

Full Length Research Paper

Comparative chemical composition of leaves of some antidiabetic medicinal plants: *Azadirachta indica*, *Vernonia amygdalina* and *Gongronema latifolium*

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As part of an investigation into the antidiabetic mechanism of some indigenous medicinal plants, the proximate, vitamins and mineral elements and phytochemical compositions of *Azadirachta indica*, *Vernonia amygdalina* and *Gongronema latifolium* were quantitatively determined using standard methods and compared. Of the 3 plants *G. latifolium* had highest ($p < 0.05$) crude protein and fat contents but lowest in fibre composition, whereas *A. indica* with highest fibre content had lowest crude protein composition. Fat and ash were lowest in *V. amygdalina*. Also *G. latifolium* showed highest composition ($p < 0.05$) of vitamins A, E and niacin content compared to *A. indica* and *V. amygdalina*. *A. indica* and *V. amygdalina* have higher ($p < 0.05$) vitamin C and riboflavin composition. Flavonoids, saponins and polyphenols were significantly predominant ($p < 0.05$) in *V. amygdalina* relative to *A. indica* and *G. latifolium*, whereas alkaloids and HCN were highest ($p < 0.05$) in *A. indica*. However, *G. latifolium* had the highest composition of tannins among the three plants. There appear to be a complement of biochemicals in the leaves of these plants which may account for reported hypoglycemic and antihyperglycemic action.

Key words: Medicinal plants, phytochemical composition, *Azadirachta indica*, *Vernonia amygdalina*, *Gongronema latifolium*.

INTRODUCTION

Arising from their biodiversity and perhaps the rich complement of phytochemicals and secondary metabolites, plants have from antiquity been used as sources of medicament against various ailments (Farombi, 2003). In rural areas where access to modern health facilities is limited by the level of development, plants/herbs remain the mainstay of the health care system (TMP, 2007). Additionally, current research in medicinal plants is beginning to lend credence to their efficacy and potency and in most instances over and above the existing conventional and chemotherapeutic options particularly as it concerns degenerative disease complexes including diabetes mellitus.

Azadirachta indica A. Juss (neem), *Vernonia amygdalina* Del., (African bitter leaf) and *Gongronema latifolium* (Utasi) are medicinal plants primarily and secondarily

indigenous to Nigeria/Africa and used extensively in the management and treatment of a number of ailments amongst which is diabetes mellitus. As one of the 13 panatropical tribes of the family Asteraceae (Compositae) (Johri and Singh, 1997), *V. amygdalina* is a small tree (1-3 m high) that grows throughout tropical Africa and has been domesticated in some parts of West Africa, e.g. Nigeria, where it is locally known as bitter leaf (Igile et al., 1995). It also occurs as an herb or shrub in tropical America, Madagascara and Asia (Johri and Singh, 1997). Besides its use as a vegetable in the popular bitter leaf soup, all parts of the plant has found usefulness in folk-medicine (Igile et al., 1994; Igile et al., 1995; Babalola et al., 2001; Ojiako and Nwanjo, 2006; Abosi and Raseroka, 2003; Izevbogie et al., 2004).

Scientific and pharmacologic studies have revealed antihyperglycemic action of the roots (Nimenibo-Uadia, 2003) and leaves (Akah and Okafor, 1992; Akah et al., 2004) and hypoglycemic effect of the leaves (Gyang et al., 2004) of this plant. Extracts from the leaves have

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been reported to possess hypolipidemic and antihyperlipidemic properties (Atangwho et al., 2007a) and to protect the kidneys (Atangwho et al., 2007b) and the liver (Atangwho et al., 2007c) of alloxan diabetic rats against complications.

A. indica belongs to the family Meliaceae and has a long history of use in folkmedicine as a treatment against various ailments (NRC, 1992). Reported pharmacological and biological properties are also numerous (Sonia and Srinivasan, 1999). The hypoglycemic actions of its leaves, stem bark and seeds have been articulated in a review by Biswas et al. (2002), and Ebong et al. (2008) indicate recently in their studies, the relative antidiabetic efficacy of its extracts when combined with that of *V. amygdalina*, over and above the individual extracts.

On the other hand, *G. latifolium*, an edible rainforest climbing plant native to South-Eastern Nigeria, is an Asclepiadaceae (Morebise et al., 2002). It is used widely as a staple vegetable and spice in tradomedicine (Morebise and Fafunso, 1998; Morebise et al., 2002) for healthy glycemic control and to support the pancreas (Okafor and Ham, 1999; Ugochukwu et al., 2003). In the United State, it is used as a constituent of herbal tea blend for maintenance of healthy glycemic control.

Scientific studies have been carried out and the antihyperglycemic, antioxidant, antilipidemic and antihypercholesterolemic activities of the leaves of *G. latifolium* in both normal and STZ diabetic rats have been reported (Ugochukwu and Babady, 2003; Ugochukwu et al., 2003).

Despite these reports, detailed scientific reports/studies as per antidiabetic efficacies and modes of the antidiabetic action of these plants are not available. Interest in the plants has concentrated more on their screening for hypoglycemic action to the neglect of investigations into the antidiabetic mechanisms of the plant. However, besides the roles played in human and animal nutrition, knowledge of proximate, phytochemical and micronutrient composition is fundamental to the understanding of modes of action of medicinal plants in general. It is the diverse composition of these components in plants that places them at advantage position over and above chemotherapy in management of complex diseases such as diabetes mellitus (Tiwari and Rao, 2002).

We therefore in this study quantitatively analysed the proximate, phytochemical and micronutrient (mineral elements and vitamins) composition of the leaves of *A. indica*, *V. amygdalina* and *G. latifolium* as part of an investigation into the antidiabetic mechanism of these plants.

MATERIALS AND METHODS

Collection of plant materials

Matured fresh leaves of *A. indica*, *V. amygdalina* and *G. latifolium* were respectively collected from the Endocrine Research Farm, University of Calabar, University of Calabar Staff Village and Uruawatt Market, Calabar, Southern Nigeria in February. These samples

were authenticated by Dr. E. G. Amanke, a plant Ecologist, Department of Botany, University of Calabar, Calabar and voucher specimens deposited in a herbarium in the Department of Botany of the same University.

Preliminary treatment/processing

The leaves were chopped into smaller bits with a knife, and wet weight of each dried on a moisture extraction oven (Carbolite, England) to a constant weight of 65°C. The dried leaves were then separately ground into powder in an electric mill (National, Food Grinder, Model MK 308, Japan). These pulverised samples were thereafter packed in air-tight plastic containers and stored in the refrigerator (2 - 8°C), from where aliquots were withdrawn and used for individual analysis.

Chemical analyses

The ash, crude fat, crude protein (nitrogen x 6.25) and crude fibre were determined using the standard methods of the Association of Official Analytical Chemists (AOAC, 2000). The Perkin Elmer Atomic Absorption spectrophotometer (Model 306, UK) was used for the determination of Mn, Se, Zn, Fe, Cu, Mg and Cr using the methods of AOAC (2000). Vitamins compositions were determined spectrophotometrically, again using the standard methods of AOAC (2000). Quantitative phytochemical compositions of the leaves were determined using the methods variously described by Harbone (1998), Trease and Evans (1996) and Sofowara (2006).

Statistical analysis

The results were analysed by one-way Anova, using SPSS Microsoft Excel package. All data is expressed as Mean \pm SE (Mean of 3 determinations) and difference between groups considered significant at $p < 0.05$.

RESULTS

The composition of the leaves of *A. indica*, *V. amygdalina* and *G. latifolium* viz: proximate composition (crude fibre, ash, fat and protein), vitamins, mineral elements and phytochemicals are shown in Tables 1a-d respectively. Of the three, *G. latifolium* had the highest crude protein ($25.55 \pm 0.35\%$) and fat ($6.13 \pm 0.03\%$) content but lowest in fibre composition ($13.69 \pm 0.25\%$) whereas *A. indica* with highest fibre content ($20.11 \pm 0.45\%$) had lowest protein composition ($13.42 \pm 0.12\%$). *V. amygdalina* demonstrated the lowest content of fat and ash ($3.53 \pm 0.09\%$ and $10.01 \pm 0.06\%$ respectively). Results of vitamin composition showed that *G. latifolium* was significantly highest ($p < 0.05$) in vitamins A, E (393.00 ± 0.38 ; 44.03 ± 0.13) and niacin (0.18 ± 0.00) compared to the other two plants. However, *A. indica* and *V. amygdalina* were significantly highest ($p < 0.05$) in vitamin C and riboflavin compositions respectively. Mineral elements composition of the leaves were similar except striking lowest contents of Mn (0.04 ± 0.00) in *G. latifolium*, Cu (0.06 ± 0.01) in *A. indica* but a highest composition of Cr (0.58 ± 0.00) in *A. indica*. Flavonoids (0.87 ± 0.02), saponins (2.15 ± 0.01) and polyphenols

Table 1. Quantitative proximate (a) vitamins (b) mineral elements (c) and phytochemical compositions of leaves of *A. indica*, *V. amygdalina* and *G. latifolium*

(a) Proximate composition.

Medicinal plant	Crude protein (%)**	Crude fibre (%)**	Fat (%)**	Ash (%)
<i>A. indica</i>	13.42 ± 0.12	20.11 ± 0.45	5.17 ± 0.09	11.93 ± 0.09
<i>V. amygdalina</i>	23.25 ± 0.12	16.05 ± 0.19	3.53 ± 0.09	10.01 ± 0.06*
<i>G. latifolium</i>	25.55 ± 0.35	13.69 ± 0.25	6.13 ± 0.03	11.63 ± 0.38

(b) Vitamins composition.

Medicinal plant	Vit. A, (IU/100g)**	Vit. E (IU/100g)**	Vit. C (mg/100)**	Riboflavin (%)	Thiamine (%)	Niacin (%)**
<i>A. indica</i>	330.20±0.05	33.60±0.39	396.00±2.54	0.95±0.03	0.18±0.00	0.58±0.00
<i>V. amygdalina</i>	348.57±0.39	37.37±0.39	202.40±5.08	1.00±0.00*	0.18±0.00	0.48±0.00
<i>G. latifolium</i>	393.00±0.38	44.03±0.13	299.20±0.51	0.96±0.00	0.18±0.00	0.81±0.00

(c) Mineral elements.

Medicinal plant	Mn (mg/100 g)	Se (mg/100 g)	Zn (mg/100 g)	Fe (mg/100 g)	Cu (mg/100 g)	Mg (%)**	Cr (mg/100 g)
<i>A. indica</i>	0.06±0.03	0.02±0.03	0.06±0.01	0.14±0.01	0.06±0.01*	0.69±0.01	0.58±0.00*
<i>V. amygdalina</i>	0.07±0.03	0.01±0.00	0.04±0.01	0.14±0.01	0.10±0.00	0.43±0.00	0.04±0.01
<i>G. latifolium</i>	0.04±0.00*	Trace	0.05±0.04	0.28±0.07	0.10±0.00	1.06±0.01	0.04±0.01

(d) Phytochemical composition.

Medicinal plant	Flavoids (%)**	Tannins (%)**	Saponins (%)	Polyphenol (%)**	Alkaloids (%)**	HCN (%)**
<i>A. indica</i>	0.39±0.02	0.63±0.01	0.56±0.01	0.35±0.00	2.84±0.03	19.89±0.02
<i>V. amygdalina</i>	0.87±0.02	0.37±0.03	2.15±0.01*	0.42±0.00	2.13±0.04	13.87±0.04
<i>G. latifolium</i>	0.54±0.02	2.04±0.02*	0.66±0.03	0.33±0.00	1.97±0.04	13.20±0.02

Result is expressed as mean of three determinations ± SEM.

*p < 0.05 compared to the other 2 groups.

**Any two of the 3 groups compared are significantly different at p < 0.05.

were significantly ($p < 0.05$) highest in *A. indica*. However, tannins (2.04 ± 0.02) were highest in *G. latifolium*. There appear, therefore, to be a complement of biochemicals in the leaves of these plants.

DISCUSSION

Many traditional plants remedies are known in folk-medicine and used for treatment and management of diabetes mellitus (Aktar and Ali, 1984), and some have been validated by scientific studies to actually exert biological action against diabetes or its complications. About 400 of such traditional plant remedy have been reported (Bailey and Day, 1989). The medicinal properties of these

plants have been attributed to the biochemicals resident in the plant materials. Hence we in this study determined the proximate, micronutrient and phytochemical compositions of 3 known antidiabetic plants namely *A. indica*, *V. amygdalina* and *G. latifolium*.

In addition to their role played in human and animal nutrition, knowledge of proximate, micronutrients and phytochemical composition is fundamental to the understanding of modes and mechanisms of action of medicinal plants in general. Although the role of phytochemical and micronutrients in diabetes therapy has been documented at least in part by some authors (Shane-McWhorter, 2001; Yeh et al., 2003; Ahmed et al., 2005), the proximate evaluation, phytochemical and micronutrient composition of these leaves have not been

compared in previous literature. Relatively high amounts of crude protein and fibre compared to low fat content were demonstrated in the three samples. This trend agrees with the reports of Igile et al. (1995) and Ejoh et al. (2007) for *V. amygdalina* and Udosen (1995) and Okafor et al. (1996) for *V. amygdalina* and *G. latifolium*. Dietary fibre has positively been implicated in the management of diabetes and post-prandial hyperglycemia. It delays gastric emptying or increase viscosity of GIT content thereby suppressing digestion and carbohydrate absorption. This mechanism is selectively advantageous in that the threat or risk of hypoglycaemia, hyperinsulinemia and undue weight gain is absolved (Tiwari and Rao, 2002). The low fat content compares with the results of Ejoh et al. (2007) who concluded that leafy vegetables are poor sources of lipids and that this is worsened when the vegetables are processed.

The roles of micronutrients-antioxidants vitamins and minerals in management of diabetes mellitus have extensively been reviewed by Bathel et al. (1999). The qualitative and quantitative presence of these vitamins (A, E, C, riboflavin and niacin) and minerals elements (Se, Zn, Cu, Mg and Cr), were demonstrated in these leaves, with leaves of *G. latifolium* showing highest concentrations of vitamins A, E and niacin, while *A. indica* and *V. amygdalina* showed highest concentrations of vitamin C and riboflavin. These vitamins and mineral elements have variously been shown to possess antioxidant activities, particularly A, E and C (Yeh et al., 2003; Shane-McWhorter, 2001). This in part may explain the reported antioxidant properties of *G. latifolium* (Ugochukwu et al., 2003) *V. amygdalina* (Igile et al., 1994) and *A. indica* (Madhi et al., 2003) and may also give an insight into the antidiabetic properties of these plants. It is interesting however to note the relative abundance and distribution of the various types of antioxidants in the leaves. It may also support the report of a higher efficacy in antidiabetic action when combined as medicinal plants as against individual usage (Ebong et al., 2008). Majority of antioxidants enzymes or defence systems of the body and processes involved in lipid metabolism in general make use if mineral elements (Gorman, 1992), and an imbalance in these mineral element would usually lead nutritional disorders and complications of nutritionally related diseases like diabetes. For instance, copper deficiency leads to elevated serum cholesterol and triacylglycerol; selenium deficiency increases cholesterol synthesis (Gorman, 1992), noted complications of diabetes. The presence of these mineral elements in the medicinal plants would, therefore, be relevant in exerting their antihyperglycemic action and amelioration of the attendant macrovascular complications. Hence an insight into the mode of action of these medicinal plants as antidiabetics.

Phytochemical constituents demonstrated in the leaves were flavonoids, saponins, polyphenols (being highest in *V. amygdalina*), alkaloids (highest in *A. indica*) and tan-

nins (highest in *G. latifolium*). The flavonoids and polyphenols components of plants are well known antioxidants (Tiwari and Rao, 2002). For instance, Ginkgo biloba is reported to owe its antidiabetic property to flavone glycosides, typical anti oxidants (Shane-McWhorter, 2001). Besides these antioxidant properties, the phenols are reported to inhibit alpha-amylase, sucrase, as well as the action of sodium glucose-transporter 1 (SGLUT-1) of the intestinal brush border, hence their antidiabetic action (Tiwari and Rao, 2002). Isoflavones, tannins, chlorogenic acids and crude saponins are also reported by these same authors to possess potent SGLUT-1 mediated inhibition of glucose transport, hence antidiabetic activity. *Magnifera indica L.*, a plant belonging to the same species with one in this study, is reported to exert its antidiabetic action via carbohydrate modulation on the GIT due to the presence of these phytochemicals (Bnouham et al., 2006).

It is clear from this study that, these plants owe their antidiabetic properties to their selective chemical composition, and that proper knowledge of the proximate, phytochemical and micronutrient composition is fundamental to understanding the mode/mechanism of antidiabetic action of these medicinal plants.

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