

Phytochemical Constituents of some Medicinal Plants

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Abstract: *In this study, five medicinal plants materials were analysed in order to investigate the presence of phytochemicals and to determine amount of tannin, glucosides, hydrogen cyanide, steroid, soluble carbohydrate, flavonoid and alkaloid in the five selected medicinal plants. The five test plant materials were Azadirachta indica leaves, Garcinia cola seeds, Zingiber officinale stem, Gongronema latifolia leaves and Carica papaya leaves. Phytochemical analysis done on Azadirachta indica A Juss, Garcinia cola Henkel, Zingiber officinale Rose, Gongronema latifolia L. and Carica papaya L. revealed the presence of tannin, soluble carbohydrate, hydrogen cyanide, steroids, flavonoids, alkaloids as well as glucosides in all the extracts tested. It also showed the levels of tannin, soluble carbohydrate, hydrogen cyanide, steroids, flavonoids, alkaloids and glucosides in the plants tested ranged from 0.01 mg/100 g – 1.97 mg/100 g. G. latifolia (1.23 mg/100 g) had the highest concentrations of tannin followed by C. papaya, (1.19 mg/100 g) while A. indica (0.41 mg/100 g) had the least concentrations in all the extracts. Soluble carbohydrate and hydrogen cyanide were present in small concentrations in all the extracts evaluated. Steroid was moderately high in G. latifolia (1.97 mg/100 g) and A. indica (1.69 mg/100g) than the other three extracts. G. cola (1.70 mg/100 g) had the highest quantity of flavonoid followed by C. papaya (1.54 mg/100g), while Z. officinale (0.87 mg/100g) had the least quantity. The levels of alkaloids in all the plant extracts tested were moderately high. Again, all the plant extracts tested had small levels of glucosides. Our finding provided evidence that crude, aqueous and organic solvent extracts these tested plants contain medicinally important bioactive compounds and it justifies their use in the traditional medicines for the treatment of different diseases and as pesticides used especially by the peasant farmers who cannot afford the costly synthetic agrochemicals to control plant pathogens that attack their crops.*

Keywords: Phytochemical, tannins, flavonoids, Azadirachta indica, Garcinia cola seeds, Zingiber officinale, Gongronema latifolia and Carica papaya

1. Introduction

The humid especially the rainforest ecological zones are endowed with abundant flora of families of plants and herbs with untapped pesticides potentials (Amadioha, 2003). Stoll (2000) listed an array of plant families and genera possessing antimicrobial properties, amongst which were Azadirachta indica, Zingiber officinale, Garcinia cola, Carica papaya, Gongronema latifolium and host of others. Stoll (1998) reported the bactericidal properties of Azadirachta indica A Juss (neem), a fast growing tree of the family Meliaceae and also a medicinal plant with insecticidal, nematocidal, antifungal and bactericidal properties. It occupies a foremost status among all the plants exploited so far for bio-efficacy against pests and diseases (Kumar and Pamar, 1996). The primary antimicrobial constituents are Azadirachtin A and B. In addition, Neem contains a good number of other chemical substances which include Salannin, Meliantriol, Azadirachtannin A, Cinnamoyl, Isoazadirohide, Nimbin/Nimbidin, which seem to have anti viral effects as well as Vilasinim as isolated from the leaf and Azadirone from the seed. Garcinia kola Henkel (bitter cola) is a perennial tree in the family Guttiferae with whorled leathery leaves. The seeds are chewed as stimulants and for other various medicinal values. Traditionally, the seeds are believed to repel snakes. Zingiber officinale Rose (Ginger) is rhizome of the family Zingiberaceae. The rhizome yields essential oil, oleoresin, consisting 1-3% volatile of which serve as the active ingredient against microorganisms and pests (Benjilali et al., 1984).

Medicinal plant materials have been successfully used for the

treatment of fungal and bacterial infections in humans (Akinyosoye and Oladummoye, 2000), suggesting that some plant materials may also possess antifungal and antibacterial constituents which are useful in controlling plant diseases (Amadioha, 1998). Previous reports (Akpomedaye and Ejechi, 1998; Ejechi and Ilondu, 1999; Ejechi et al., 1999) show that spices, herbs and other plant materials possess antifungal activity. Akinyosoye and Oladunmoye (2000) have reported the antifungal efficacy of stem and leaf-extracts of Mirabilis jalapa in reducing mycelia growth of four different strains of fungi. The legendary medicinal qualities of the neem tree have been known for a long time and the aqueous leaf extract have systemic action (Egunjobi and Onoyemi, 1981; Sowunmi and Akinusi, 1983). Medicinal plants contains some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (RNS Yadav and Munin Agarwala, 2011). Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. It is well-known that plant produces these chemicals to protect them but recent research demonstrates that they can also protect humans against diseases (Stafford and Warren, 1993). There are more than thousand known phytochemicals. Some of the well-known phytochemicals are lycopene in tomatoes, isoflavones in soy and flavanoids in fruits. They are also chemical compounds that occur naturally in plants, are responsible for color and organoleptic properties, such as the deep purple of blueberries and smell of garlic. The term is generally used to refer to those chemicals that may have biological significance but are not established as essential nutrients. Scientists estimate that there may be as many as 10,000

different phytochemicals having the potential to affect diseases such as cancer, stroke or metabolic syndrome. Although certain phytochemicals are available as dietary supplements, some scientists speculate that potential health benefits of phytochemicals may best derive from consumption of whole foods. These phytochemicals are very useful in pharmaceutical industries. Among the great variety of secondary metabolites produced by the higher plants, alkaloids occupy a special place; they have remarkable effects on the physiology of animals and throughout history have been used in numerous ways mostly as medicines (Stafford and Warren, 1993). Alkaloids are extremely

2. Materials and Method

This experiment was conducted at the Department of Crop Science analytical laboratory, University of Nigeria, Nsukka. Nsukka is located in the derived Savannah Zone (06° 52' N, 07° 24' E and altitude of 447.26 meters above sea level).

2.1 Determination of the phytochemicals on the Five Plant samples

The assay carried out was based on the procedure outlined by Trease and Evans (1996) in order to assess the phytochemicals present in the 5 test plant parts; *Azadirachta indica* leaves, *Garcinia cola* seeds, *Zingiber officinale* stem, *Gongronema latifolia* leaves and *Carica papaya* leaves.

2.2 Sources of plant materials

The fresh leaves of *Azadirachta indica* was obtained from botanical garden, Department of Botany, University of Nigeria, Nsukka. *Gongronema latifolia* and *Carica papaya* were obtained from Department of Crop Science farm, of the same University while fresh *Zingiber officinale* stems and *Garcinia cola* seeds were bought from Ogige Main Market, Nsukka, Nigeria

2.3 Preparation of the plant extracts

The fresh leaves of *Azadirachta indica*, *Gongronema latifolia*, *Carica papaya*, and stems of *Zingiber officinale* and seeds of *Garcinia cola* were washed separately under tap water, rinsed with sterile distilled water and allowed to dry in a glass house. The dried leaves, stems and seeds were mashed and ground using electric milling machine to a fine powder.

2.4 Hot water extraction

Dried finely powder of each plant materials (5 g) was put in beaker separately and 200 ml of distilled water was added. The mixtures were heated on a hot plate with continuous stirring at 30°-40°C for 20 minutes, allowed to cool and filtered through cheese cloth. The filtrates obtained were used for the phytochemical analysis. The water extract was kept in refrigerator for further use.

2.5 Ethanol extraction

Each powder (200g) was soaked in 600 ml of analytical ethanol. These mixtures were left to stand for 24 hours after

difficult to be defined for they do not represent a homogenous group of compounds, either from the chemical or physiological point of view (Claus et al., 1971). However, typical alkaloids (base-like) are basic, contain one or more nitrogen atoms, usually within a heterocyclic ring and they usually have a marked physiological action on animals (Stafford and Warren, 1993).

This research therefore was to investigate the presence of phytochemicals and to determine amount of tannin, glucosides, hydrogen cyanide, steroid, soluble carbohydrate, flavonoid and alkaloid in the five selected medicinal plants. which were filtered with cheese cloth and the supernatant obtained were concentrated to dryness in an oven (100 to 105°C). The dry supernatant of each was used as the crude plant extracts

2.6 Qualitative Phytochemicals

The extracts were tested for the presence of bioactive compounds.

2.6.1 Test for carbohydrate

a. Molisch test

The extracts (0.1 g) was boiled with 2ml of distilled water and filtered. To the filtrate, few drops of naphthol solution in ethanol (Molisch reagent) were added. Concentrated sulphuric acid in a Pasteur pipette was then gently poured down the side of the test tube to form a lower layer. A purple interfacial ring indicated the presence of carbohydrate.

2.6.2 Test for Alkaloids

Twenty millilitres (20 ml) of 3% sulphuric acid in 50% ethanol was added to 2g of the extracts and heated on a boiling water bath for 10 minutes, cooled and filtered. The filtrates (2 ml) was tested with a few drops of Mayer's reagent (potassium mercuric iodide solution), Dragendorff's reagent (bismuth potassium iodide solution), Wagner's reagent (iodine in potassium iodide solution) and picric acid solution (1%). The remaining filtrate was placed in 100ml separator funnel and made alkaline with dilute ammonia solution. The aqueous solution was separated and extracted with two 5 ml of diluted sulphuric acid. The extract was tested with a few drops of Mayer's Wagner', Dragendorff's reagent and picric acid solution. Alkaloids gave milky precipitate with few drops of Mayer's reagent; reddish brown precipitate with few drops of Wagner's reagent; yellowish precipitate with few drops of picric acid and brick red precipitate with few drops of Dragendorff reagent.

2.6.3 Test for Glucosides

Dilute sulphuric acid (5 ml) was added to 0.1g of the extracts in a test tube and boiled for 15 minutes in a water bath, then cooled and neutralized with 20% potassium hydroxide solution. 10 ml of a mixture of equal parts of Fehling's solutions A and B were added and boiled for 5 minutes. A more dense brick red precipitate indicated the presence of glucoside.

2.6.4 Test for Tannins

The powdered extracts (1 g) was boiled with 20 ml of water, filtered and used for the ferric chloride test;

2.6.5 Ferric chloride Test

Few drops of ferric chloride were added to 3 ml of the filtrate. A greenish black precipitate indicated the presence of tannins

2.6.6 Test for Flavonoids

Ethyl acetate (10 g) were added to 0.2 g of the extracts and heated on a water bath for 3 minutes. The mixture was cooled, filtered and the filtrate was used for the following tests;

2.6.7 Ammonium Test

The filtrate (4 ml) was shaken with 1ml of dilute ammonia solution. The layers were allowed to separate and the yellow colour in the ammonium layer indicated the presence of flavonoids.

2.6.8 1% Aluminium Chloride Solution Test

Another 4 ml portion of the filtrate was shaken with 1ml of 1% Aluminium chloride solution. The layers were allowed to separate. A yellow colour in the aluminium chloride layer indicated the presence of flavonoids

2.6.9 Test for Steroids

Ethanol (9 ml) was added to 1g of the extracts and refluxed for a few minutes and filtered. The filtrate was concentrated to 2.5 ml in a boiling water bath. Hot distilled water (5 ml) was added to the concentrated solution, the mixture was allowed to stand for 1 hour and the waxy matter was filtered off. The filtrate was extracted with 2.5 ml of chloroform using separated funnel. To 0.5ml of the chloroform extracted in the test tube was carefully added 1ml of concentrated sulphuric acid to form a lower layer. A reddish brown interface showed the presence of steroids.

3. Quantitative Phytochemicals

The amount of tannin, glucosides, hydrogen cyanide, steroid, soluble carbohydrate, flavonoid and alkaloid in the aqueous extracts were determined.

3.1 Glucosides determination

To 1g of each dried ground 5 test plant part; Azadirachta indica leaf, Garcinia kola seed, Zingiber officinale stem, Gongronema latifolia leaf and Carica papaya leaf were added to 2.5 mls of 15% lead acetate and filter. chloroform (2.5 ml) were added to the filtrates, shaken vigorously and the lower layer collected and evaporated to dryness. Glacial acetic acid (3ml) was also added together with 0.1 ml of 5% ferric chloride and 0.25ml of concentrated H₂SO₄. The mixture was shaken and put in the dark for 2 hours. Absorbance was measured at 530 nm.

3.2 Tannin determination

Dried ground (1 g) of each 5 test plant parts: Azadirachta indica leaf, Garcinia cola seed, Zingiber officinale stem, Gongronema latifolia leaf and Carica papaya leaf were weighed out and massarated with 50 ml of methanol. The mixture was filtered and 5 ml of the filtrate was pipetted out. The filtrate was added to 0.3 ml of 0.1N ferric chloride in 0.1 N HCL, together with 0.3 ml of 0.0008 M potassium ferricyanide. Absorbance was measured at 720 nm.

3.3 Hydrogen Cyanide determination

Dried ground (1 g) of each 5 test plants parts; Azadirachta indica leaf, Garcinia cola seed, Zingiber officinale stem, Gongronema latifolia leaf and Carica papaya leaf were weighed out separately and massarated with 50 ml of distilled water and left to stand for 24 hours. The mixture was filtered and 1ml of the filtrate was pipetted out. Alkaline picrate solution (4 ml) was added on each sample, boiled for 5 minutes and allowed to cool. Absorbance was measured at 490 nm.

3.4 Soluble Carbohydrate determination

Dried ground (1 g) of each 5 test plants parts; Azadirachta indica leaf, Garcinia cola seed, Zingiber officinale stem, Gongronema latifolia leaf and Carica papaya leaf were weighed out separately, massarated with 50ml of distilled water and were filtered. The filtrate (1 ml) was pipetted out for individual sample. Saturated picric acid (2ml) was added to the mixture and absorbance was measured at 530 nm.

3.5 Steroid determination

Dried ground (1 g) of each 5 test plants parts: Azadirachta indica leaf, Garcinia cola seed, Zingiber officinale stem, Gongronema latifolia leaf and Carica papaya leaf were weighed out separately, massarated with 20ml of ethanol and filtered. The filtrate (2 ml) was pipetted out. Colour reagent (2ml) was added and the mixture were left to stand for 30 minutes. Absorbances were measured at 550 nm.

3.6 Flavoid determination

Dried ground (1 g) of each 5 test plants parts; Azadirachta indica leaf, Garcinia cola seed, Zingiber officinale stem, Gongronema latifolia leaf and Carica papaya leaf were weighed out separately, massarated with 20ml of ethylene, filtered and 5ml of the filtrate were pipetted out. Dilute ammonia (5 ml) were added, shaken and the upper layer of the mixtures were collected. Absorbances at 490 nm were measured.

3.7 Alkaloid determination

Dried ground (1 g) of each 5 test plants parts; Azadirachta indica leaf, Garcinia cola seed, Zingiber officinale stem, Gongronema latifolia leaf and Carica papaya leaf were weighed out separately, massarated with 20ml of 20% H₂SO₄ in ethanol (1.1) and were filtered. The filtrate (1 ml) was pipetted out and 5ml of 60% H₂SO₄ and 5ml of 0.5% formaldehyde in 60% H₂SO₄ were also added. They were

mixed together, allowed to stand for 3 hours and absorbance at 550 nm was measured.

4. Results

4.1 Phytochemical analysis of Azadirachta indica, A Juss leaves, Garcinia kola Henkel seeds, Zingiber officinale Rose stems, Gongronema latifolia leaves and Carica papaya leaves

Table 1: Presence of phytochemical in the various plant extracts

Phytochemicals (mg/100g)	G. latifolia a	A. indica a	C. papaya a	G. cola a	Z. officinale e
Tannin	+++	+	++	++	+
Soluble Carbohydrate	++	+	+	+	+
Hydrogen	+	+	+	+	+
Steroid	++	++	+	+	++
Flavonoid	++	+	++	++	+

4.3 The levels of phytochemicals in the five plant extracts

The result showed that the levels of tannin, soluble carbohydrate, hydrogen cyanide, steroids, flavonoids, alkaloids and glucosides in the plant extracts tested ranged from 0.01 mg/100 g – 1.97 mg/100 g (Table 2). G. latifolia (1.23 mg/100 g) had the highest concentrations of tannin, followed by C. papaya (1.19 mg/100 g) while A. indica (0.41 mg/100 g) had the least concentrations. Soluble carbohydrate and hydrogen cyanide were present in small

Table 2: Levels of the various phytochemicals (mg/100 g)

Phytochemicals (mg/100g)	G. latifolia	A. indica	C. papaya	G. cola	Z. officinale
Tannin	1.23	0.41	1.19	0.94	0.64
Soluble Carbohydrate	0.62	0.24	0.83	0.24	0.32
Hydrogen Cyanide	0.02	0.01	0.09	0.01	0.03
Steroid	1.97	1.69	0.30	0.43	0.37
Flavonoid	1.04	0.94	1.54	1.70	0.87
Alkaloid	1.57	1.60	1.74	1.54	1.27
Glucoside	0.64	0.65	0.48	0.41	0.51

5. Discussion

The phytochemical analysis conducted on Azadirachta indica and Carica papaya leaves, Zingiber officinale stem and Garcinia kola seeds revealed the presence of some bioactive compounds known to exhibit medicinal and physiological activities (Cowan, 1999). They were tannin, soluble carbohydrates, hydrogen cyanide, steroids, flavonoid, alkaloid and glucoside. Tannins bind to produce rich protein and interfere with protein synthesis. They are known to exert anti-microbial activities by iron deprivation, hydrogen bonding or specific interactions with vital proteins such as enzymes in microbial cells (Scalbert, 1991). They are also observed to have remarkably activity in cancer prevention (Li et al., 2003). Motar et al., (1985) showed tannins to be useful in treatment of inflamed or ulcerated tissues. Thus, the

4.2 The qualitative phytochemical analysis

The result of the qualitative phytochemical analysis done on Azadirachta indica, A Juss, Garcinia kola Henkel, Zingiber officinale Rose, Gongronema latifolia and Carica papaya were shown in Table 1. The results revealed the presence of tannin, soluble carbohydrate, hydrogen cyanide, steroids, flavonoids, alkaloids as well as glucosides in all the extracts tested.

Alkaloid	++	++	++	++	++
Glucoside	+	+	+	+	+

- Not present
- + Present in small concentration
- ++ Present in moderate high concentration
- +++ Present in very high concentration
- ++++ Abundantly present

concentrations in all the extracts evaluated. Steroid was moderately high in G. latifolia (1.97 mg/100 g) and A. indica (1.69 mg/100g) than the other three extracts. G. cola (1.70 mg/100 g) had the highest quantity of flavonoid followed by C. papaya (1.54 mg/100 g), while Z. officinale (0.87 mg/100g) had the smallest quantity. The levels of alkaloid in all the plant extracts tested were moderately high. Again, all the plant extracts tested had small levels of Glucosides.

presence of tannin in the five plant extracts supported the traditional medicinal uses of these extracts in the treatment of ailments caused by microorganisms. The biological functions of flavonoids include protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatotoxins, viruses and tumor (Farquar, 1996; Okwu and Omodamiro, 2005; Okwu, 2004). Flavonoids represent the most common and widely distributed groups of plant phenolics. They are potent water soluble super anti oxidants and free radical scavengers which prevent oxidative cell damage, have strong anti –cancer activity and protects against all stages of carcinogenesis (Manimi et al., 1994). Flavonoids in intestinal tract lower the risk of heart disease. As anti- oxidants, flavonoids from these plant extracts provide anti-inflammatory action (Okwu, 2001 A and B). This may be the reason behind the use of G. cola extracts in the treatment of intestinal problems in herbal medicinal (Okwu, 2004). These observations also support the usefulness of G. cola in folklore remedies for the treatment of various infections (Hodek et al., 2002). Steroids have been reported to have antibacterial properties and they are very important compounds due to their relationship with compounds such as sex hormones (Stafford and Warren, 1993). Alkaloids in these plants ranked the most efficient therapeutically significant plant substance. Pure isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agents for agents for their analgesic, antispasmodic and bacterial effect (Stray, 1998). Alkaloids isolated from Ruta graveolens L. leaves have fungicidal activity against Colletotrichum and Fusarium species (Oliva et al., 2003).

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