MEDWELL PUBLISHING
Research Journal of Medical Sciences 3 (2): 70-74, 2009
ISSN: 1815-9346


# Evaluation of Flavonoids and Total Phenolic Contents of Stem Bark and Leaves of *Parkia biglobosa* (Jacq.) Benth. (Mimosaceae)-Free Radical Scavenging and Antimicrobial Activities

[1]H. Millogo-Kone, [1]M. Lompo, [1]F. Kini, [1-3]S. Asimi, [1, 2]I.P. Guissou and [2]O. Nacoulma
[1]Département Médecine et Pharmacopée Traditionnelles,
Centre National de la Recherche Scientifique et Technologique (CNRST),
Institut de Recherche en Sciences de la Santé (IRSS), Pharmacie (MEPHATRA/PH), Burkina Faso
[2]Département Biochimie/Microbiologie, Université de Ouagadougou, UFR/SVT, Burkina Faso
[3]Département Production Forestière, CNRST, Laboratoire de Microbiologie, Burkina Faso

**Abstract:** The total phenolics and the flavonoids contents of stem barks and leaves of *Parkia biglobosa* harvested at different periods of the year, on 2 different areas of Burkina Faso (Yako and Lèna), were determined. The total phenolics were extracted according to Harborne's method and also by the means of a soxhlet apparatus. The determination of the phenolics contents was conducted with 8 extracts using tannic acid as standard. The flavonoids contents of the 8 extracts were also determined, using quercetin as a reference substance. The results showed that the total phenolics contents vary significantly all year round at the same area and also from Lèna to Yako. They also revealed that the flavonoids contents of the leaves harvested at Yako ranged from 1.49-1.80% throughout the year. The results also confirmed that the leaves contain more flavonoids than the barks. Antioxidant activity was determined by the means of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay. The results showed effectiveness with a radical inhibition ranging from 25.53±3.56-55.449±4.53 mg $mL^{-1}$. The antibacterial assays on gram positive *Staphylococcus aureus* showed that the hydroalcoholic extract of stem bark harvested in May at Yako was the most effective of all the extracts.

**Key words:** *Parkia biglobosa*, total phenolics, flavonoids, antioxidant, antimicrobial

## INTRODUCTION

Many diseases, including cancer, cardiovascular diseases, cataracts, arterosclerosis, diabetes, arthritis, immune deficiency diseases and aging are associated with oxidative damage (Pietta *et al*., 1998; Lee *et al*., 2000; Middleton *et al*., 2000). Many plants contain antioxidant compounds that may function as free radical scavengers, complexers of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation (Andlauer and Furst, 1998). Occurring naturally in plants, phenolic compounds including flavonoids, tannins and phenolic acids are currently of growing interest due to their biological effects in human health (Jayaprakasha *et al*., 2003; Motalleb *et al*., 2005; Sachetti *et al*., 2005; Rajeshwar *et al*., 2005; Hinneburg *et al*., 2006).

*Parkia biglobosa* (Jacq.) Benth., a widespread savana tree, is very used in traditional medicine. Its stem bark and leaves are used for the treatment of many infectious diseases: dental caries, pneumonia, bronchitis, violent stomachaches, severe cough, diarrhea, wounds, otitis, dermatosis, amoebiasis, bilharziosis, leprosis, ankylosis, tracheitis, conjunctivitis (Kerharo and Adam, 1973; Nacoulma, 1996; Arbonnier, 2002). *Parkia biglobosa* is a plant recognized to be very rich in phenolic compounds notably in tannins (Nacro and Millogo, 1993; Millogo-Kone *et al*., 2006). Simple phenols and phenolic acids have been identified in the crude extract after acid hydrolysis (Millogo-Kone *et al*., 2001).

Our study was aimed at evaluating the total phenolic and the flavonoid contents, the antimicrobial and the antioxidant activities of samples of leaves and stem barks harvested on 2 different areas of Burkina Faso, at different periods.

## MATERIALS AND METHODS

**Plant material:** The leaves and stem barks of *Parkia biglobosa* were purchased from 2 different areas (Yako and Lèna) in Burkina Faso. They were identified and

**Corresponding Author:** H. Millogo-Kone, Département Médecine et Pharmacopée Traditionnelles,
Centre National de la Recherche Scientifique et Technologique (CNRST),
Institut de Recherche en Sciences de la Santé (IRSS), Pharmacie (MEPHATRA/PH), Burkina Faso

authenticated by Prof Jeanne MILLOGO, a botanist of the Department of Botanic, University of Burkina Faso. Voucher specimen was deposited at the Herbarium of this University.

**Extracts:** The leaves and stem barks were dried at room temperature and finely powdered. The aqueous extracts were obtained by boiling 10 g of plant material in 100 mL of distilled water for 10 min. The crude ethanolic extracts were obtained by pouring 100 mL of boiling ethanol:water (70:30) v:v onto 10 g of plant material, according to Harborne (1989) method. The mixture was allowed to macerate for 18 h, then filtered with Whatman filter paper No. 1.

Ten grams of plant materials were also extracted by ethanol-water (70:30) v:v using a soxhlet extractor apparatus. The extracts were freeze dried.

For flavonoid extracts, 1 g of plant material was placed in a test tube, added with 10 mL of methanol and maintained in a boiler for 15 min at 60°C. The mixture was filtered with Whatman filter No. 1.

**Determination of the total phenolic contents:** The standard calibration (3.33-20 mg $mL^{-1}$) curve was plotted using tannic acid. The absorbances were read at 280 nm using agilent 8453 UV-Visible spectrophotodensitometer. Aqueous methanol 60% was used as a control. Different concentrations of the extracts were prepared and read at this wavelength. The contents of phenolic compounds were expressed as tannic acid equivalents (%).

**Determination of the flavonoid contents:** The determination of the flavonoid contents was conducted with alumine trichloride ($AlCl_3$) according to the method of Dowd adapted by Arvouet-Grand *et al.* (1994). Quercetin was used as a standard. Five mL of each concentration of the extracts were added with 5 mL of $AlCl_3$ 2%. After 10 min of contact, the Optic Density was read at 415 nm.

**Determination of the DPPH radical scavenging activity:** The capability of extracts to scavenge the 1.1-diphenyl-2-picrylhydrazyl (DPPH) free radicals was determined by Gyamfi *et al.* (1999) method. Fifity µL of each extract were mixed with 450 µL of Tris-HCL buffer and 1.0 mL of 0.1 mM 1.1-diphenyl-2-picrylhydrazyl in methanol. The controls contained all the reaction reagents except the extract. After a 30 min incubation in darkness and at ambient temperature, the resulting absorbance was recorded at 492 nm. The percentage inhibition values were calculated using the following equation (Lompo *et al.*, 2007):

$$\text{DPPH scavenged (\%)} = \frac{(A_{ctl} - A_{test})}{A_{ctrl}} \times 100$$

where:

$A_{ctrl}$ = The absorbance of the control reaction
$A_{test}$ = The absorbance in the presence of extract

$IC_{50}$ expressed the antioxidant activity defined as the concentration in mL that inhibits the formation of DPPH radicals by 50%.

**Antimicrobial assay:** The extracts were tested against clinical isolate strains *Staphylococcus aureus* obtained from pus (1 strain), blood (1 strain), vaginal excretions (2 strains), sperms (2 strains), urines (1 strain) and stools (1strain). The biological activities were determined using the agar well diffusion method. Fifity µL of each extract were deposited in a 6 mm diameter well in the inoculated petri dishes. The petri dishes were incubated for 24 h and the growth inhibition zone diameters were measured in millimeters. A collection strain *Staphylococcus aureus* ATCC 25293 was also tested as a control with the hydroalcoholic extract of the stem bark. Gentamicin, a broad spectrum antibiotic, was used as positive control at a concentration of 5µg $mL^{-1}$.

**Statistical analysis:** Data are expressed as X±SE (n = 7). Significant differences are determined by the student t-test from PHARM/PCS (Tallarida and Murray, 1987).

## RESULTS

As shown in Table 1, the best polyphenolic content was obtained with the stem barks harvested on May at Lèna (12.29±0.03%). The results revealed that the total phenolic contents of stem bark harvested at the same period at Yako is 2.59±0.20%. They also showed that on the same area (Yako) the contents of total phenolics vary from one period to another. The values obtained are 2.59-3.43-1.18%, respectively.

The Table 1 confirms that the leaves contain more flavonoids than the barks. The best flavonoid content was obtained with the samples of leaves harvested at Yako in October. However this Table 1 shows that there is no significant difference ($p<0.01$) between the contents in flavonoids throughout the year and from one area to another.

In Table 2, the antimicrobial activity of the ethanolic extract has been evaluated by measuring the diameters of inhibitory zones in mm. The ethanolic extracts of the stem bark and the leaf harvested at Lèna showed marked inhibition against *Staphylococcus aureus* (14-13 and 15 mm of inhibitory zone diameters at a concentration of

Table 1: Total phenolics and flavonoid contents

| Samples | Total phenolic contents Flavonoid contents (%) | Eq. Tannic acid (%) |
|---|---|---|
| **Samples harvested at Yako** | | |
| Stem barks harvested on May | 2.59±0.20 | 0.24±0.01 |
| Stem barks harvested on August | 3.43±0.22 | 0.29±0.02 |
| Stem barks harvested on October | 1.18±0.05 | 0.17±0.02 |
| Leaves on May | 4.01±0.07 | 1.67±0.06 |
| Leaves on August | 2.57±0.25 | 1.49±0.08 |
| Leaves on October | 3.69±0.08 | 1.80±0.06 |
| **Samples harvested at Lèna** | | |
| Stem barks on May | 12.29±0.03 | 0.93±0.02 |
| Leaves on May | 3.88±0.18 | 1.41±0.09 |

Table 2: Antimicrobial activity of Stem Bark (S.B.) and leaf ethanolic extracts against *Staphylococcus aureus* (diameters of inhibitory zones in mm)

| | Extracts (mg mL$^{-1}$) | | | |
|---|---|---|---|---|
| Samples | 20 | 10 | 5 | 2.5 |
| **Samples harvested at Yako** | | | | |
| S.B. (May) | 15.5±3.53 | 13.5±2.12 | 11.5±2.12 | 9.5±2.12 |
| S.B. (August) | 14.5±0.70 | 12.5±0.70 | 12.0±0.00 | 10.0±0.0 |
| S.B. (October) | 11.0±1.00 | 9.5±0.700 | 8.0±0.000 | - |
| Leaves (May) | 13.5±0.50 | 12.0±0.00 | 10.5±0.50 | 8.5±0.50 |
| Leaves (August) | 10.5±0.50 | 8.0±0.500 | - | - |
| Leaves (October) | 11.0±0.00 | 10.0±0.00 | 9.5±0.50 | - |
| **Samples harvested at Lèna** | | | | |
| S.B. (May) | 14.0±0.00 | 12.5±0.50 | 11.0±1.00 | 9.0±0.00 |
| Leaves (May) | 13.0±0.00 | 12.0±0.00 | 11.0±0.50 | 9.0±0.00 |

Table 3: *In vitro* antioxidant activity of leaf and stem bark

| Samples | $IC_{50}$ (mg mL$^{-1}$) |
|---|---|
| **Samples harvested at Yako** | |
| $S.B._{D}$ | 54.449±4.53 |
| $L._{D}$ | 25.53±3.560 |
| $L._{HA}$ | 45.24±3.760 |
| $S.B._{HA}$ | 127.41±6.540 |
| Quercetin | 1.78±0.320 |

20 mg mL$^{-1}$, respectively). The results also showed that the hydroalcoholic extract of stem bark harvested at Yako in May was the most effective of the extracts; it exerts the strongest inhibition of *Staphylococcus aureus* with 15.5 mm of inhibitory zone diameter, even if it is not the richest in total phenolics nor in flavonoids. The results also revealed that the hydroalcoholic extract of leaves harvested at Yako in May was a good inhibitor of *Staphylococcus aureus*.

Gentamicin gave 31±0.2 mm inhibitory diameter zone with *Staphylococcus aureus* at a concentration of 5 μg mL$^{-1}$.

Results with the stem bark ethanolic extract against *Staphylococcus aureus* ATCC 25293 gave a Minimal Inhibitory Concentration (MIC) and a Minimal Bactericidal Concentration (MBC) of 1.25 mg mL$^{-1}$. With the clinical isolate *Staphylococcus aureus*, the MIC is 0.62 mg mL$^{-1}$.

Having displayed a strong antimicrobial activity, the stem bark and also the leaf harvested at Yako have been evaluated for their *in vitro* antioxidant activity by using 1, 1, DPPH. Table 3 showed that the aqueous extract of the leaf exhibits the best antioxidant activity ($IC_{50}$ = 25.53 mg mL$^{-1}$). Quercetin, used as a standard substance, gave an $IC_{50}$ = 1.78 mg mL$^{-1}$. The stem bark hydroethanolic extract, even though presenting the strongest antimicrobial activity, showed a weak antioxidant activity (127.41 mg mL$^{-1}$) as compared to the other extracts.

## DISCUSSION

The results showed that the total phenolic contents of the leaf and that of the bark, at the same area, vary all year round. They also showed that according to the nature of the soil the total polyphenolics contents vary from one area to the other at the same period (Covelo and Gallardo, 2001; Mpofu *et al*., 2006). BUNASOLS (2002a, b) revealed that the microclimates and the soils differ from Lèna (Province of Houet) to Yako (Province of Passore) 400 km farther.

The maximum content in flavonoids was observed in October where UV irradiation is very important in West African Region. Studying the seasonal variation in flavonoid contents of green pepper and other vegetables, Kana *et al*. (2005) demonstrated that Flavonoid contents were increased in October to December and the tendency to decrease in May to June. Chaves *et al*. (1997) demonstrated that UV irradiation was the major inducer of the enhanced flavonoid secretion in the exudate. These results support an ecophysiological role of the flavonoids in the exudate to protect the plant against the damaging effects of UV irradiation.

Indeed, plants synthesize chemical compounds for their own needs, to adapt to their environment. They synthesize flavonoids and stock them in the epidermic cells of the leaf, to protect themselves against the damaging effects of U.V lights (Kozaki and Takeba, 1996). Some researchers have related the antimicrobial and the antioxidant activities of plants to their content in total phenolics (Arvouet-Grand *et al*., 1994; Zheng and Wang, 2001; Dorman *et al*., 2004; Randhir *et al*., 2004; Hinneburg *et al*., 2006). However, Motalleb *et al*. (2005) and Hinneburg *et al*. (2006) demonstrated that there is no relationship between the total phenolics content and the antioxidant activity even if it was demonstrated that phenolic substances are responsible for the antioxidant activity of plant materials (Rice-Evans *et al*., 1996). Our results showed that the samples harvested at Lèna in May contain more phenolics than any other one but they do not exert the strongest antimicrobial activity. Also the best antioxidant activity was not obtained with the sample containing the greatest amount of phenolics. The antimicrobial and the antioxidant activities should be related to the type and the ratio of phenolic compounds contained in the extracts.

Antimicrobial assays revealed that all these extracts inhibit the growth of pathogenic *Staphylococcus aureus*. These results give support to the ethnomedical uses of *Parkia biglobosa* in the treatment of many infectious diseases.

Also pharmacological properties such as antitumor, anti-inflammatory and hypotensive antioxidant activity have been linked to the presence of phenolic compounds in the plant (Huang *et al*., 1992).

## CONCLUSION

The results revealed that a good antibacterial activity and a good antioxidant activity can be obtained with the leaves, the removal of which is less traumatizing for the tree. That could be a contribution to the protection of our environment.

## ACKNOWLEDGEMENT

Prof Jeanne MILLOGO, botaniste, University of Ouagadougou. Dr Bognounou Ouetian, ethnobotanist. Mr Yaro Boubakar, technician in chemistry.

## REFERENCES

Andlauer, W. and P. Furst, 1998. Antioxidative power of phytochemicals with special reference to cereals. Cereal Foods World, 43: 356-359.

Arbonnier, M., 2002. Trees, bushes of dried zones of West Africa, Ed., pp: 574. ISBN: CIRAD,Pont-sur-Yonne, 392.

Arvouet-Grand, A., B. Vennat, A. Pourrat and Legret, 1994. Standardisation of an extract of Propolis and identification of the major compounds. J. Pharm. Belg., 49: 462-468.

BUNASOLS, 2002a. Technical Report of Morphological Studies of HOUET and TUY Regions, 126: 40-53.

BUNASOLS, 2002b. Technical Report of Morphological Studies of PASSORE Region, 127: 17-29.

Chaves, N., J.C. Escudero and C. Gutierrez-Merino, 1997. Role of ecological variables in the seasonal variation of flavonoid content of *Cistus ladanifer* Exudate. J. Chem. Ecol., 23 (3): 579-603. DOI: 10. 1023/B:JOEC.0000006398.79306.09. http://www.springerlink.com/content/104273/?k=Role+of+ecological+variables+in+the++++++++++seasonal+variation+of+flavonoid+content+of+Cistus+ladanifer+Exudate&sortorder=asc&v=condensed&Copyright=1997.

Covelo, F. and A. Gallardo, 2001. Temporal variation in total leaf phenolics concentration of *Quercus robur* in forested and harvested stands in northwestern Spain. Can. J. Bot., 79 (11): 1262-1269. DOI: 10.1139/cjb-79-11-1262. http://rparticle.web-p.cisti.nrc.ca/rparticle/AbstractTemplateServlet?journal=cjb&volume=79&year=&issue=&msno=b01-109&calyLang=eng.

Dorman, H.J.D., O. Hachmayer, M. Kosar and R. Hiltunen, 2004. Antioxidant properties of aqueous extracts from selected *Lamiaceae* sp. grown in Turkey. J. Agric. Food Chem., 52: 762-770. DOI: 10.1021/jf034908v. PubMed: 14969528. http://pubs.acs.org/doi/abs/10.1021/jf034908v.

Gyamfi, M.A., M. Yonamine and Y. Aniya, 1999. Free radical scavenging action of medical herbs from Ghana. *Thonningia sanguinea* on experimentally induced liver injuries. Gen. Pharmacol. Vasc. S., 32: 661-667. DOI: 10.1016/S 0306/3623 (98)00238-9. http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T71-3WT2NB3-5&_user=10&_coverDate=06%2F30%2F1999&_rdoc=5&_fmt=high&_orig=browse&_srch=doc-info(%23toc%235045%231999%23999679993%23105752%23FLA%23display%23Volume)&_cdi=5045&_sort=d&_docanchor=&_ct=13&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=a48dbba175a861e11f2a3069c5d1ee64.

Harborne, J.B., 1989. General procedures and measurement of total phenolics. In: Methods in Plant Biochem. Academic Press, Londres, 1: 1-28.

Hinneburg, I., H.J.D. Dorman and R. Hiltunen, 2006. Antioxidant activities of extracts from selected culinary herbs and spices. Food Chem., 97: 122-129. DOI: 10.1016/j.foodchem.2005.03.028. http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T6R-4G7DY3V-2&_user=10&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=8e194cbb5eea6717930f6b2445cb14df.

Huang, M.T., C.T. Ho and C.Y. Lee, 1992. Phenolic compounds in food and their effects on health II: Antioxidants and cancer prevention, American Chemical Society Symposium Series 507. Washington: American Chemical Society, pp: 2-7.

Jayaprakasha, G.K., T. Selvi and K.K. Sakariah, 2003. Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extract. Food Res. Int., 36: 117-122. DOI: 10.1016/50963-9969(02)00116-3. http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T6V-4603PN7-2&_user=10&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=371af7af235c6bb0cf8751a78f0b1611.

Kana, I., T. Yoko and A. Sayumi, 2005. Seasonal variation in flavonoid contents of green pepper and other vegetables. J. Jap. Soc. F. Sci. Technol., 52 (4): 190-195. Accession No.: 05A0387831.

Kerharo, J. and J.G. Adam, 1973. Senegalese traditional pharmacopoeia. Medicinal and toxic plants. Edit. Vigot Frères, pp: 579-580.

Kozaki, A. and G.O. Takeba, 1996. Photorespiration protects $C_3$ plants from photooxidation, Nature, 384: 557-560. DOI: 101038/384557. http://www.nature.com/nature/journal/v384/n6609/abs/384557a0.html.

Lee, K.G., A.E. Mitchell and T. Shibamoto, 2000. Determination of antioxidant properties of aroma extracts from various beans. J. Agric. Food Chem., 48: 4817-4820. DOI: 10.1021/jf000237e. PMID: 11052738. http://pubs.acs.org/action/doSearch?searchText=Determination+of+antioxidant++properties+of+aroma+extracts+from+various+beans&searchSub=Search&publication=40026026.

Lompo, M., J. Bubois and I.P. Guissou, 2007. *In vitro* preliminary study of free radical scavenging activity of extracts from *Khaya senegalensis* A. Juss. (Meliaceae). J. Biol. Sci., 7 (4): 677-680. http://www.ansijournals.com/3/detail.php?id=1&jid=jbs&theme=3&issueno=175&articleno=55585#.

Middleton, E., C. Kandaswany and T.C. Theoharides, 2000. The effects of plant Flavonoids on mammalian cells: Implications for inflammation, heart diseases and cancer. Pharmacol. Rev., 52: 673-751. PMID: 11121513. http://www.africanethnomedicines.net/ojs2/index.php/ajtcam/search/results.

Millogo-Kone, H., I.P. Guissou, N. Idika, A. Adepoju-Bello, H.A.B. Coker and P.U. Agomo, 2001. Identification of phenolic acids and free phenols of the stem barks of *Parkia biglobosa* (Jacq.) Mimosaceae-Comparative study of the activity of the total and hydroalcoholic extracts with that of the gentamicin against pathogenic bacteria. West Afr. J. Pharm., 15: 74-76.

Millogo-Kone, H., I.P. Guissou, O. Nacoulma and A.S. Traore, 2006. Study of the antibac-terial activity of the stem bark and leaf extracts of *Parkia biglobosa* (Jacq.) Benth. on *Staphylococcus aureus*, AJTCAM, 3: 74-78. http://www.africanethnomedicines.net/ojs2/index.php/ajtcam/search/results.

Motalleb, G., P. Hanachi, S.H. Kua, O. Fauziah, R. Asmah, 2005. Evaluation of phenolic content and total antioxidant activity in *Berberis vulgaris* fruit extract, J. Biol. Sci., 5 (5): 648-653. http://www.ansijournals.com/3/detail.php?id=1&jid=jbs&theme=3&issueno=163&articleno=55192.

Mpofu, A., H. D. Sapirstein and T. Beta, 2006. Genotype and environmental variation in phenolic content, phenolic acid composition and antioxidant activity of hard spring wheat. J. Agric. Food Chem., 54 (4): 1265-1270. DOI: 10.1021/jf052683d. PMID: 16478246. http://pubs.acs.org/doi/abs/10.1021/jf52683d?prevSearch=Mpofu%2C+A.%2C+Sapirstein%2C+H.+D.%3B+Beta%2C+T&searchHistoryKey.

Nacoulma, O., 1996. Burkina Faso medicinal plants and traditional medical practics. Case of Central Region, 2: 179-180.

Nacro, M. and J. Millogo, 1993. Burkina Faso Tinctorial Plants and Plants Rich in Tannins, Ed. Scientifika, Amiens, 106: 152.

Pietta, P., P. Simonetti and P. Mauri, 1998. Antioxidant activity of selected medicinal plants. J. Agric. Food Chem, 46: 4487-4490. DOI: 10.1021/jf980310p. http://pubs.acs.org/doi/abs/10.1021/jf980310p?prevSearch=Pietta%2C+P.%2C+Simonetti%2C+P.%2C+Mauri+P&searchHistoryKey=.

Rajeshwar, Y., S.G.P. Kuma, M. Gupta and Mazumder, 2005. Study of *in vitro* antioxidant of methanol extract of *Mucuna pruriens* (Fabaceae) seeds. Eur. Bull. Drug. Res., 13: 1. http://scholar.google.com/scholar?q=Study+of+in+vitro++antioxidant+of+methanol+extract+of+Mucuna+pruriens+(Fabaceae)+seeds&hl=en&client=firefox-a&rls=org.mozilla:en-US:official&hs=HMg&um=1&ie=UTF-8&oi=scholart.

Randhir, R., Y.T. Lin and K. Shetty, 2004. Phenolics, their antioxidant and antimicrobial activity in dark germinated fenugreek sprouts in response to peptide and phytochemical elicitors. Asia Pac. J. Clin. Nutr., 13 (3): 295-307. PMID: 15331344. http://apjcn.nhri.org.tw/server/APJCN/Volume13/vol13.3/finished/index.htm#top.

Rice-Evans, C., N. Miller and G. Paganga, 1996. Structure-antioxydant activity relationships of flavonoids and phenolic acids. Free Radic. Biol. Med., 20: 933-956.

Sachetti, G., S. Maietti, M. Muzzoli, M. Scaglianti, S. Manfredini, M. Radice, R. Bruni, 2005. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chem., 91: 621-632. DOI: 10.1016/j.foodchem.2004.06.031. http://hinari-gw.who.int/whalecomwww.sciencedirect.com/whalecom0/science?_ob=ArticleURL&_udi=B6T6R-4DDXRSS-2&_user=2778664&_coverDate=08%2F01%2F2005&_rdoc=6&_fmt=high&_orig=browse&_srch=doc-info(%23toc%235037%232005%23999089995%23564512%23FLA%23display%23Volume)&_cdi=5037&_sort=d&_docanchor=&_ct=25&_acct=C000049744&_version=1&_urlVersion=0&_userid=2778664&md5=be34ca86eeb86107c67481ab2f927f2c.

Tallarida, R.J. and R.B. Murray, 1987. Pharmacological Calculation System based on Manual of Pharmacological Calculations with Computer Program 2nd Edn. Springer-Verlag-New York Inc.

Zheng, W. and S. Wang, 2001. Antioxydant activity and phenolic composition in selected herbs. J. Agric. Food Chem., 49: 5165-5170. DOI: 10.1021/jf010697n. http://pubs.acs.org/doi/abs/10.1021/jf010697n.