

Comparative evaluation of *in vitro* antioxidant properties of *Cajanus cajan* seed and *Moringa oleifera* leave extracts

*Ilesanmi, Funmilayo Florence¹, Akinloye, Oluseyi Adeboye² and Ilesanmi, Olayinka Stephen³

¹ Department of Biochemistry, Nigerian Stored Products Research Institute, P.M.B 5044, Nigeria.

² Department of Biochemistry, College of Natural Sciences, University of Agriculture, P. M. B. 2240, Abeokuta, Ogun State, Nigeria

³ Department of Community Medicine, University College Hospital, Ibadan. Nigeria

ABSTRACT

Aims: This study was carried out to compare the antioxidant potentials of hydro-methanolic extracts of seeds of *Cajanus cajan* and the leaves of *Moringa oleifera*. These plants have been associated with alleviating oxidative stress related conditions.

Place and Duration of Study: Department of Biochemistry, Federal University of Agriculture, Abeokuta and Covenant University Sango, Nigeria, between August 2012 and September, 2012.

Methodology: : Different analyses for determining antioxidant potentials were used to compare the antioxidant potentials of the plants: hydroxyl radical scavenging test, ascorbic acid value, total polyphenols, total flavonoids content, ferric reducing antioxidant power, 2,2-azino-bis (3-ethylbenzthiazoline-6- sulphonic acid (ABTS) scavenging test, 2,2-diphenyl-2-picrylhydrazyl (DPPH) and inhibition of lipid peroxidation (lipoprotein).

Results: The total phenolic contents of *Cajanus cajan* and *Moringa oleifera* were 83.0 ± 0.02 and 541.0 ± 0.02 mg gallic acid equivalents per gram dry weight (mg/GAE/g DW) respectively. Also, the results obtained for total flavonoids in *Cajanus cajan* and *Moringa oleifera* were 46.0 ± 0.01 and 645.0 ± 0.10 mg pyrocatechol equivalent per gram dry weight respectively. It was observed that *Moringa oleifera* leaves extract has significantly higher antioxidant potentials than the *Cajanus cajan* seeds extract ($p < .05$)

Conclusion: Data suggest that *Moringa oleifera* leaves extract had higher antioxidant potentials in comparison with that of *Cajanus cajan* seeds. Further research in determining the benefits of *Moringa Oleifera* leaves and *Cajanus cajan* seeds extracts due to their antioxidant potential in animal model is therefore recommended.

Keywords: antioxidant, *Moringa oleifera*, Pigeon pea, flavonoids.

1. INTRODUCTION

Moringa oleifera is the most widely cultivated species of a monogeneric family of Moringaceae. It has been given several names including horseradish tree, drumstick tree, benzolive tree, kelor, marango, mlonge, moonga, mulangay, nébéday, saijhan, sajna and ben oil tree. [1, 2] It is a rapidly-growing tree utilized worldwide. *Moringa oleifera* is considered one of the world's most useful trees, as almost every part of the tree has some nutritional, medicinal and other beneficial properties. [3] The medicinal properties have been attributed to phytochemical compositions of its various parts; the roots, bark, leaves, flowers, fruits, and seeds.[4, 5] Though, the dry leaves of *Moringa oleifera* are widely utilized in the developing countries as a good source of protein, calcium and anti-oxidizing agents. The leaves, especially young shoots, are eaten as salads, in vegetable curries, and as pickles. The leaves can be eaten fresh, cooked, or stored as dried powder for many months without refrigeration, and reportedly without loss of nutritional values.[3]

Cajanus cajan millsp. (Pigeon pea), a herbaceous plant of the botanical family Fabaceae occurs in several varieties. It is known as pigeon pea (English), Otili (Yoruba), and Waken turawa (Hausa). Other names of *Cajanus cajan* include, Pigeon pea, red gram, Congo pea, gungo pea, no eye pea, dhal, gandul, gandure, frijol de árbol, and pois cajan. In developing tropical countries Pigeon peas are widely consumed as an excellent source of protein. The seeds and the pods are eaten as vegetables and additives in soups. Preparations of the leaves are used to treat jaundice and the extracts of pigeon pea are commonly used all over the world for the treatment of diabetes, dysentery, hepatitis, measles and as a febrifuge to stabilize the menstrual period.[6] Nowadays, pigeon pea leaves are used for the treatment of wounds, bedsores and malaria [7]. Protective effects of extracts from pigeon pea leaf against hypoxic-ischemic brain damage and alcohol-induced liver damage have also been reported.[8] Chemical constituent investigations have indicated that pigeon pea leaves are rich in flavonoids and stilbenes, which are considered to be responsible for its beneficial efficacies [9, 10].

Moringa oleifera leave extracts and *Cajanus cajan* seed extracts are widely used as a result of their antioxidant properties. Antioxidants are compounds that inhibit or terminate the oxidation process which results in generation of free radicals and reactive oxygen species. They help to scavenge free radicals within the human body. Free radicals impair the proper functioning of the immune system and they have been implicated in various disease conditions such as diabetes mellitus, cancer, atherosclerosis, arthritis and neurodegenerative diseases as well as ageing process.[11, 12] The antioxidant ability of *Moringa oleifera* leave extracts and *Cajanus cajan* seed extracts have been documented.[13] It was suggested that pigeon pea leaf extracts may be valuable natural antioxidant sources and are potentially applicable in both medicine and food industry.[13] The extent of the antioxidant properties of both *Moringa oleifera* leave extracts and *Cajanus cajan* is yet to be fully elucidated and compared with a substance with standard antioxidant properties. The aim of this work is therefore to investigate and compare the antioxidant properties of the *Cajanus cajan* seed extracts and *Moringa oleifera* leaves extracts with antioxidant properties of Ascorbic acid.

2. MATERIAL AND METHODS

2.1 Plant materials

The leaves of *Moringa oleifera* were collected from a biological garden in Ibadan Oyo state Nigeria and the herbarium specimens were deposited in Forestry Research Institute of Nigeria, Ibadan, Nigeria and the *Cajanus cajan* seeds were purchased from Bodija Market Ibadan. The plant materials were identified and authenticated by taxonomists. (Ugbogu O. A.

& Shosanya O. S. Voucher Specimen no.108486) at Forestry Research Institute of Nigeria, Ibadan, Nigeria.

Chemicals

2-Thiobarbituric acid (TBA), trichloroacetic acid, ascorbic acid and 2, 2-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma (Deisenhofen, Germany). The other chemicals were of analytical grades.

2.1.1 Preparation of plant extracts

Fresh leaves of *Moringa oleifera* collected, were air-dried in a shed and milled into powder. The powder (50g) was extracted in a round bottom flask with 80% methanol at room temperature for 48 hours with 1:10 sample to solvent ratio. After 48 hours, the extract was filtered through filter paper and dried using rotary evaporator. The seeds of *Cajanus cajan* were also milled to powder and then defatted using n-hexane in a soxhlet apparatus. The residue was later subjected to 80% methanolic extraction. The stock solution was prepared by dissolving 0.1g of extract in 10ml of distilled water.

2.2 ANTIOXIDANT ANALYSIS

2.2.1 Quantification of total phenolic content

The Folin–Ciocalteu method was used for total phenolic content determination.[14] The Folin–Ciocalteu reagent (2.5 ml) was diluted with water (1:10, v/v), and mixed with 2 ml of 75 g/L aqueous solution of sodium carbonate. The resultant solution was added to 0.5 ml of the extract. The mixture was kept for 5 min at 50°C before measuring the absorbance at 760 nm. The total phenolic content was expressed as mg gallic acid equivalent (GAE) /g of extract.

2.2.2 Estimation of Flavonoid Content

Total flavonoids contents were estimated both in methanolic extracts of *Moringa oleifera* leaves and *Cajanus cajan* seed using pyrocatechol as standard.[15] Briefly, 0.5mL aliquot extract was added to 75µL of 5% NaNO₂ (sodium nitrite) solution. After 6 minutes, 150µL of a 10% AlCl₃ 6H₂O solution was added and the mixture was allowed to stand for another 5 minutes. Then, 0.5mL of 1 molar NaOH and 2.5mL of distilled water was added. The solutions were mixed and absorbance was measured at 510 nm using Labo Med Inc. Spectrophotometer (USA). All experiments were carried out in triplicates. Total flavonoids content was calculated as mg/pyrocatechol equivalent/gram of dry samples.

2.2.3 Total antioxidant capacity (TAC)

TAC was measured according to the method described by Oboh with slight modification [16]. The reacting mixture contained 75 µl of (2g of 2, 4 Dinitro phenyl hydrazine, and 230 mg of thiourea and 270 mg of CuSO₄.5H₂O in 100ml of 5 M H₂SO₄), 50 µl of extract, 350 µl of distilled water and 100 µl of 13.3% (w/v) TCA. The mixture was incubated for 3hrs at 37°C after which 500 µl of 65% (v/v) H₂SO₄ was added. Absorbance was read at 520nm and ascorbic acid content was calculated from the formula:

(Absorbance of sample/Absorbance of standard) x concentration of standard (1)

2.2.4 Ferric reducing antioxidant potential (FRAP) test

The FRAP test was conducted according to the method of Oboh with slight modification [16]. 50µl aliquot extract was mixed with 450µl of 200mM sodium phosphate buffer (PH 6.6) and 250µl 1% potassium ferricyanide was added. The mixture was incubated at 50°C for 20 minutes. 250 µl of 10% Trichloroacetic acid was added. The mixture was centrifuged at 2000 rpm for 10mins. 10µl of the supernatant was mixed with 500 µl 0.1% FeCl₃. Absorbance of the reaction mixture was measured at 700 nm using spectrophotometer. All tests were run in

triplicates. Increase in the absorbance of the reaction mixture indicated increased reducing power.

2.2.5 DPPH Radicals Scavenging Potentials

The scavenging effect of extract on DPPH free radical was measured accordingly [17]. 2ml of each sample solution was added to 2 ml of 0.1mM DPPH dissolved in 95% ethanol. The mixture was shaken and left for 30 min at room temperature, and the absorbance of resulting solution was read at 517nm. Ascorbic acid was used as positive reference. The experiment was carried out in triplicates. The scavenging effect was expressed as shown in the following equation:

$$\% \text{ inhibition} = [(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100 \quad (2)$$

Where A_{blank} = absorbance of blank and A_{sample} = absorbance of extract samples. A lower absorbance represents a higher DPPH scavenging potential.

2.2.6 Hydroxyl Radical Scavenging potential

Hydroxyl radical scavenging potential is commonly used to evaluate the free radical scavenging effectiveness of various antioxidant substances [16]. It was measured by studying the competition between deoxyribose and the extracts for hydroxyl radicals generated from the $\text{Fe}^{2+}/\text{EDTA}/\text{H}_2\text{O}_2$ system (Fenton reaction). The hydroxyl radical attacks deoxyribose, which eventually results in the formation of thiobarbituric acid reacting substances (TBARS). Reaction mixture contained 60 μL of 1.0mM FeCl_3 , 90 μL of 1.0mM 1,10-phenanthroline, 2.4mL of 0.2M phosphate buffer (pH 7.8), 150 μL of 0.17 M H_2O_2 , and 1.5mL of extract. After incubation at room temperature for 5min, the absorbance of reaction mixture was noted at 560nm. The hydroxyl radicals scavenging potential was calculated according to the following equation and compared with ascorbic acid as standard:

$$\% \text{ Inhibition} = [(AB - AE)/AB] \times 100 \quad (3)$$

Where AB was the absorbance of blank (without extract) and AE was the absorbance of tested samples.

2.2.7 ABTS radicals scavenging potentials

ABTS radical scavenging potential of extracts was determined according to a standard method [18]. A stock solution of ABTS radicals was prepared by mixing 5.0 ml of 7 mM ABTS solution with 88 μl of 140 mM potassium persulfate, and kept in the dark at room temperature for 16 hrs. An aliquot of stock solution was diluted with phosphate buffer (5 mM, pH 7.4) containing 0.15 M NaCl in order to prepare the working solution of ABTS radicals to an absorbance of 0.70 ± 0.02 at 734 nm. A 65 μl aliquot of sample solution was mixed with 910 μl of ABTS radical working solution, incubated for 10 min at room temperature in the dark, and absorbance was measured at 734nm. The percent reduction of ABTS* to ABTS was calculated according to the following equation:

$$\text{ABTS (\%)} = [(A_0 - A_1)/A_0] \times 100 \quad (4)$$

A_0 = absorbance of control, A_1 = absorbance of sample

2.2.8 Inhibition of lipid peroxidation

The reaction mixtures contained 0.2% lecithin in 0.1mol/l phosphate buffer solution (PBS)(pH 7.4), 25 μl of extract was dissolved in 0.1mg/ L PBS (pH7.4) and 50 μL 25mmol/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, the total volume was 1ml . The mixture was incubated at 37°C in a water bath for 1 hour. Reaction was stopped by adding 250 μl 20% TCA. Followed by addition of 250 μl of 0.8% TBA, the tubes was placed in boiling water bath for 20 minutes and then crushed in ice bath before centrifuging at 2500rpm for 10mins. The rate of inhibition of the sample was

assessed by measuring optical density of the supernatant at 532nm using spectrophotometer.

The antioxidative potential at the end of the analysis was expressed as reduction percent for peroxidation (RP %)

RP% = [(peroxidation indicator value without antioxidant) – (peroxidation indicator value with antioxidant) / (peroxidation indicator value without antioxidant)] x 100 (5)

184

185

2.3 Statistical Analysis

187

Data were entered and analyzed using SPSS 21 for Window software (SPSS Inc., Chicago, IL). Mean value of Polyphenols mg/g Dry Weight and Flavonoids mg/g Dry Weight were compared using independent t-test. Comparison of anti-oxidant properties of Ascorbic acid, *Cajanus cajan*, and *Moringa oleifera* using different antioxidant Assay was done using one way analysis of variance (ANOVA). *P*-value less than .05 were considered statistically significant.

194

195

3. RESULTS AND DISCUSSION

197

3.1 Extract yield, total Polyphenolic and total flavonoids

198

The methanolic extraction yielded 21.24% and 26.66% dry weight for *Cajanus cajan* and *Moringa oleifera* respectively.

Table [1] shows the content of phenolic compounds that were measured in terms of gallic acid equivalents. The total phenolic contents in the *Cajanus cajan* and *Moringa oleifera* was 83.0 ± 0.02 and 541.0 ± 0.02 mg Gallic acid equivalents per gram of extract respectively.

The flavonoids content of *Cajanus cajan* and *Moringa oleifera* was 46.0 ± 0.01 and 645.0 ± 0.10mg pyrocatechol equivalent/g DW respectively. The higher total phenolic content and total flavonoids content were obtained from *Moringa oleifera* leave extract.

207

208

Table 1. Polyphenols and flavonoids contents of methanolic extracts of the leaves of *Moringa oleifera* and *Cajanus cajan* seeds.

210

211

Samples	Polyphenols mg/g Dry Weight Mean ± Standard Deviation	Flavonoids mg/g Dry Weight Mean ± Standard Deviation	p-value
<i>Moringa oleifera</i> leaves	541.0 ± 0.02	645.0 ± 0.10	<0.001
<i>Cajanus cajan</i> seeds	83.0 ± 0.02	46.0 ± 0.01	<0.001

212

3.2 Antioxidant Analysis

214

3.2.1 DPPH free radical scavenging Potential

215

The DPPH analysis showed that the antioxidant property of *Moringa oleifera* is significantly different from that of *Cajanus cajan* as observed in Table 2. Methanol extract of *Moringa*

217

leaves exhibited higher radical scavenging potential than the seed of *Cajanus cajan* (67.157 ± 0.08 and $26.440 \pm 5.07\%$ inhibition). The positive control, ascorbic acid showed maximum scavenging effect, ($73.863 \pm 0.54\%$ inhibition).

3.2.2 Hydroxyl (OH[•]) radical scavenging potential

Hydroxyl (OH[•]) free radical scavenging potential of the *Moringa oleifera* extracts was found to be higher than that of the reference compound ascorbic acid and *Cajanus cajan*. The EC₅₀ values of ascorbic acid, *Moringa oleifera* and *Cajanus cajan* were 168.35 ± 13.41 , 428.37 ± 17.46 and 60.0 ± 6.41 mg/ml, respectively.

3.2.3 Total ascorbic acid content

Table [2] showed that total ascorbic acid content in *Moringa oleifera* extract (28.02 ± 0.136 mg/g DW) was higher than what was obtained in *Cajanus cajan* (12.48 ± 0.591 mg/g DW) while reference ascorbic acid (100 ± 0.00 mg/g) had maximum reducing ability.

3.2.4 Ferric reducing antioxidant potentials

In the reducing potential determination, the presence of reducing agents in the sample resulted in the reduction of Fe³⁺ to Fe²⁺ by donating an electron. Amount of Fe²⁺ complex was monitored by measuring the formation of Perl's blue at 700 nm. The extracts could reduce the most Fe³⁺ ions, as indicative from the results. The values for ferric reducing antioxidant power revealed that the moringa extract (34.70 ± 0.06) had a significantly higher reducing power than *Cajanus cajan* (33.0 ± 00) ($P < 0.05$). The reference compound ascorbic acid had the highest reducing ability (50.00 ± 0.11).

3.2.5 Antioxidant potential determined by the ABTS method

ABTS analysis of extracts demonstrated antioxidant capacities to scavenge radical action. The ABTS analysis revealed that *Moringa oleifera*, *Cajanus cajan* and Ascorbic acid had (99.86 ± 0.00 mg/L), (99.587 ± 0.27 mg/L) and (69.57 ± 69.57 mg/L) antioxidant values respectively.

3.2.6 Inhibition of lipid peroxidation

The result of inhibition of lecithin revealed that *Moringa oleifera* had lower lipid peroxidation inhibition value (3.458 ± 2.37) compared to either *Cajanus cajan* (9.631 ± 2.65) or the reference ascorbic acid (44.173 ± 2.50).

Table 2. Comparison of Antioxidant properties of Ascorbic acid, *Cajanus cajan* and *Moringa oleifera* using different Anti-oxidants parameters

Mean $\pm$ Standard Deviation				
Anti-oxidant parameters*	Ascorbic acid	Cajanus cajan	Moringa oleifera	p value
DPPH	73.863 ± 0.54	26.440 ± 5.07	67.157 ± 0.08	<0.001
HO [•]	168.35 ± 13.41	60.00 ± 6.41	428.37 ± 17.46	0.043
Total Ascorbic Acid	100.00 ± 0.00	12.48 ± 0.59	28.02 ± 0.14	<0.001
ABTS	69.570 ± 0.95	99.587 ± 0.25	99.860 ± 0.00	<0.001
LIPID PEROXIDATION	44.173 ± 2.51	9.631 ± 2.65	3.458 ± 2.37	< 0.001
FRAP	50.00 ± 0.11	33.00 ± 00	34.700 ± 0.06	<0.001

254 *DPPH: 2, 2-diphenyl-2-picrylhydrazyl
255 ABTS: 2, 2-azino-bis (3-ethylbenzthiazoline-6- sulphonic acid
256 FRAP: Ferric Reducing Antioxidant Power
257

258 3.3 DISCUSSION

259
260 This study compared the antioxidant properties of *Cajanus cajan* seed extracts and *Moringa*
261 *oleifera* leaves extracts with antioxidant properties of Ascorbic acid. The *in vitro* total
262 antioxidant analysis of the plant extracts revealed appreciable antioxidant potential
263 compared with the standard ascorbic acid. However, a higher antioxidant property was
264 found in *Moringa oleifera* leaf. The extent of the antioxidant properties seems to be due to
265 the presence of polyphenols and flavonoids.

266
267 Phenolic compounds like flavonoids have been reported to possess significant antioxidant
268 properties.[19-22] The phenolic content values obtained in this work shows that *Moringa*
269 *oleifera* leaves and *Cajanus cajan* seeds were similar to previous study [23]. Flavonoids in
270 the leaves of *Cajanus cajan* was previously estimated.[7, 13] Phenolic compounds have
271 been found to be one of the most effective antioxidant constituents in plant foods, including
272 fruits, vegetables and grains. [24, 25]

273
274 Among the oxygen radicals, the hydroxyl radical is the most reactive which severely
275 damages adjacent biomolecules such as proteins, DNA, nucleic acid and almost any
276 biological molecule it touches. This damage causes aging, cancer and several diseases. In
277 addition; this radical species is considered as one of the quick initiators of the lipid oxidation
278 process, abstracting hydrogen atoms from unsaturated fatty acids.[26, 27] Therefore, the
279 removal of hydroxyl radical is probably one of the most effective defenses of a living body
280 against various diseases.

281 4. CONCLUSION

282
283
284 It was observed that *Moringa oleifera* leaves extract has higher antioxidant potentials than
285 the *Cajanus cajan* seeds extract. The antioxidant potentials of these extracts were directly
286 proportional to the phenolic contents indicating that phenolic compounds are the major
287 contributors to the antioxidant properties of *Moringa oleifera* leaves and *Cajanus cajan*
288 seeds extract. Further research in determining the benefits of *Moringa Oleifera* leaves and
289 *Cajanus cajan* seeds extracts due to their antioxidant potential in animal model is therefore
290 recommended

291 292 ACKNOWLEDGEMENTS

293
294 We acknowledge Dr Rotimi S O for the technical assistance rendered in the analysis. Also,
295 our appreciation goes to the Management of Nigerian Stored Products Research Institute for
296 providing some of the equipment used in the analysis.
297

298 **COMPETING INTERESTS**

299

300 Authors have declared that no competing interests exist.

301

302 **AUTHORS' CONTRIBUTIONS**

303

304 AOA and IFF designed the study and wrote the first draft of the manuscript, IOS performed
305 the statistical analysis and managed the literature searches.

306

307

308 **COMPETING INTERESTS**

309

310 Authors have declared that no competing interests exist.

311

312

313

314

315

316

317

318

319

320

321

322

323

324

325

326

327

328

329

330

331

332

333

334

335

336

337

338

339

340

341

342

343

344

345

346

347 **REFERENCES**

- 348
- 349
- 350 1. Rajangam J, Azahakia R, Manavalan S, Thangaraj T, Vijayakumar A, Muthukrishnan
- 351 N. Status of production and utilisation of Moringa in Southern India. In: The Miracle Tree/ the
- 352 Multiple Attributes of Moringa. Dakar: CWS. Tamil Nadu, India Division of Vegetable
- 353 Sciences, Horticultural College and Research Institute, Tamil Nadu Agricultural University,
- 354 2001.
- 355 2. Olson M. Introduction to the Moringa family. In The Miracle Tree – Moringa oleifera:
- 356 Natural Nutrition for the Tropics. Fuglie LL, editor. Dakar: Church World Service; 2001
- 357 3. Luqman S, Srivastava S, Kumar R, Maurya AK, Chanda D. Experimental
- 358 Assessment of Moringa oleifera Leaf and Fruit for Its Antistress, Antioxidant, and
- 359 Scavenging Potential Using In Vitro and In Vivo Assays. Evidence-based complementary
- 360 and alternative medicine: eCAM. 2012; 2012:519084. PubMed PMID: 22216055. Pubmed
- 361 Central PMCID: PMC3247066. Epub 2012/01/05. eng.
- 362 4. Anwar F, Latif S, Ashraf M, Gilani AH. Moringa oleifera: a food plant with multiple
- 363 medicinal uses. Phytother. Res. 2007; 21: 17-25.
- 364 5. Kumar PS, Mishra D, Ghosh G, Panda GS. Medicinal uses and pharmacological
- 365 properties of Moringa oleifera. Int. J. Phytother. 2010; 2: 210-216.
- 366 6. Ahsan R, Islam M. In vitro antibacterial screening and toxicological study of some
- 367 useful plants (Cajanus cajan). Euro J Sci Res 2009.41:227-232.
- 368 7. Aiyelaja A, Bello O. Ethnobotanical potentials of common herbs in Nigeria: A case
- 369 study of Enugu state. Educat. Res. Rev. 2006;1: 16-22.
- 370 8. Pal D, Sarkar A, Gain S, Jana S, Mandal S. CNS depressant activities of Cocos
- 371 nucifera in mice. Acta Pol Pharm. 2011; 68: 249-254.
- 372 9. Parrotta J. Healing plants of Peninsular India. Wallingford, UK and New York: CABI
- 373 Publishing; 2001.
- 374 10. Lans C. Comparison of plants used for skin and stomach problems in Trinidad and
- 375 Tobago with Asian Ethnomedicine. J Ethnobiol Ethnomed. 2007; 33: 22-28.
- 376 11. Gey K. The antioxidant hypothesis of cardiovascular disease: Epidemiology and
- 377 mechanisms. Biochemical Society Transactions. 1990; 18: 1041-1045.
- 378 12. Dawidowicz AL, Wianowska D, Baraniak B. The antioxidant properties of alcoholic
- 379 extracts from Sambucus nigra L. (antioxidant properties of extracts). Lebensmittel-
- 380 Wissenschaft + [i.e. und] Technologie. Food science + technology. Science + technologie
- 381 alimentaire. 2006 04; 39 (3):308-315.
- 382 13. Wu N, Fu K, Fu Y, Zu Y, Chang F, Chen Y, et al. Antioxidant Activities of Extracts
- 383 and Main Components of Pigeonpea [Cajanus cajan (L.) Millsp.] Leaves. Molecules. 2009;
- 384 14(3):1032-1043. PubMed PMID: doi: 10. 3390/molecules14031032.

- 385 14. Vázquez G, Fontenla E, Santos J, Freire MS, González-Álvarez J, Antorrena G.
386 Antioxidant activity and phenolic content of chestnut (*Castanea sativa*) shell and eucalyptus
387 (*Eucalyptus globulus*) bark extracts. *Industrial Crops and Products*. 2008; 28(3):279-285.
- 388 15. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in
389 mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*. 1999 3//;
390 64(4):555-559.
- 391 16. Oboh G, Ademiluyi AO, Akinyemi AJ. Inhibition of acetylcholinesterase activities and
392 some pro-oxidant induced lipid peroxidation in rat brain by two varieties of ginger (*Zingiber*
393 *officinale*). *Experimental and toxicologic pathology: official journal of the Gesellschaft fur*
394 *Toxikologische Pathologie*. 2012 May; 64(4):315-319. PubMed PMID: 20952170. Epub
395 2010/10/19. eng.
- 396 17. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of
397 xanthone on the auto oxidation of soybean in cyclodextrin emulsion. *J Agric Food Chem*
398 1992; 40: 945-948.
- 399 18. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant
400 activity applying an improved ABTS radical cation decolorization assay. *Free radical biology*
401 *& medicine*. 1999 May; 26(9-10):1231-1237. PubMed PMID: 10381194. Epub 1999/06/25.
402 eng.
- 403 19. Ng TB, Liu F, Wang Z. Antioxidative activity of natural products from plants. *Life Sci*.
404 2000; 66(8):709-723.
- 405 20. Cakir A, Mavi A, Kazaz C, Yildirim A, Kufrevioglu O. Antioxidant activities of the
406 extracts and components of *Teucrium orientale* L. var. *orientale*. *Turk J Chem* 2006; 30: 483-
407 494.
- 408 21. Aiyegoro O, Okoh A. Phytochemical Screening and Polyphenolic Antioxidant Activity
409 of Aqueous Crude Leaf Extract of *Helichrysum pedunculatum*. *Int J Mol Sci* 2009; 10: 4990-
410 5001.
- 411 22. Imaga NOA, Gbenle GO, Okochi VI, Adenekan SO, Edeoghon SO, Kehinde MO, et
412 al. Antisickling and toxicological profile of *Parquetina nigrescens*. *J. Med. Plants Res*. 2010;
413 4(8):639-643.
- 414 23. Amal A. Mohamed, Ashraf A. Khalil, Hossam E. S. El-Beltagi. Antioxidant and
415 antimicrobial properties of kaff maryam (*Anastatica hierochuntica*) and doum palm
416 (*Hyphaene thebaica*). *Grasas Y Aceites*. 2010; 61(1):67-75.
- 417 24. Velioglu Y, Mazza G, Gao L, Oomah B. Antioxidant activity and total polyphenolics
418 in selected fruits, vegetables, and grain products. *J Agric Food Chem*. 1998;46(10):4113-
419 4117.
- 420 25. Imaga NOA, Gbenle GO, Okochi VI, Akanbi SO, Edeoghon SO, Oigbochie V, et al.
421 Antisickling property of *Carica papaya* leaf extract. *Afr J Biochem Res*. 2009; 3(4):102-106.
- 422 26. Aruoma OI. Free radicals oxidative stress and antioxidants in human health and
423 disease. *J Amer Oil Chem Soc*. 1998; 75: 199-211.

424 27. Pin-Der-Duh X, Pin-Chan-Du X, Gow-Chin Yen X. Action of methanolic extract of
425 mung hulls as inhibitors of lipid peroxidation and non-lipid oxidative damage. Food Chem
426 Toxicol. 1997; 37: 1055-1061.
427