EVALUATION OF ANTIOXIDANT ACTIVITY OF TEA BLENDS FORMULATED USING PINEAPPLE PEELS, GINGER AND LEMON GRASS.

Supported by tetfund

Samoh .F. Teghtegh [a*]; Ikyenge .B. Aloo [b]; Issa .O. Mohammed [c]; Ologunde .I. Femi [d]; Ioryina .V. Nguemo [e]; Istifanus .L. Mbaku'esu [f]; Akoso Vershima [g]

[a] Chemistry Department, University of Ilorin, Ilorin, Kwara State, Nigeria
[b] Chemistry Department, Benue State University, Makurdi, Benue State, Nigeria
[c] Teeside University, Middlesbrough, United Kingdom
[d] Charles River Laboratories, Tranent, Scotland, United Kingdom
[e] Freshlife Foods and Beverages Limited, Gboko, Benue State, Nigeria
[f] Department of Microbiology, Federal University of Lafia, Lafia, Nasarawa State, Nigeria
[g] Natural Science Unit, National Institute of Languages, Aba, Abia State, Nigeria
***Corresponding author email**: samoh.ft@unilorin.edu.ng

Received: September 14, 2023 **Accepted:** November 28, 2023

Abstract: The insufficient intake of fruits and vegetables in diets is identified as a modifiable health risk, emphasizing the need to promote their increased consumption for their health benefits. This study explores the antioxidant potential of tea blends incorporating pineapple peels, ginger, and lemongrass at the ratios of (2:0.1:0.1), (2.0:0.25:0.25), and (2.0:0.5:0.5) respectively. Material and methods used for this study detail the sample collection and preparation process, focusing on the antioxidant assays to include DPPH, total flavonoid, and vitamin C estimation. Results show variations in antioxidant activity, flavonoid, sugar, and vitamin C contents among pineapple peels, ginger, lemon grass and their blends with the tea blend ratio of 2.0:0.1:0.1 having the highest antioxidant activity. The subsequent investigation into composite tea blends demonstrates how different ingredient ratios influence the antioxidant, flavonoid, sugar, and vitamin C content in the final product. The findings underscore the potential of these blends as functional beverages with varying health benefits suggestive for those monitoring their sugar intake and stress levels. Hence, the study is able to address the critical role antioxidants from these materials can play in combating oxidative stress, a contributor to various diseases.

Keyword: Antioxidants, Food-Security, Phytochemicals, Tea-Blends, Waste-Utilization

Introduction

Tea, originating from China as a traditional beverage, holds the distinction of being the world's oldest and most widely embraced non-alcoholic caffeinated drink. The infusion is crafted through the brewing of processed leaves from the tea plant, scientifically known as *Camellia sinensis* (Kumar and Shruthi, 2014). Globally, tea stands as the second most consumed beverage, trailing only water in popularity. The prevalent varieties, namely black, green, and oolong, all trace their origins back to the *Camellia sinensis* plant, a member of the Theaceae family. Annually, an estimated 3.0 million metric tons of dried tea are produced, with green tea accounting for 20%, oolong tea for 2%, and the remainder being black tea. Notably, Asian countries predominantly favor green and oolong tea, while black tea finds widespread consumption in India and Western countries (Anonymous, 2002)

Nigeria possesses a rich variety of fruits that are consumed not only for their nutritional benefits but also for their medicinal values. The prevalent antioxidants found in these fruits include polyphenols, vitamins A, B, C and E, with carotenoids present in some fruits to a lesser extent. The majority of polyphenols are flavonoids, primarily in ester and glycoside forms (Fleuriet and Macheix, 2003). Notably, vitamin C and phenolics are categorized as hydrophilic antioxidants, while carotenoids are considered lipophilic antioxidants (Klein and Kurilich, 2000). Various analytical methods have been employed to explore the antioxidant properties of tropical fruits (Jimenez – Escrig et al., 2001).

The imbalance between oxidative mechanisms and antioxidants in an organism's metabolic system, attributed to the excessive production of free radicals, has been linked to diseases associated with oxidative stress (Oyedemi and Afolayan, 2011). Antioxidants play a crucial role in preventing or delaying oxidative damage caused by reactive oxygen species. These include reactive free radicals like superoxide, hydroxyl, peroxyl, alkoxyl, and non-radicals such as hydrogen peroxide and hypochlorous. Antioxidants function by inhibiting initiation and breaking chain propagation or by suppressing the formation of free radicals through binding to metal ions, reducing hydrogen peroxide, and quenching superoxide and singlet oxygen (Shi et al., 2001).

The insufficient intake of fruits and vegetables in the diet has been identified as a modifiable health risk factor contributing to the global burden of chronic diseases (WHO, 2005). To address this challenge, promoting increased consumption of fruits and plants is crucial, as numerous studies have demonstrated their health benefits (Stangeland et al., 2009).

The byproducts generated from fruit processing pose socio-economic and environmental challenges due to their low industrial and commercial value. Pineapple, for instance, results in approximately 75% byproducts during industrial processing, with the peel being the largest proportion, often overlooked for commercial use (Roda et al., 2016). Interestingly, research indicates that the peel of some fruits, such as pineapple, contains a higher quantity of antioxidants compared to the edible portion, showcasing its nutritional value (Guo et al., 2003; Ajila et al., 2007). The bioactive compounds in pineapple peel, including sugars, organic acids, essential minerals, cellulose, hemicellulose, pectin, lignin, enzymes, flavonoids, and vitamins A and C, make it a promising ingredient for various applications, including preservatives and substitutes for synthetic antioxidants (De Oliveira et al., 2009; Lu et al., 2014; Yang et al., 2016; Caleja et al., 2017).

Ginger, belonging to the Zingiberaceae family, is a plant with significant medicinal, ethno-medicinal, and nutritional values. The rhizome of ginger contains polyphenol compounds with high antioxidant activity, making it a potential preventive agent against various diseases (Kumar et al., 2014; Stoner, 2013).

Lemon grass (Cymbopogon citrates) is an aromatic herb with phytochemical-rich leaves, including alkaloids, glucosides, phenols, saponins, flavonoids, tannins, terpenoids, and resins. With its origins in North and West tropical Africa, Lemongrass has been historically used in folk remedies for various ailments, and research has identified its antioxidant, antiseptic, bactericidal, and sedative properties (Amer et al., 2018; Manvitha & Bidya, 2014; Uraku et al., 2015; Naik et al., 2010). Pineapple peels, ginger, and lemon grass have been known for their individual antioxidant properties, but their combined application and effects in a tea blend remain largely unexplored. As the demand for natural and functional beverages rises, investigating the antioxidant potential of these complementary ingredients can contribute valuable insights into the development of health-promoting tea blends.

Material and Methods

Sample collection

Fresh pineapples were acquired from the railway market in Makurdi, lemon grass leaves were gathered from the surroundings of the staff quarters of Benue State University, and fresh ginger rhizomes were purchased from Wurukum Market in Makurdi, Benue State. Subsequently, these raw materials were brought to the laboratory for additional processing.

Samples preparation

Pineapple fruits were soaked in water with added vinegar. After soaking for 5 minutes, they were scrubbed with a vegetable brush, rinsed under running water, and peeled to remove dark eyes. The pineapple peels were then dried in an oven at 60 °C for 24 hours. The dried peels were grounded using a Khafaga Glass Blender, 2 g powder was measured and packed into an empty tea bag, heat-sealed, and labeled.

Fresh ginger fruits were washed, scrubbed with a vegetable brush, sun-dried for a week, pounded, ground, and finally placed in an oven at 60 °C for a day. 2 g powder was measured and packed into an empty tea bag, heat-sealed, and labeled.

Fresh lemon grass leaves were washed with running tap water. Once free of water, they were dried in an oven at 60 °C. The dried leaves were pulverized using a mortar and pestle, and then grounded using a blender. 2 g powder was measured and packed into an empty tea bag, heat-sealed, and labeled.

Fig1: dried and powdered pineapple peels

Fig 2: dried and powdered Ginger

Fig 3: dried and powdered Lemon grasses

Fig 4: Packaged Samples in Tea Bags for Analysis

Formulation of Tea Blends

The prepared powdered materials were blended in ratios and packed into a tea bag as shown in figures 1 - 4, sealed and ready for infusion. The three blends were mixed in the following ratios of Pineapple peels, Lemon grass and Ginger respectively in grams: (2.0:0.1:0.1), (2.0:0.25:0.25), (2.0:0.5:0.5).

Prepared Sample Infusions

The Six tea samples were each infused at a temperature (100 °C) and for a time duration of 5 min. The infused liquid was freeze dried and kept for further analysis.

Antioxidants Assays

DPPH ANTIOXIDANT ASSAY

Reagents: 1, 1-diphenyl 2-picryl-hydrazyl (DPPH), Methanol, Ascorbic acid

Procedure

An amount of 0.1mM working solution of DPPH in Methanol was prepared. 1mg/ml of the sample was prepared in appropriate solvent. The concentration of the samples was varied to 100 – 500 µg/mL by serial dilution. The reaction mixture contained 1000 µL of the sample and 500 µL of DPPH reagent. The mixture was allowed to incubate at room temperature for 30 min in dark. The absorbance of the reaction mixture was taken at 518 nm against the reagent blank, methanol. The control involved methanol and DPPH reagent. Ascorbic acid was used as standard to compare the % inhibition.

Calculation: % Inhibition= Absorbance Ctrl - Absorbance Sample/Absorbance Ctrl × 100

DETERMINATION OF TOTAL SUGAR

Method: Phenol Sulfuric acid Method

Principle: In hot acidic medium, glucose is dehydrated to hydroxymethyl furfural. This forms a green coloured product with the phenol and has absorption maximum at 490 nm.

Materials

1. 2.5N Hcl
2. Phenol 5%(w/v) ie 5g in 100 ml of distilled water
3. Sulfuric acid 96% reagent grade
4. Solid sodium carbonate
5. Standard glucose: Stock: 100mg in 100 ml of distilled water

Working standard: 10 ml of stock diluted to 100 ml with distilled water (10%v/v) of the stock solution.

Procedure

0.10g of the sample was weighed into 15 ml test tube and heated to boil by adding 5ml of 2.5N HCl in water bath for 1hr. After cooling, the solution was neutralized with sodium carbonate until effervescence ceases. The volume was made up to 10 ml and centrifuge for 5min at 3000rpm. 1ml of the supernatant was taken into a test tube and 1ml of phenol solution added to it. 1ml of distilled water was set as blank while glucose was used as standard (10-100µg/ml) and treated similarly as standard. 2ml of 96% sulfuric acid was added to all tubes and shaken after which the mixture was incubated for 30min. The absorbance of the resulting solution was taken at 490 nm against the reagent blank. The amount of total sugar was calculated from the standard curve obtained

ESTIMATION OF TOTAL FLAVONOID

Total flavonoid content was measured by aluminium chloride colorimetric assay described by Talari et al. (2012). 1ml of extracts or standard solution of Quarcetin (20 – 100 µg/ml) was added to 10 ml volumetric flask containing 4 ml of distilled water. To the above mixture, 0.3 ml of 5 % NaNO2 was added. After 5 minutes, 0.3 ml of 10 % AlCl3 was added. At 6th min, 2 ml of 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. Total flavonoids content of the samples were expressed as mg of Quarcetin equivalent per 100 g of fresh (Singh et al., 2004; Şakar et al., 2008; Ayodeji et al.,2016; Ademoye et al.,2018 and Zaïri et al.,2020)

DETERMINATION OF VITAMIN C (ASCORBIC ACID)

An amount of 0.1 g of the sample was taken into a 15 ml test tube. This was extracted with 1 ml of 4 % trichloroacetic acid (TCA). This was stirred with vortex mixer and allowed to stay for 15 minutes. The component was centrifuged at 2000 rpm for 5 minutes. 500 microliter of Vitamin C color reagent (Dichlorophenolindophenol) was added to 250 microliter of the supernatant. The orange color that developed was measured at 700 nm. Blank was prepared the same way as sample but TCA used in place of sample supernatant. The standard was prepared by using ascorbic acid at various concentration. The vitamin C content in each sample was calculated from the standard curve prepared using the standard (Okwu & Omodamiro 2005).

Results and discussion

Table 1: Total Antioxidant, Total Flavonoid, Total Sugar and Vitamin C Content in Pineapple Peels, Ginger and Lemon Grass

Sample (g)	Total Antioxidant 200mg/ml	Total Flavonoid mg/100g	Total Sugar µg/ml	Vitamin C content mg/100g
Pineapple	69.2653	23.8334	14.6917	25.7623
Ginger	92.4731	169.0000	2.3311	27.2787
Lemon Grass	92.2043	14.6666	0.6837	27.0738

Table 2: Total Antioxidant, Total Flavonoid, Total Sugar and Vitamin C Content of the composite Tea Blends from Pineapple Peels, Ginger and Lemon Grass at different ratios

Composite Tea Blends			Total Antioxidant 200mg/ml	Total Flavonoid mg/100g	Total Sugar µg/ml	Vitamin C content mg/100g
Pineapple (g)	Ginger (g)	Lemon Grass (g)				
2	0.1	0.1	91.8459	62.1666	21.2768	8.5902
2	0.25	0.25	90.3226	41.8333	17.1582	11.7459
2	0.5	0.5	88.0825	36.6667	16.1697	49.0000

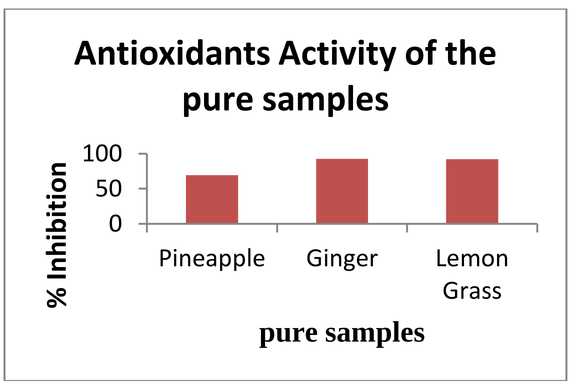

Fig 5: **Total Antioxidant in Pineapple Peels, Ginger and Lemon Grass**

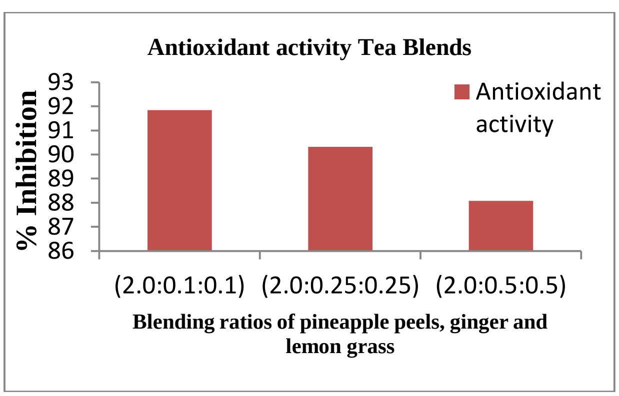

Fig 6: **Antioxidant Activity of Tea Blends from Pineapple Peels, Ginger and Lemon Grass**

The Total anti-oxidants, flavonoids, reducing properties and vitamin C content of Pineapple peels, Ginger, lemon grass and their ratio blends were analyzed. Table 1 presents data on the total antioxidant, total flavonoid, total sugar, and vitamin C content in pure powders of dried pineapple peels, ginger, and lemon grass.

The antioxidant content of the samples reveals interesting variations. While pineapple peels exhibit a total antioxidant value of 69.2653, ginger and lemon grass had the values of 92.4731 and 92.2043 respectively shown in Table 1 and Figure 5. Ginger and lemon grass show higher total antioxidant content compared to pineapple. This indicates their potential in combating oxidative stress and promoting health. Furthermore, Ginger stands out in terms of flavonoid content, boasting a remarkable 169.0000, in contrast to pineapple's 23.8334 and lemon grass's 14.6666. Flavonoids are known for their antioxidant properties and potential health benefits. Ginger exhibits the highest total flavonoid content, significantly surpassing pineapple and lemongrass. This suggests that ginger may offer more diverse flavonoid compounds with potential health-promoting effects (Joshipura et al., 2001).

In terms of sugar content, pineapple leads with 14.6917, followed by ginger with 2.3311 and lemongrass with 0.6837. The total sugar content varied widely among the samples. Pineapple had the highest sugar content, followed by ginger and lemon grass. This information is essential for those monitoring sugar intakes, and it also impacts the taste profile of each sample. The vitamin C content is notably consistent among the samples, with ginger having the highest value of 27.2787, followed closely by lemon grass (27.0738) and pineapple (25.7623). This suggests that all three samples can contribute significantly to the daily vitamin C intake. Vitamin C is an essential nutrient known for its antioxidant properties and role in immune function. Pineapple, with its significant vitamin C content, can be a valuable addition to a diet focused on immune support. Researchers and nutritionists can use this data for further investigations into the health-promoting properties of these plant materials. Also food and beverage industries can use this information to develop products with enhanced nutritional profiles.

Table 2 presents data on composite tea blends formulated from Pineapple Peels, Ginger, and Lemon Grass, showcasing the various ratios of these ingredients and their corresponding Total Antioxidant, Total Flavonoid, Total Sugar, and Vitamin C contents. The blend ratios are presented in table 2, with ratio values of (2:0.1:0.1), (2:0.25:0.25), and (2:0.5:0.5), indicating the proportion of each ingredient in the blend. The Total Antioxidant content varies across the different blends. In the first ratio (2:0.1:0.1), the Total Antioxidant content is 91.8459. As the ratio of Ginger and Lemon Grass increases in subsequent ratios, the Total Antioxidant content decreases, reaching 88.0825 in the last row with ratio (2:0.5:0.5) as shown in figure 6. This suggests a potential correlation between the ratios of ingredients and the antioxidant capacity of the tea blends. Similar to the Total Antioxidant activity, the Total Flavonoid content shows variation with different ingredient ratios. The highest Total Flavonoid content is observed in the first row (62.1666), while the content decreases in the subsequent rows. This variation might be indicative of the influence of ingredient ratios on the flavonoid composition of the tea blends. In the table 2 above, there is a consistent decrease in Total Sugar content as the ratio of Ginger and Lemon Grass increases. The lowest Total Sugar content is observed in the last row with ratio (2:0.5:0.5), suggesting that the choice of ingredients and their increasing proportions has a reducing impact on the sweetness of the tea blends. The Vitamin C content also exhibits variability across the different ratios. The highest Vitamin C content is found in the third row with ratio (2:0.5:0.5) at 49.0000, while the first two rows show lower Vitamin C content. This indicates that increasing the ratios of Ginger, and Lemon Grass can increase the Vitamin C levels in the composite tea blends.

Hence, the formulated tea blends shows increased antioxidant activity, vitamin C, and decreasing flavonoid and sugar contents, suggestive of a tea blend best for those monitoring their sugar intake and stress level. While tea blend ratio (2.0:0.5:0.5) is best for those monitoring their sugar intake, tea blend ratio (2.0:0.1:0.1) is best for those not monitoring their sugar intake. While both blends had comparatively close antioxidant activity.

Conclusion

The antioxidant properties of complementary tea blends from Pineapple Peels, Ginger, and Lemon Grass were evaluated. The blends revealed a nuanced relationship between ingredient ratios and the content of total antioxidant activity, this activity was observed to be dependent on the total flavonoid, total sugar, and vitamin C present in the individual amount of the sample materials added to pineapple peels. The blend ratio of (2.0:0.1:0.1) is adjudge to be the best tea blend rich in antioxidants and require the smallest amount of additional sample materials (Ginger and Lemon Grass) in its formulation. The findings underscore the potential of these blends as functional beverages with varying health benefits, depending on their composition.

Acknowledgement

The authors acknowledges the Centre for Food Technology and Research (CEFTER), Benue State University, Makurdi, Benue State, Nigeria for their financial support towards the purchase of raw materials used in preparation of the tea blends at the occasion of CEFTER Food Week held at Benue State University.

References

Ajila, C.M., Bhat, S.G., & Prasada Rao, U.J.S. (2007). Valuable components of raw and ripe peels from two Indian mango varieties. Food Chemistry, 102, 1006–1011.

Amer H.H. Alzobaay* and Baidaa H. Kadhim.(*2018*). Phytochemical Screening, Chemical Composition and Antibacterial Activity of Lemongrass (*Cymbopogon citratus*) Leaves Extracts. *Indian Journal of Natural Sciences*; *9 (51) 0976 – 0997*

Amin, I.; Zamaliah, M.M, and Chin, W.F. (2004). Total antioxidant activity and Phenolic contented of selected vegetables. *Food Chem*; 87: 581-586.

Bajaj, Y.P.S. (1989). Biotechnology in agriculture and forestry: medicinal and aromatic plants. Springer-Verlag. Berlin. (p. 6).

De Oliveira, A.C., Valentim, I.B., Silva, C.A., Bechara, E.J.H., de Barros, M.P., Mano, C.M., & Goulart, M.O.F. (2009). Total phenolic content and free radical scavenging activities of methanolic extract powders of tropical fruit residues. Food Chemistry, 115, 469–475.

Guo, C., Yang, J., Wei, J., Li, Y., Xu, J., & Jiang, Y. (2003). Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. Nutrition Research, 23, 1719–1726.

Jimenez- Escrig, A., Rincon M., Pulido, R. and saura-Calixto, F.(2001). Guava Fruit (Psidium Guajara L.) as a new source of antioxidant dietary fiber. *Journal of Agricultural and food chemistry*; 9: 5489-5493.

Joshipura KJ, Hu FB, Manson JE, Stampfer MJ, Remm EB, Speizer FE, Colditz G, Ascherio A, Rosner B, Spiegelman D, and Willett WC (2001). The effect of fruit and vegetable intake on risk for coronary heart disease. Ann Intern Med 134:1106–14.

Klein B.P and Kurilich, A.C. (2000). Proceessing effects on dietary antioxidants from plant foods. *Hort Science*; 35(4): 580-584

Kumar, N.V., Murthy, P.S., Manjunatha, J.R., & Bettadaiah, B.K. (2014). Synthesis and quorum sensing inhibitory activity of key phenolic compounds of ginger and their derivatives. Food Chemistry, 159, 451–457.

Lu, X.-H., Sun, D.-Q., Wu, Q.-S., Liu, S.-H., & Sun, G.-M. (2014). Physico-chemical properties, antioxidant activity and mineral contents of pineapple genotypes grown in China. Molecules, 19, 8518–8532.

Manvitha, K., & Bidya, B. (2014). Review on pharmacological activity of Cymbopogon citratus.

International Journal of Herbal Medicine, 1(6), 5-7.

Naik, M. I., Fomda, B. A., Jaykumar, E., & Bhat, J. A. (2010). Antibacterial activity of lemongrass (Cymbopogon citratus) oil against some selected pathogenic bacteria. Asian Pacific Journal of Tropical Medicine, 535-538.

Okwu, D.E and O.D. Omodamiro. (2005). Effects of hexane extract and phytochemical content of Xylopia guinea pig. Bio-res.,3 (in press)

Oyedeun S.O Arowosegbe,S. and Afolayan,A.J.(2013).Preliminary studies on vitro antioxidant potential and vitamin composition on selected dietary fruits consumed in alice region of south Africa. *International journal of Pharmacology.* 9 (1): 33-41.

Plazzotta, S., Manzocco, L., & Nicoli, M.C. (2017). Fruit and vegetable waste management and the challenge of fresh-cut salad. Trends in Food Science & Technology, 63, 51–59.

Roda, A., De Faveri, D.M., Giacosa, S., Dordoni, R., & Lambri, M. (2016). Effect of pre-treatments on the saccharification of pineapple waste as a potential source for vinegar production. Journal of Cleaner Production, 112 Pt 5, 4477–4484.

Rui Hai Liu (2003). Health benefits of fruits and vegetables are from additives and synergistic combination of phytochemicals. The American Journal of Clinical Nutrition 78(3) :5175-5205

Shi, H., Noguchi, N., & Niki, E. (2001). Natural antioxidants. In J. Pokorny, N. Yanishlieva, & M. Gordon (Eds.), Antioxidants in food Practical application, (1st ed). Cambridge: CRC Press Woodhead Publishing Ltd

Stangeland T., Remberg, S.T and Lye, K.A (2009). Total antioxidant activity in 35 Ugandan fruits and vegetable. *Food Chem*., 113: 85-91

Stoner, G.D. (2013). Ginger: Is it ready for prime time? Cancer Prevention Research, 6, 257–262.

Uraku, A. J., Onuoh, S. C., Edwin, N., Ezeani, N., Ogbanshi, M. E., Ezeali, C., Nwali, B. U., & Ominyi, M. C. (2015). Nutritional and Anti-Nutritional Quantification Assessment of Cymbopogon citratus Leaf. Pharmacology & Pharmacy, 6, 401-410.

WHO (2005). Preventing Chronic Diseases; A vital Investment: WHO Global Report. World Health Organization, Geneva, Switzerland, ISBN: 9241563001.