IJMPR 2022, 6(4), 21-27

International Journal of Modern Pharmaceutical Research
www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Research Article

SJIF Impact Factor: 5.273

PHYSICOCHEMICAL SCREENING AND ANTIOXIDANT PROPERTIES OF *LORENTHUS BENGWENSIS* (COCOA MISTLETOES) AND *CARICA PAPAYA* (PAWPAW LEAF)

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Received on: 15/03/2022
Revised on: 05/04/2022
Accepted on: 25/04/2022

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ABSTRACT

This study was aimed at assessing the phytochemical content, minerals composition, proximate and free radical scavenger ability of *Lorenthus bengwensis (*Cocoa Mistletoes*)* and *Carica papaya (*Pawpaw leaf*)*. Antioxidant properties (Inhibition of Fe^{2+}-induced lipid peroxidation, total phenol and total flavonoid contents, ferric reducing antioxidant property, nitric oxide radical scavenging ability using *in vitro* model were evaluated. These results revealed the nutritional profile of the plants as a good source of plant protein, carbohydrates, basic phytochemicals, free radical scavengers, macro and micro minerals. The antioxidant activity and inhibitory effect of both extracts against lipid peroxidation could be attributed to the phenolic phytochemicals present in the extracts which are potentials that could be exploited by food and pharmaceutical industries.

KEYWORDS: Phytochemicals; Cocoa Mistletoes; *Carica papaya*; proximate analysis; scavenging free radical ability.

INTRODUCTION

Plants continue to be a major source of medicines, as they have been throughout human history. The use of medicinal plants all over the world predates the introduction of antibiotics and other modern drugs **(**Dick G. Papaya, 2003**)**. A medicinal plant is any plant with one or more of its organs containing substances that can be used for therapeutic purposes or which are the precursors for the synthesis of useful drugs.They are of great importance to the health of individuals and communities; the medicinal values of certain plants lie in some chemical substances that produce definite physiological action on the human body. The most important of these bioactive constituents of plants are flavonoids, tannins, alkaloids and foods plants sometimes added to foods (Kokowaro, 1976). These active principles or ingredients occur naturally in such plants.

The medicinal plants are rich in secondary metabolites, which are potential sources of drugs and essential oils of therapeutic importance. Medicinal plants are widely used in various ailments, because of their safety besides being economical, effective and their easy availability (Siddiqui, 1993). According to a survey (1993) of the World Health Organization (WHO), the practitioners of traditional system of medicine treat about 80% of patients in India, 85% in Burma and 90% in Bangladesh. Globally, the early part of the 20th century brought an evolution of the pharmaceutical industry. With the progress of chemical techniques, crude drugs came to be replaced by pure chemical drugs and the developed countries witnessed a decline in popularity of medicinal plant therapy. However, during the recent part the interest has swung again and there is a resurgence of interest in the study and use of medicinal plants. Mixtures of medicinal plants are prescribed by the traditional healers for diseases ranging from common colds to malaria, arthritis, ulcers, etc (Obiajunwa *et al*.,2002). Several studies have been carried out on edible wild plants (Özcan *et al*.,1998 and Al-Kharusi *et al*.,2009) but, limited studies on mineral contents of condiments were made (Akgül,1993). Proximate analysis, phytochemical screening and free radical scavengers' ability in plants gives valuable information and helps to access the quality of the sample. It provide information on moisture content, ash content, volatile matter, content, ash, fixed carbon, percentage inhibition of DPPH, superoxide anion free radicals and Fe^{2+} chelation etc. Ash is the inorganic residue remaining after water and organic matter has been removed by heating, which provides a measure of the total amount of minerals within the drug Minor elements have very important functions and it is believed a key component of proteins such as haem protein and hemoglobin, which play a role in biochemical functions and essential enzyme system even in low doses. Başgel & Erdemoğlu (2005) reported the daily mineral intakes by consuming herbal tea for a 70 kg person and the reported amounts of minerals per day are 500 mg Ca, 300 mg Mg, 15 mg Fe, 5 mg .Studies originally showed that optimal intakes of

elements such as sodium, potassium, magnesium, calcium, manganese, copper, zinc and iodine could reduce individual risk factors, including those related to cardiovascular disease for both human beings and animals (Anke et al.,1987; Mertz,1982 and Sanchez-Castillo *et al*.,1998).

Amongst such medicinal plants is Pawpaw *(Carica papaya)*. *Carica papaya Linnaeus*, (pawpaw), belongs to the family of Caricaceae. Papaya is not a tree but an herbaceous succulent plants that possess self-supporting stems. (Dick Gross, 2003). Papaya is a large perennial herb with a rapid growth rate. The plants are usually short-lived, but can produce fruit for more than 20years. The papaya has a rather complicated means of reproduction. The plants are male, hermaphrodite, or female (Bruce and Peter, 2008).The male trees are uncommon, but sometimes occur when homeowners collect their own seeds. Hermaphrodite trees (flowers with male and female parts) are the commercial standard, producing a pear shaped fruit. These plants are self-pollinated (Jari,2009). *Carica papaya* plants produce natural compounds (annonaceous acetogenins) in leaf bark and twig tissues that possess both highly anti-tumour and pesticidal properties. It was suggested that a potentially lucrative industry based simply on production of plant biomass could develop for production of anti-cancer drugs, pending Food and Drug Agency approval, and natural (botanical) pesticides (Mc Langhlin, 1992). The high level of natural self-defence compounds in the tree makes it highly resistant to insect and disease infestation (Peter, 1991). *Carica papaya L*. leaf tea or extract has a reputation as a tumour-destroying agent. (Walter Last, 2008) The papaya fruit, as well as all other parts of the plant, contain a milky juice in which an active principle known as papain is present.

Mistletoe is a general term for woody shoot parasites in several plant families, especially in *Loranthaceae* and *Viscaceae* families. The common European mistletoe grows on various trees, usually apples and junipers. It is an evergreen plant with small, greenish flowers and berries. Similar mistletoe, American mistletoe, found in the United States, grows on deciduous trees, particularly red marple elm, from eastern Texas to Florida and northward to Missouri and New Jersey (Redmond and Redmond, 2008). The leafless flowering dwarf mistletoes depend entirely on the host tree for nourishment. These scrubs are lethal parasites of conifers, such as pine, spruce, fir and hemlock. The plant leaves and berries contain toxic chemicals that can be poisonous and the plant should be kept out of reach of young children who may be tempted to eat the berries (Redmond and Redmond, 2008).

In view of the above, the present study was planned to determine the physicochemical and antioxidant properties of Mistletoes and dry pawpaw leaf.

MATERIALS AND METHODS

Collection of Samples

The mistletoes and pawpaw plants were obtained from Ifaki-Ekiti local farm.

Experimental Protocol

Determination of Calcium, Potassium, Chloride and Sodium

Plants Sample levels of Ca, K, Cl and Na were determined using Biolyte *Spin 6 Plus Series* Electrolyte Analyzer. Biolyte *Spin 6 Plus Series* Electrolyte Analyzer, is a fast, accurate, convenient and practical clinical instrument, based on advanced ion-selective Electrode (ISE) technology and sensor technology, with advantage of easy operation and accurate measurement.

Determination of sample Copper, Zinc, Manganese, Chromium, Molybdenum, Iron and Selenium

Plants sample Cu, Zn, Mn, Cr, Mo, Fe and Se were determined by Atomic Absorption Spectrophotometry (AAS) using a direct method described by Kaneko, (1999).

Principle

The method is based on the principle that atoms of an element, when aspirated into the Atomic Absorption Spectrophotometer (AAS), vapourized and absorbed light of the same wavelength as that emitted by element when in the excited state. The amount of light absorbed in the flame is proportional to the concentration of the element in the sample.

Determination of sample Sulphur

Sulphur concentration in the sample was determined in the Technicon auto analyser by the method described by Tel and Rao, (1982). One hundred microliter (100ul) of samples were measured and mixed with 25ml of the extraction solution. The mixture was shaken for 5 minutes and was centrifuged for 10 minutes at 4,500rpm. After centrifugation, the supernatant was filtered into a set of clean plastic vials and the working standards of 0,2,4,6 and 8 ppm were prepared. The auto analyzer was calibrated and the standard solutions and sample were read in the auto analyser.

Estimation of Phosphorus

Phosphorus was estimated using spectrophotometric method described by Munoz *et al*., (1983). Inorganic phosphorus in a sample reacts with molybdate in acid medium forming a phosphomolybdate complex that can be measured by spectrophotometry.

Estimation of Magnesium

Magnesium was estimated using spectrophotometric method described by Chromya *et al*., (1973).Magnesium in the sample react with xylidyl blue in alkaline medium forming a coloured complex that can be measure by spectrophotometry method, ethylene glycol tetraacetic acid (EGTA) is included in the reagents to remove calcium interference.

Methods for antioxidant properties of the sample analyses

Determination of total phenol

The total phenol content of the extract determine by the method of (Singleton et. al., 1999). 0.2mll of the extract was mix with 2.5ml of 10% Folin ciocalteau's reagent and 2ml of 7.5% Sodium carbonate. The reaction mixture will be subsequently incubated at 45oC for 40mins, and the absorbance was measure at 700nm in the spectrophotometer, garlic acid would be used as standard phenol.

Determination of total flavonoid

The total flavonoid content of the extract was determined using a colorimeter assay developed by (Bao. J.Y.,2005). 0.2ml of the extract was added to 0.3ml of 5% $NaNO_3$ at zero time. After 5min, 0.6ml of 10% AlCl3 was added and after 6min, 2ml of 1M NaOH was added to the mixture followed by the addition of 2.1ml of distilled water. Absorbance was read at 510nm against the reagent blank and flavonoid content was expressed as mg rutin equivalent.

Determination of ferric reducing property

The reducing property of the extracts was determined by assessing the ability of the extract to reduce $FeCl_3$ solution as described by Oyaizu (1986). A 2.5mL aliquot was mixed with 2.5mL of 200 mmol l^{-1}sodium phosphate buffer(pH 6.6); thereafter, 2.5mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min and then 2.5mL of 10 % trichloroacetic acid was added. This mixture was centrifuged at 2000rpm for 10 min. 5mL of the supernatant was mixed with an equal volume of distilled water and 1mL of 0.1% ferric chloride was added. The absorbance was measured at 700 nm and ferric reducing antioxidant property was subsequently calculated using ascorbic acid as standard.

Determination Fe^{2+} Chelation

The ability of the extract to chelate Fe^{2+} was determined using a modified method of Minotti & Aust (1987) by Puntel et al (2005). Briefly, 150mM $FeSO_4$ will be added to a reaction mixture containing 168ml of 0.1M Tris-HCl pH 7.4, 218ml saline and extract and the volume is made up 1ml with distilled water. The reaction mixture will be incubated for 5min, before the additional of 13ml of 1, 10-phenantroline the absorbance will be read at 510nm.

Determination of free radical scavenging ability

DPPH free radical scavenging ability

The free radical scavenging ability of the extracts against DPPH (1, 1-diphenyl–2 picrylhydrazyl) free radical was evaluated as described by Lin, et al., (2010). 1.0mL of the extract was mixed with 1mL of 0.4 mmol l^{-1} methanolic solution containing DPPH radicals. The mixture was left in the dark for 30mins and the absorbance was measured at 516nm. The DPPH free radical scavenging ability was subsequently calculated with respect to the reference (which contains all the reagents without the test sample).

Determination of NO radical scavenging ability

Sodium Nitropruside in aqueous solution at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be estimated by use of Greiss reagent. Scavengers of NO compete with oxygen, leading to reduce production of NO. Briefly 5mM sodium nitroprusside in phosphate-saline was mixed with the extract, before incubation at 25oC for 150min. Thereafter the reaction mixture was added to Greiss reagent. Before measuring the absorbance at 546nm, relative to the absorbance of standard solution of potassium nitrate treated in the same way with Greiss reagent. (Jagetia and Baliga 2004).

ABTS scavenging ability

2, 2'-azino-bis (3-ethylbenthiazoline-6-sulphonic acid) (ABTS) scavenging ability The ABTS scavenging ability of the extract was determined according to the method describe by Re et al., (1999). The ABTS was generated by reacting an (7mM).

ABTS aqueous solution with $K_2S_2O_8$ (2.45 mM/l, final conc.) in the dark for 16hours and adjusting the absorbance at 734nm to 0.700 with ethanol 0.2 of the appropriate dilution of the extract was then added to 2.0ml of ABTS solution and the absorbance was read at 732nm after 15mins. The TROLOX equivalent antioxidant capacity was subsequently calculated.

Phytochemical screening

Chemical tests were carried out on each extract to screen for phytochemical constituents as described by Sofowora (2006), Trease and Evans (1989) and Harborne (1984).

Proximate analysis

Proximate analyses were carried out according to the procedure of Association of Official Analytical Chemist (AOAC, 1990). The percentages moisture content, ash content, crude fat, crude fibre and protein were determined using this procedure.

RESULT AND DISCUSSION

Nutritional analyses were carried out of the selected plants to know the nutritional composition of these frequently consumed species in the traditional medicines. The results of dry pawpaw leaf and mistletoes for Mineral composition in Table 4.0.0 revealed that the sample is highly rich in sodium, calcium, potassium, magnesium and slightly rich in manganese, zinc, iron, copper, cobalt, chromium and lead but cadmium contents is very low in mistletoes while was not detected in dry pawpaw leaf.

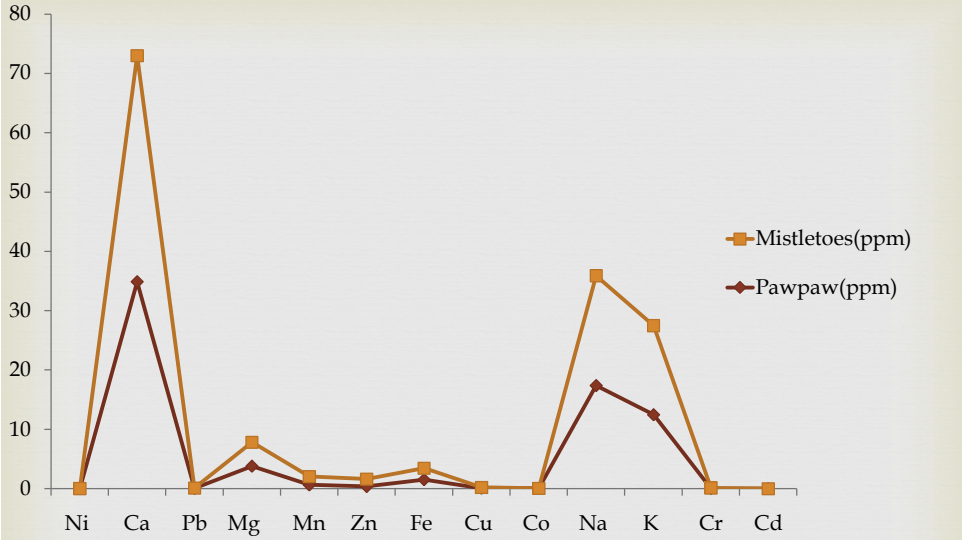

Figure 1.Mineral composition of composition of dry pawpaw leaf and mistletoes.
n= 3 in each group, values are mean±SEM

Mineral composition in Table 4.0.0 revealed that the sample is highly rich in sodium, calcium, potassium, magnesium and slightly rich in manganese ,zinc, iron ,copper, cobalt, chromium and lead but cadmium contents is very low in mistletoes while was not detected in dry pawpaw leaf. Calcium was high in Pawpaw leaf and mistletoes. Calcium constitutes a large proportion of the bone, human blood and extracellular fluid; it is necessary for the normal functioning of cardiac muscles, blood coagulation and milk clotting, and the regulation of cell permeability. It also plays an important part in nerve-impulse transmission and in the mechanism of the neuromuscular system (FAO/WHO, 1974). Magnesium were found to be moderate in Pawpaw leaf and mistletoes. In humans, Mg is required in the plasma and extracellular fluid, where it helps maintain osmotic equilibrium. It is required in many enzyme-catalyzed reactions, especially those in which nucleotides participate where the reactive species is the magnesium salt, e.g., Mg ATP2–. It can also prevent some heart disorders and lower blood pressure. Lack of Mg is associated with abnormal irritability of muscle and convulsions and excess Mg with depression of the central nervous system (Prasad and Symposia, 1981). Iron is an essential element for human beings and animals and is an essential component of hemoglobin. It facilitates the oxidation of carbohydrates, protein and fat to control body weight, which is a very important factor in diabetes. It is essential for hemoglobin formation, but excess is harmful (Critchley, 1986). The dietary limit of Fe in the food is 10-60 mg/day. Low Fe content causes gastrointestinal infection, nose bleeding and myocardial infarction. The role of iron in the body is clearly associated with hemoglobin and the transfer of oxygen from lungs to the tissue cells. Iron deficiency is the most prevalent nutritional deficiency in humans and is commonly caused by insufficient dietary intake, excessive menstrual flow or multiple births. The element iron has many functions in the body. This element is used by the body to make tendons and ligaments. Certain chemicals in our brain are controlled by the presence or absence of iron. It is also important for maintaining a healthy immune system. Phosphorous maintain blood sugar level, normal heart contraction is dependent on phosphorous (Linder and Manria, 2008) also important for normal cell growth and repair, needed for bone growth, kidney function and cell growth. It plays an important role in maintaining the body's acid-alkaline balance (Johns and Duquette, 1999).

The results of dry pawpaw leaf and mistletoes for proximate composition in Table 4.1.0 revealed that the sample is rich in protein, fat, ash content and fiber content while carbohydrate is highly rich in mistletoes compare to pawpaw leaf which have a very low content of carbohydrate.

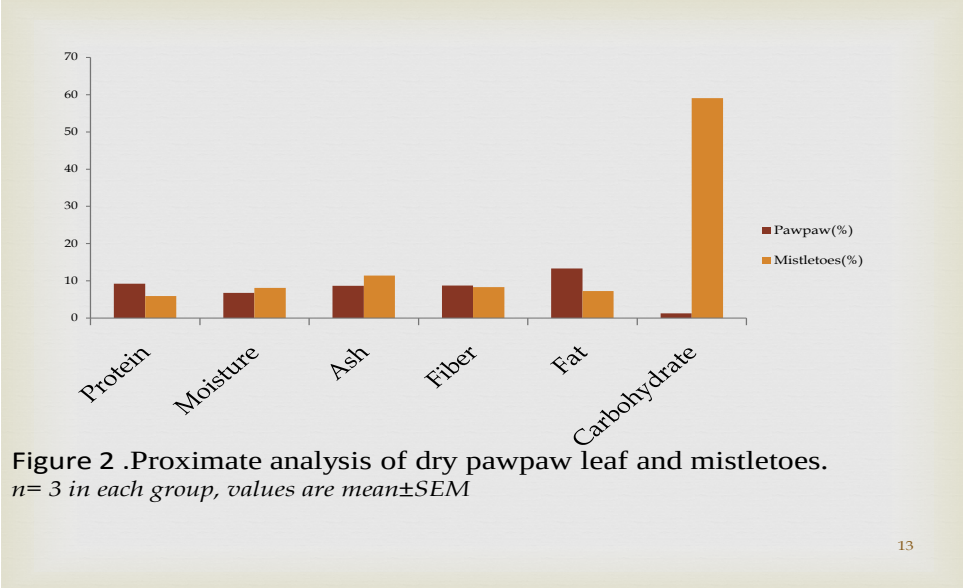

Figure 2 .Proximate analysis of dry pawpaw leaf and mistletoes.
n= 3 in each group, values are mean±SEM

The results of dry pawpaw leaf and mistletoes for proximate composition in Table 4.1.0 revealed that the samples are rich in protein, fat, ash content and fiber content while carbohydrate is highly rich in mistletoes compare to pawpaw leaf which have a very low content of carbohydrate. As a nutritive value of food, fibers in the diet are necessary for digestion and for effective elimination of wastes, and can lower the serum cholesterol, the risk of coronary heart disease, hypertension, constipation, diabetes, and colon and breast cancer. Intake of such medicinal plants in traditional recipes showed evidence that dietary fiber is associated with enhanced insulin sensitivity and therefore may have a role in the prevention and control of the risk of coronary heart diseases (Vadivel and Janardhanan, 2005). Thus, these medicinal plants can be considered as a valuable source of dietary fiber in human nutrition. Moisture content depends on the environmental conditions such as humidity, temperature, harvest time, and climate as well as storage conditions. Thus, it is important for food scientists to be able to reliably measure moisture contents. Abubakar et al.(2007) suggested a strong correlation between moisture contents and fiber, which could be of interest to human health as the fibrous are easily digested and disintegrated. The results of the fat analysis indicated that the spice contains a moderate amount of fat.Pawpaw leaf and mistletoes have a good amount of fat and sufficient amount of fiber and moisture with suitable mineral element showing high nutritive value. This in turn indicates that the herb seem to be good for younger people, diabetic and the risk of coronary heart disease people.

Table 1.1: Phytochemical screening of dry pawpaw leaf and mistletoes. Both contain alkaloids, steroids, tannin, flavonoid and glycosides but absence of anthraquinone and Phlobatanin, Saponin and alkaloid were also absent in dry pawpaw leaf while present in mistletoes.

Table 1.1: Phytochemical screening of dry pawpaw leaf and mistletoes.

Parameters	dry pawpaw leaf	mistletoes
Saponin	-	+
Tannin	+	+
Steroid	+	+
Flavonoid	+	+
Anthraquinone	-	-
Alkaloid	-	+
Phlobatanin	-	-
Keller killiani	+	+
Lieberman test	+	+
Salkwoski test	+	+
Legal test	+	+

+= Present, - = Absent
n= 3 in each group, values are mean±SEM

The percentage inhibition of DPPH, superoxide anion free radicals and Fe^{2+} chelation of dry pawpaw leaf and mistletoes were high as the amount of the total phenolic compound and flavonoid were slightly low in these antioxidant properties studied as obtained in Table 1.2.

Table 1.2: Free radical scavenging ability of dry pawpaw leaf and mistletoes.

Parameters	dry pawpaw leaf (mg/g)	mistletoes (mg/g)
FLAVONOID	0.59±0.02	0.48±0.04
TOTAL PHENOL	6.87±0.01	1.11±0.01
FRAP	48.78±0.23	34.48±0.04
DPPH %	65.83±0.07	59.48±0.04
NO %	68.78±1.23	55.09±0.52
Fe^{2+} CHELATION%	67.63±0.27	64.15±0.27

n= 3 in each group, values are mean±SEM

The presence of secondary metabolites (tannins, alkaloids, flavonoids, cardiac glycosides, saponins) in the plant is of great importance as they contribute to its medicinal value as well as exhibiting physiological activity. It is interesting to note that alkaloids and flavonoids are commonly associated with various pharmacological activities of natural products .Flavonoids have been reported to have excellent free radical scavenging properties, disease prevention and therapeutic properties (Bharani *et al*., 1995). Tona *et al*., (1999) reported that tannins have been reported to have several pharmacological activities such as spasmolyticactivity in smooth muscle cells. These are also known as antimicrobial agents as they prevent the growth of microorganisms by precipitating microbial protein (Sharma and Sharma, 2010). The cardiac glycosides are naturally cardioactive drugs used in the treatment of congestive heart failure and cardiac arrhythmia. Recently it has been discovered that cardiac glycosides exert pleiotropic effects on many aspects of cell metabolism (Riganti *et al*., 2001). Saponins is present only in mistletoes plant. Saponins inhibit Na+ efflux by blockage of the entrance of the Na^+ outof the cell. This leads to higher Na^+ concentration in cells, activating Na^+ - Ca_2^+antiporter producing elevated cytosolic Ca_2^+ which strengthens the contractionof heart muscle and thereby reducing congestive heart failure. It should benoted that steroidal compounds are of particular interest in pharmacy due totheir relationship with sex hormones (Shahidi *et al*., 1992). The antioxidant activity of thepawpaw leaf and mistletoes may be attributed to the presence of phenolic compounds as it is wellknown that the plant phenolics in general are highly effective in free radicalscavenging and are antioxidants and have been known to show medicinalactivity as well as for exhibiting physiological functions (Sofowora, 1993). The present studyindicates that mistletoes and pawpaw leaf *possesses* potent radicals cavenging activity and protective effect against hydroperoxide generation.

CONCLUSION

In conclusion, the present investigation on mistletoes and pawpaw leaf clearly demonstrates the presence of large number of secondary metabolites and their antioxidant nature. However, further studies involving isolation and purification of thea ctive principle(s) are warranted for its better utilization as a therapeutic agent.

REFERENCES

1. Abubakar MS, Musa AM, Ahmed A, Hussaini IM. The Perception and Practice of Traditional Medicine in the Treatment of Cancers and Inflammations by the Hausa and Fulani Tribe of Northern Nigeria. Journal of Ethnopharmacology, 2007; 3(3): 625–629.
2. Anke M, Groppel B, Kronemann H, Bratter P, Schramel P (Eds.). Significance of newer essential trace elements (like Si, Ni, As, Li, V,) for the nutrition of man and animals: Trace elem anal chem med Biol., Berlin, 1984; 3: 421.
3. Başgel S, Erdemoğlu SB. Determination of mineral and trace elements in some medicinal herbs and their infusions consumed in Turkey. Sci Tot Env, 2005; 359: 82–89.
4. Bharani A, Ganguly A and Bhargava KD, Salutary effect of *Terminalia arjuna* inpatients with severe refractory heart failure, Int. J. Cardiol., 1995; 49: 191-199.
5. Bruce S. and Peter CA. Handbook of environmental physiology of fruit crops. 1st Ed., 2008; 217.
6. Dick Gross"Papaya": A tantalising taste of the Tropics. Maricopa County Master Gardener Volunteer information, University of Arizona Cooperative Extension. www.papaya Maricopa-hort@ag.arizo.edu.
7. Jari, S. Papayas are yummy easy to grow. University of Hawaii- Manoa College of Tropical Agric. & Human Resources, 2009.
8. Johns T and Duquette M. Deficiency of phosphorus in man. Am. J. of Clin. Nutr., 1991, 53: 448 - 56. 46.
9. Khammash A. "Holly Parasite orChristmas Mistletoe" Jordan Times Weekender. 2005 Copyright, 2005.
10. Kokowaro J O. Asparagus africanus.Medicinal plants of East Africa, 1976; 239.

11. Linder, Manria C. Nutritional Biochemistry and metabolism with clinical applications. Appleton and Lange, Norwalk, 1991; 2: 191 - 212.
12. McLanghlin, J. L., Ratanyake, S. Rupprecht, J. K. and, Potter, W. M. Evaluation of various parts of the pawpaw tree, Asimina triloba (Annonaceae), as commercial source of the pesticidal annonaceous acetogenins. J. Econ. Entomol, 1992; 85: 2353-2356.
13. Mertz W. Food fortification in United States. Nutritional reviews, 1982; 41(11): 2807-2812.
14. Nagulendran KR, Velavan S, Mahesh R and Hazeena Begum V *In vitro* antioxidant activity and total polyphenolic content of *Cyperus rotundus* rhizomes, Eur. J. Chem., 2007; 4: 440-449.
15. Peter, R. N. Pawpaw (Asimina). In: J. N. Moore and J. R. Ballington (eds). Genetic resources of temperate fruit and nut trees. Acta Hort, 1991; 290: 567-600.
16. Polhill R, Wiens D. Mistletoes of Africa. The Royal Botanic Garden, Kew, UK., 1998; 370.
17. Prasad A. S. Zinc deficiency: Has been known of for 40 years but ignored by global health organisations. *British Medical J.,* 2003; 326(7386): 409–10.
18. Prasad A.S. Zinc: an overview. Nutrition, 1995; 11: 93-99.
19. Prasad A.S., Beck F.W., Grabowski S.M., Kaplan J., Mathog R.H. Zinc deficiency: changes in cytokine production and T-cell subpopulations in patients with head and neck cancer and in non-cancer subjects. *Proc Assoc Am Physicians,* 1997; 109: 68-77.
20. Prasad AS Symposia from the XII International Congress on Nutrition. Prog Clin Biol Res, 1981; 77: 172–177.
21. Redmond WA. Redmond, W.A.: Microsoft Corporation; 2007. "Mistletoe" Microsoft ® Student, 2008.
22. Riganti C, Campia I, Kopecka J, Gazzano E, Doublier S, Aldieri E, Bosia A and GhioD, Pleiotropic effects of cardioactive glycosides, Curr. Med. Chem., 2011; 18: 872- 885.
23. Sanchez-Castillo CP, Dewey PJS, Aguirre A, Lara JJ, Vaca R, Leon de la Barra. The mineral content in Mexican fruits and vegetables. J Food Comp Anal, 1998; 11: 340– 356.
24. Shahidi F, Janitha PK and Wanasundara PD, Phenolic antioxidants. Crit. Rev. Food Sci. Nut., 1992; 32: 67-103.
25. Sharma A., Mathur R. and Dixit V.P. Prevention of hypercholesterolemia and Shoback, edited by David G. Gardner, Dolores *Greenspan's basic & clinical endocrinology* (9th ed.). New York: McGraw-Hill Medical. Chapter, 1995; 17.
26. Sharma MC and Sharma S, Phytochemical and Pharmacological Screening of Combined *Mimosa pudica* Linn and *Tridax procumbens* for *in vitro* AntimicrobialActivity, Int. J. Micro. Res., 2010; 1: 171-174.
27. Siddiqui HH Safety of herbal drugs-an overview. Drugs News & Views, 1993; 1(2): 7–10.
28. Sofowara A, Medicinal Plants and Traditional Medicine in Africa, Spectrum Books Ltd., Ibadan, Nigeria, 1993; 289-300.
29. Tona L, Kambu K, Mesia K, Cimanga K, Apers S, de Bruyne T, Pieters L, Totte J and Vlietinck AJ, Biological screening of traditional preparations from some medicinal plants used as anti diarrhoeal in Kinshasa, Congo, Phytomedicine, 1999; 6: 1999, 59-66.
30. Vadivel V, Janardhanan K. Nutritional and ant nutritional characteristics of seven South Indian wild legumes. Plant Food Hum Nutri, 2005; 60: 69-75.
31. Walter Last: Cancer remedies. www.health-science-spirit.com/cancer6-remedies, 2008.
32. WHO survey. In medicinal plants (Eds. Haq. I.). Karachi: Hamdard Foundation Press, 1993.