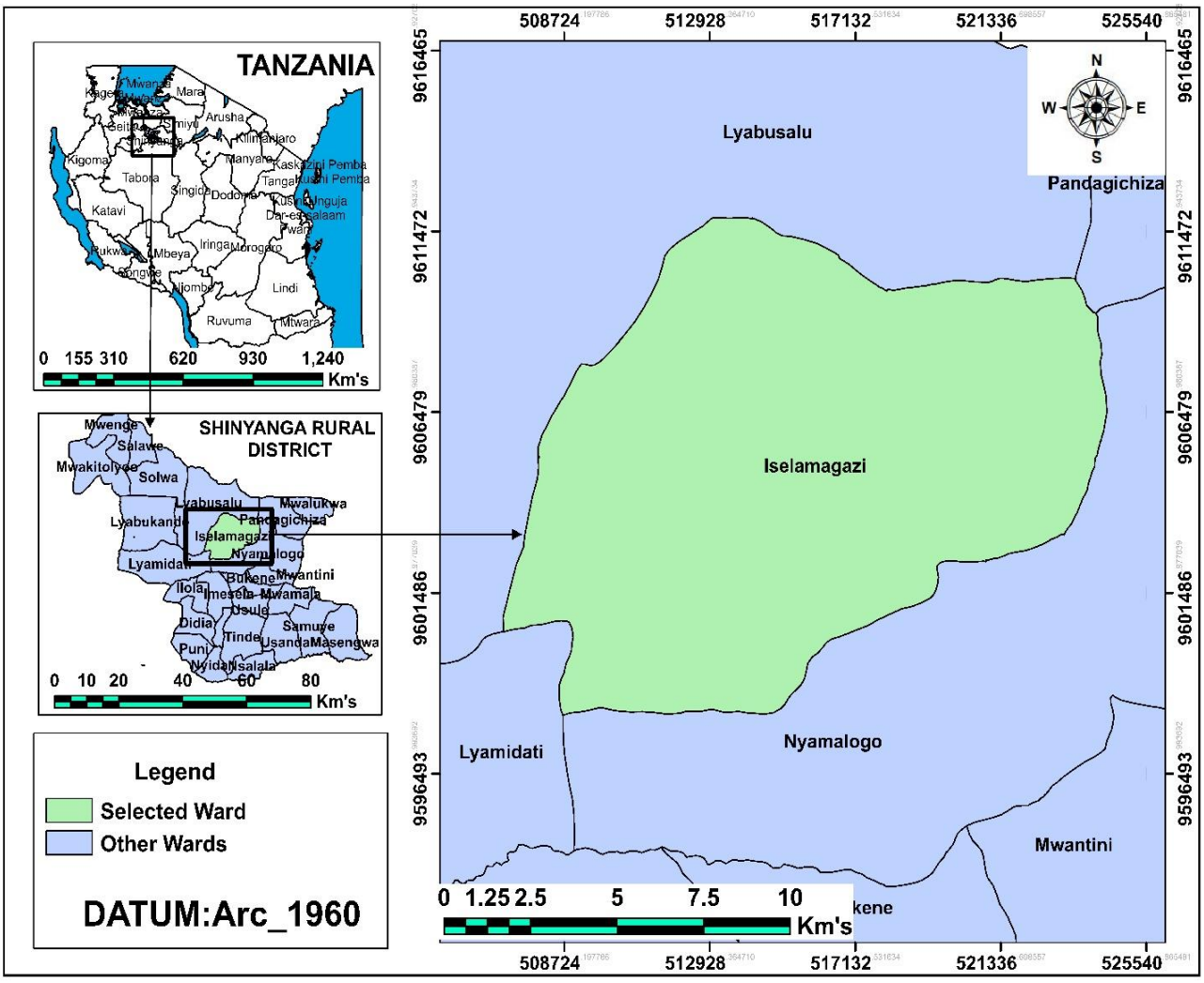

Figure 1: Map of the sampling area (Source: IRA 2022)

Data Analysis

Extract of the wild fruits were evaluated calorimetrically using the spectrophotometer UV-Visible model 6305 Jenway, UK and their results were presented on the table for interpretation and discussion. Ethnomedicinal information from the questionnaire was tabulated to enable interpretation and discussion. Photographs of the fruits (1-8) were presented for readers. Antioxidants were calculated using the equations used by (Rane *et al*., 2019): %DPPH RSA = (Absorbance control – Absorbance sample/Absorbance control) × 100. Total phenolic calculated by equation TP (mg GAE/g) = A_{765}/0.702) × 80.

Total flavonoid calculated by the equation TF (mg RUE/g) = (A_{425}÷0.47) ×1000.

RESULTS AND DISCUSSION

Antioxidant Capacity: Radical scavenge ability of DPPH Phenolic content and

Flavonoids

Extract of fruits evaluated for radical scavenging ability (D% DPPH), phenolic contents and flavonoid were presented in Table 1 with the total phenolic ranging from 19.513-175.983mg GAE/g. and total flavonoid ranging from 9.171-176.99 mg RUE/g. The results showed that all fruit investigated in the study had very high scavenging abilities. They also had very high antiradical activities which ranged from 0.368-44.77 TAU in total phenolic and ranged 0.233-5.68 TAU in total flavonoid. The results suggest that the wild fruits can potentially be used as natural antioxidants because extract showed to have significant difference for DPPH scavenging ability and antiradical activity. *Adansonia digitata showed* the highest antioxidant capacity while *Trema orientalis had* the lowest antioxidant capacity among the fruits investigated in the study (Table 1 and Figure 2).

All natural plants contain phenolic compounds. Phenolic compounds are said to exhibit antioxidant activity which play roles in inactivating lipid free radicals (reactive oxygen substance) as well as preventing hydro peroxides decomposition which results in producing free radicals. Flavonoid which is part of phenolic compound has demonstrated to be very effective antioxidants too.

The mechanism of antioxidant activity in fruits and other food exhibiting antioxidant is to scavenge free radical by converting to a form which is not reactive (ROS, RNS). To evaluate this mechanism of antioxidant activity , sympathised organic radical usually used is 22-dyphenyl -1 picylhydrazyl (DPPH) and 22-azino – bis (-ethlbenzothiazolite -6- sulphonic acid(ABTS) radicals, which are similar free radicals that are formed during respiration (aerobic). After performing different dilution for each extract and mixing them with DPPH solution. The percentage of DPPH destruction was plotted and the concentration of extract was presented as EC50. The EC50 is the lowest dilution that can quench 50% of DPPH.

Table 1: Calorimetric results showing Antioxidant Capacity of the studied fruits.

	Anti-radical activity						Antioxidant capacity					
Fruit	Phenolic (TAU)			Flavonoid (TAU)			Total phenolic mg GAE/g			Total flavonoid mg RUE/g		
F1	38.01	0.564	0.533	0.568	0.570	0.566	164.105	164.544	163.065	165.112	77.129	77.269
F2	31.73	0.51	0.479	0.514	0.520	0.515	175.983	176.033	174.943	176.99	82.712	82.852
F3	21.66	0.542	0.511	0.546	0.25	0.23	53.934	52.100	52.894	54.941	25.348	25.488
F4	44.77	0.451	0.42	0.455	3.89	3.54	89.809	87.250	88.769	90.816	42.21	42.25
F5	36.27	0.447	0.416	0.451	4.24	3.89	131.138	130.003	130.098	132.145	61.634	61.774
F6	18.56	0.432	0.401	0.436	3.76	3.41	143.622	142.456	142.582	144.629	67.502	67.642
F7	14.94	0.399	0.368	0.403	5.68	5.33	19.513	17.898	18.473	20.52	9.171	9.311
F8	12,54	0.402	0.371	0.406	5.14	4.79	20.927	19.500	19.927	21.974	9.854	9.994

Table 2: Ethnomedicinal data of the studied fruits in Shinyanga region of Tanzania

S/N	Local name	Scientific name	Plant part	Medicinal use
F5	Zambarau	*Syzygium cordatum*	Leaf, root, back, fruit	Treat burns, wound, cough, sexual infection, tuberculosis, malaria
F6	Mchunga	*Akocanthera schimperi*	Leaf, fruit	Treat wounds, bacterial infections
F8	Mtowo	*Azanza gackeana*	Fruit, root	Treat anemia, athma, chest pain
F7	Mkole	*Trema orientalis*	Fruit, leaf	Antidiabetic, antidiarerrhoeal
F3	Mfulu	*Vitex doniana*	Fruit, leaf	Treat malaria, stomach ache, painful menstruation, control hypertension
F4	Mnyewa	*Strychnosspinosa*	Fruit, root	Treat snakebites, venereal disease, increase breastmilk in lactating mother
F2	ubuyu	*Adansonia digitata*	Pulp, leaf, back, seed	Antiflamatory, antidiarerrhoeal, HIV relief, improve immunity
F1	Ukwaju	*Tamarindus indica*	Pulp, leaf, root	Antiflamatory, weight reduction, analgetic

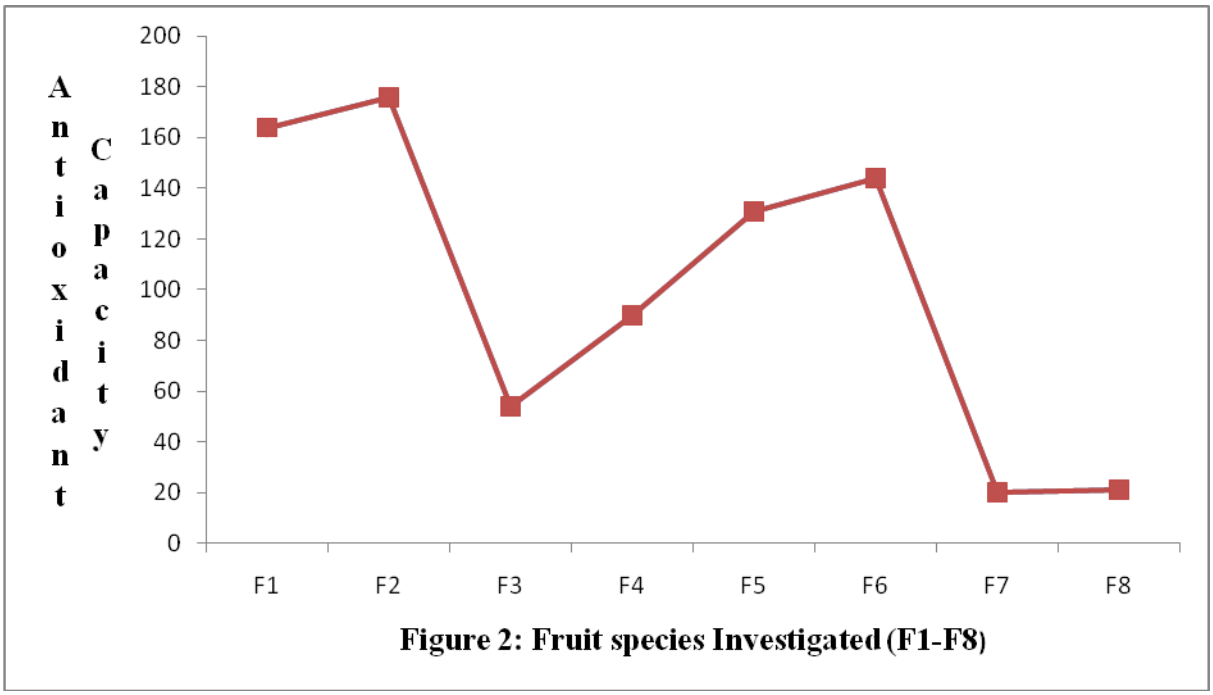

Figure 2: Fruit species Investigated (F1-F8)

1. *Syzygium cordatum* 2. *Acokanthera schimpen* 3. *Trema orientalis* 4. *Vitex doniana*

5. *Strychnos spinosa* 6. *Adansoma digitata* 7. *Tamarindus indica* 8. *Azanza gackeana*

Photography 1-8: Wild fruits investigated for antioxidant

A total of 23 diseases were reported to be treatable using wild fruits from eight fruit species as indicated in Table 2 while the whole fruit, leaf and roots were reported as parts which are mostly used for medicinal purposes. The range of antioxidant capacity and antiradical activities as summarized in Table 1 is the supporting evidence to show why the wild fruits from the eight plant species can treat such diseases listed in Table 2. Therefore, improving the knowledge of the community on the potential of the wild fruits will potentially improve their livelihood, economy and health. About eight local names which are common to most of regions in Tanzania were reported and documented although some of the names may vary from one region to another. Medicinal information collected from the study is an indication that the community is aware of the medicinal and food potential of the wild fruits (Njau, 2005) available in their environments despite of the economic potential may contribute when the fruits are cultivated commercially.

CONCLUSION AND RECOMMENDATION

The investigated fruits have indicated potentially medicinal importance to the community health as they have higher ability of fighting against harmful radical and diseases in human body, but also economically important as they can improve economy and reduce poverty in the community when domesticated and cultivated commercially. Propagating the fruits in the dry and semi-desert areas of Shinyanga region will also improve, protect soil erosion and conserve the Shinyanga environments.

The research is recommending propagation of the investigated fruits primarily to the lest of the dry areas of Shinyanga but also to other areas of Tanzania where the fruits can grow. The research is also recommending fruits utilization sensitization programmes to be conducted to Tanzania communities so as to improve health and livelihood of Tanzanians.

Declaration of conflict of interest

No conflict of interest to declare.

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REFERENCES

Battino, M. Giampieri, F. Pistollato, F. (2018). Nrf2 as regulator of innate immunity: A molecular Swiss army knife. *Biotechnology Advances*, **36**(2): 358–370.

Brand-Williams, W. Cuvelier, M. E. & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, ***28*** (1), 25–30.

Cătană, C. Atanasov, A. and Berindan-Neagoe, I. (2018). Natural products with anti-aging potential: affected targets and molecular mechanisms *Biotechnology Advances*, **36** (6): 1649–1656.

Moosavi, M. Haghi, A. Rahmati, M, (2018). Phytochemicals as potent modulators of autophagy for cancer therapy. *Cancer Letters*, **424**: 46–69.

Njau, P. (2005). Contribution of non-wood forest products (NWFPS) to the livelihood of the communities surrounding Nyang'oro range forest in Iringa district. Pp58

Onwuka, G., Clifford, H. & Onuegbu, N. (2012). Development of extraction protocol for phenolic compounds in African pear seed (*Dacryodes edulis*). *International Journal of Innovative Pharmaceutical Research,* **3**(2), 212–216.

Pękal, A., Pyrzynska, K. (2014). Evaluation of aluminium complexation reaction for flavonoid content assay. *Food Analytical Methods* **7**, 1776–1782.

Rane, M. Suryawanshi, S., Patil, R., Aware, C., Jadhav, R., Gaikwad, S. Singh, P. Yadav, S. Bapat, V. Gurav, R. & Jadhav, J. (2019). Exploring the proximate composition, antioxidant, anti-Parkinson's and anti-inflammatory potential of two neglected and underutilized *Mucuna* species from India. *South African Journal of Botany*, *124*, 304– 310.

Siddhuraju, Perumal, & Becker, K. (2007). The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L.) Walp.) seed extracts. *Food Chemistry*, *101*(1), 10–19.

Tewari, D, Stankiewicz, M, Mocan, A, (2018). Ethnopharmacological approaches for dementia therapy and significance of natural products and herbal drugs, *Frontiers in Aging Neuroscience*,**10**: 3.

Tewari, D, Nabavi, S, Nabavi, M, (2018). Targeting activator protein 1 signalling pathway by bioactive natural agents: possible therapeutic strategy for cancer prevention and intervention," *Pharmacological Research*, **128**:366–375.

Waltenberger, B, Mocan, A, Šmejkal, K, Heiss, E, and Atanasov, G. (2016). "Natural products to counteract the epidemic of cardiovascular and metabolic disorders, *Molecules*, **21** (6): 807.

Yeung, A, Goto, T, and Leung, W, (2017). The changing landscape of neuroscience research, 2006–2015: a bibliometric study. *Frontiers in Neuroscience*, **11**: 120.

Yeung, A, Goto, T, and Keung Leung, W, (2017). A bibliometric review of research trendsin neuroimaging. *Current Science*, **112** (4): 725–734.

Yeung, A, Heinrich, M, and Atanasov, A, (2018). Ethnopharmacology—a bibliometric analysis of a field of research meandering between medicine and food science?" *Frontiers in Pharmacology*, **9**:215.

Yeung, A, Aggarwal, B, Barreca, D, (2018). Dietary natural products and their potential to influence health and disease including animal model studies. *Animal Science Papers and Reports*, **36**:345–358.