Mistletoe Contains Higher Secondary Metabolites Than the Host Plant at the Host-Parasite Interface: Insights From *Tapinanthus Globiferus* Collected In Enugu, Nigeria

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Research article

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Abstract

Background: This study aims at evaluating the phytochemicals composition at the host-parasite interfaces of parasitic plant *Tapinanthus globiferus* (mistletoe) and four host plants. Wood tissues of the hosts and the parasite at the host-parasite interface were collected and analyzed to determine the presence secondary metabolites.

Results: The result showed that flavonoids, saponins, and glycosides were present in the plants and parasite samples. The results revealed higher concentration of flavonoids ($P < 0.05$) in the parasite of *C. acuminata* (1190.33 ± 48.23 mgQE/g) and *P. macrophylla* (1482.55 ± 31.35 mgQE/g) than in the host plant. Saponins was significantly ($P < 0.05$) higher in the parasites as compared to their respective host.

Conclusion: At the host-parasite interface, significantly higher phytochemicals in the wood portion of *T. globiferus* was observed as compared to the host plants wood; however, the variability in phytochemical content of *T. globiferus* is dependent on the host. Therefore, milestoe would be a better source of bioactive compounds with high medicinal values than their host plants if explored further.

Background

Enormous biologically active compounds with a variety of chemical structures and properties are all deposited in the plant kingdom.[1] Phytochemical is a broad word generally used to describes a wide diversity of compounds that are found naturally in plants. They are found in the different parts of the plants; such as roots, stem, barks, leaves, flowers, seeds and pulps.[2] Phytochemicals otherwise known as secondary metabolites present in smaller quantities in higher plants, include the flavonoids, alkaloids, terpenoids, tannins, steroids etc.[3] These bioactive secondary metabolites helps the plant to overcome temporal or continuous threats integral to their environment, while also controlling essential functions of growth and reproduction. In other words, they are essential for the survival and proper functioning of plants.[4]

Medicinal properties of plants are dependent on a number of chemically active substances that produces a definite physiological action on a biological assay. In general terms, the phytochemicals in plants play an important role in their medicinal properties. Nevertheless, medicinal plants are the richest bio-resource of drugs in traditional system of medicine and the phytochemicals are responsible for the different flavours, colours, and smell of plants. These secondary metabolites (phytochemicals) have recently become of great interest owing to their versatility.[5] Phytochemicals in plants are been utilized globally as the traditional herbal medicine. These phytochemicals are present in the different plant parts and are used for healing of certain disorder like diabetes, arthritis, cancer, etc.[6] Secondary metabolites are economically vital in the manufacturing of drugs, fragrances, dyes, pesticides and food additives. Therefore, researchers have laid more emphasis on phytochemical studies.[2]

Tapinanthus globiferus (mistletoes) are the predominant group of plant semi-parasitic ever green shrubs which belong to the family Loranthaceae. They grow on the branches of host trees or shrubs and take water and nutrients from the host's vasculature. Mistletoes include notorious parasites and are capable of destroying the trees and shrubs of economic value. However, they have been reported to attack a large number of varieties of taxonomically unrelated hosts and their attack has been proved to be fatal to various trees and shrubs.[7] This parasitic plant thrives on deciduous trees preferring those with soft bark like old apple trees, guava, cocoa, citrus and other trees.

Though Mistletoes are generally recognized as destructive agents to various valuable species, they do not seem to have received much attention from both researchers and plant scientists. Presence of various phytochemicals such as glycosides, alkaloids, viscotoxins, phenylpropannoids, tannins, lignins, lectins and sugars has been reported in the mistletoe collected from different host plant. Mistletoes found on various host trees is endowed with different antioxidant activity; however, the antioxidant capacity of the extract could vary according to the harvesting time of the plant as well as nature of the host tree[8, 9]. Very often, host trees that are attacked by mistletoes suffer from them as the triumph of mistletoes lead to poor growth and productivity and eventual death of such host plants. The reason for the successful parasitism of mistletoes may be related to the phytochemical interaction between the host and the parasite. Nevertheless, there is insufficient information on the phytochemical study of parasitic plants and their hosts in order to detect the secondary metabolites present at the host-parasite interface. The increasing interest in powerful biological activity of phytochemicals outlined the necessity of determining their composition at the host-parasite interface of *T. globiferus* and its four named hosts. This study generally aims at evaluating the phytochemical composition at the host-parasite interfaces among *Citrus sinensis*, *Pentaclethra macrophylla*, *Cola acuminata* and *Persea americana*.

Results

The qualitative phytochemistry as presented in Table 1 shows that only flavonoids, saponins and glycosides were present in the different plant host and parasites at different level of abundance. The quercetin calibration curve gave a regression equation of Abs = 0.0003[flavonoids] + 0.0479 with a 96.05% coefficient of determination to determine the flavonoids concentration (Supplementary material, Fig. 1). The absorbance of digitoxin standards which was plotted against digitoxin concentration gave a regression equation of Abs = 0.00015[glycosides] + 0.0479 and R^2 of 0.9700 for the determination of the total glycoside content (TGC) of the wood extracts (Supplementary material, Fig. 2). Similarly, the absorbance readings of diosgenin standard varied from 0.0377 to 0.1386. However, the total saponins content (TSC) of the extracts were determined from the regression equation for the calibration curve (Abs = 0.0013[saponins] + 0.0042; R^2 = 0.9459) (Supplementary material, Fig. 3).

Table 1
Qualitative Phytochemical Analysis of the Pulverised Samples

	Pulverised Samples							
Phyto-constituents	*P. americana*		*C. acuminata*		*P. macrophylla*		*C. sinensis*	
	Host	Parasite	Host	Parasite	Host	Parasite	Host	Parasite
Flavonoids	-	-	++	++	-	++	++	-
Tannins	-	-	-	-	-	-	-	-
Saponins	-	+	-	+	-	+++	++	+++
Alkaloids	-	-	-	-	-	-	-	-
Glycosides	-	-	+	++	-	++	-	-
Terpenoids	-	-	-	-	-	-	-	-
Steroids	-	-	-	-	-	-	-	-
+ = low in abundance; ++ = moderate in abundance; +++ = high in abundance; - = absent								

Flavonoids was observed to be present in both the *C. acuminata* host and milestoe parasite, however, flavonoids was significantly ($P < 0.05$) concentrated in the parasite as compared to the host plant (Table 2). Similarly, the parasite recorded considerably a significant ($P < 0.05$) amount of flavonoids in *P. macrophylla* as compared to the host plant whereby flavonoids was not detected. In contrast, flavonoids was found in the host of *C. sinensis* but was not detected in the parasite (Table 2). The total glycosides content recorded in the host and parasite of *C. acuminata* were not significantly different, while *P. macrophylla* and *P. Americana* had total glycosides content in their respective parasites but the different host had no glycosides (Table 3). Saponins was observed to be present in both the host and milestoe parasite of *C. sinensis*, however, it was significantly ($P < 0.05$) higher in the parasite as compared to the host plant (Table 4). Alternatively, saponins content was only found in the parasites of *C. acuminata, P. macrophylla* and *P. Americana* (Table 4).

Table 2
Total Flavonoids content (mgQE/g) of the host and parasites of the studied plants

Plant	Host	Parasite
C. acuminata	$849.22 \pm 9.09^{a,2}$	$1190.33 \pm 48.23^{b,1}$
P. macrophylla	$0.00 \pm 0.00^{c,2}$	$1482.55 \pm 31.35^{a,1}$
C. sinensis	$409.22 \pm 7.78^{b,1}$	$0.00 \pm 0.00^{c,2}$
P. americana	$0.00 \pm 0.00^{c,1}$	$0.00 \pm 0.00^{c,1}$

*means with different alphabet along each vertical array represents significant differences ($P < 0.05$) among the plants and parasites, while means with different numbers along each horizontal array signifies significant differences ($P < 0.05$) between the host and parasite.

Table 3
Total glycosides content (mgDE/g) of the host and parasites of the studied plants

	Host	Parasite
C. acuminata	$276.00 \pm 88.19^{a,1}$	$398.44 \pm 4.44^{a,1}$
P. macrophylla	$0.00 \pm 0.00^{b,2}$	$360.54 \pm 0.11^{b,1}$
C. sinensis	$0.00 \pm 0.00^{b,1}$	$0.00 \pm 0.00^{c,1}$
P. americana	$0.00 \pm 0.00^{b,2}$	$367.33 \pm 6.67^{b,1}$

*means with different alphabet along each vertical array represents significant differences ($P < 0.05$) among the plants and parasites, while means with different numbers along each horizontal array signifies significant differences ($P < 0.05$) between the host and parasite.

Table 4
Total saponins content (mgDE/g) of the host and parasites of the studied plants

	Host	Parasite
C. acuminata	$0.00 \pm 0.00^{b,2}$	$39.39 \pm 9.07^{c,1}$
P. macrophylla	$0.00 \pm 0.00^{b,2}$	$59.54 \pm 9.78^{ab,1}$
C. sinensis	$36.23 \pm 8.53^{a,2}$	$74.71 \pm 1.11^{a,1}$
P. americana	$0.00 \pm 0.00^{b,2}$	$45.28 \pm 13.07^{bc,1}$

*means with different alphabet along each vertical array represents significant differences ($P < 0.05$) among the plants and parasites, while means with different numbers along each horizontal array signifies significant differences ($P < 0.05$) between the host and parasite.

Discussion

The aim of this study was to evaluate the phytochemicals present in the extracts of selected plants and *T. globiferus* at the host-parasite interface. The levels of the phytochemical contents evaluated varied significantly by plant species and their parasites. The influence of the host plant on secondary metabolite levels of mistletoes is well-known. This has been associated to their parasitic habit that photosynthesizes but depends on the host for water and nutrients for photosynthesis.[14, 15] It was observed that the parasite generally contained higher concentrations of the phytochemicals evaluated than their host species. This observation is similar to the report of Lo Gullo et al.[16] who reported higher concentrations of minerals in parasites than their host species. The variation in secondary metabolites had been described by Urban et al.[17] to be as a result of the transpiration rates of mistletoes being higher than that of the host plants.[9, 15] However, the differences in phytochemicals in the wood of the host plant and the parasite at the interface could signify that they were two different plant species. More so, the differences in phytochemical contents in mistletoes from different host could also be influenced by the environment the host plant faces, such as soil fertility.[15, 18] Therefore, the inconsistent concentration of phytochemicals observed in our study could be due to the differences in species as well as in the environment.

Flavonoids defend plants against a variety of biotic and abiotic stresses by demonstrating a wide spectrum of biological functions and play a significant function in the interaction between the plant and their environment.[19] The increased flavonoid content in *C. acuminata* and *P. macrophylla* could be an indication that the parasites were more exposed to environmental stress than the host. Studies show that flavonoids could have a determinant role in response to different stresses such as drought.[20] In this regard, Nakabayashi et al.[21] reported that the over expression of some genes involved in flavonoid metabolism and over accumulation of flavonoids would caused an increase in drought tolerance. Therefore it would be reasonable to suggest that, the parasites utilizes this as a medium of survival since the transpiration rates of mistletoes are higher than that of the host plants.[17]

Saponins are usually found in tissues that are highly susceptible to attacks by bacteria, fungi or insects.[22] Hence, it is assumed that one of the roles saponins plays in plants is to act as a chemical barrier in opposition to prospective pathogens. Therefore, this would account for their antimicrobial activity.[23, 24] Due to their toxicity to various organisms, saponins can be utilised for their insecticidal, antibiotic, fungicidal, and pharmacological properties.[24]

Conclusion

At the host-parasite interface, significantly higher phytochemicals in the wood portion of *Tapinanthus globiferus* (milestoe) was observed as compared to the host plants wood, however, the variability in phytochemical content of *Tapinanthus globiferus* is dependent on the host. From these findings, milestoe grown on *C. acuminata* and *P. macrophylla* will be rich in flavonoids, while *C. sinensis* host favours the production of saponins in milestoe. Therefore, milestoe would be a better source of bioactive compounds with high medicinal values than their host plants if explored further.

Materials And Methods

Collection and Authentication of Plant Specimens

The twigs of mistletoes parasites from *C. sinensis*, *P. macrophylla*, *C. acuminata* and *P. americana* and their respective host stems were collected from farmlands in Alor-Agu, Igboeze South L.G.A, Enugu, Nigeria with the approval of the locals and authenticated and deposited by Mr. Felix Nwafor in the Department of Pharmacognosy and Environmental Medicines Habarium, University of Nigeria Nsukka, Enugu, Nigeria with voucher specimen number of PCG/UNN/00266 (*Tapinanthus globiferus*), PCG/UNN/0056 (*C. sinensis*), PCG/UNN/0080 (*P. macrophylla*), PCG/UNN/0330 (*C. acuminata*) and PCG/UNN/0012) *P. americana*. The plant specimens were cleaned, air-dried at room temperature and reduced to fine particles. The air-dried samples were pulverised and 10g of each samples were macerated with 100mL of methanol and kept at room temperature for 48hrs with agitation. The solvents were dried *in vacuo* at 40°C to obtain the dry extracts.[10]

Qualitative phytochemical analysis of the pulverised samples was done to determine the presence of secondary metabolites (flavonoids, tannins, saponins, alkaloids, glycosides, terpenoids and steroids) according to standard procedure.[11] The confirmed metabolites where then further analyzed quantitatively.

Preparation of the Calibration Curves

Quercetin

A quercetin stock solution was prepared by dissolving 10mg in 10mL methanol to give a stock solution of (1000 mg/L). The stock solution was further diluted to different concentrations of 100, 80, 60, 40 and 20m*g*/L. 1mL each of the different concentration of quercetin was mixed with 4mL of distilled water and 0.30mL of 5% (*w/v*) sodium nitrite. After 5 min, 0.30mL of 10% (*w/v*) $AlCl_3.6H_2O$ solution was added to the mixture, followed by addition of 2mL of 1.0M NaOH after another 5min and diluted to 10mL with distilled water. The absorbance of the different concentrations was then measured against the reagent blank at 510nm with a UV/Visible spectrophotometer.

Digitoxin: The standard curve was prepared by adding 0.2mg/mL digitoxin in chloroform – methanol (1:1; *v/v*). A solution of 0.1, 0.2, 0.3, 0.4 and 0.5mL each (equivalent to 0.02, 0.04, 0.06, 0.08 and 0.10mg of digitoxin respectively) was transferred to a dry Erlenmeyer flasks and evaporated to a small bulk (0.35mL) and made up to volume with distilled water. Freshly prepared Baljet's reagent (10mL) was added to each flask and allowed to stand for 1 h at room temperature and the resulting mixture diluted with 20mL of distilled water. The absorbance of the colour developed was determined using a spectrophotometer against a suitable blank at 495 nm.

Diosgenin

Diosgenin standard (0.20mg/mL) was dissolved in methanol and 0.1, 0.2, 0.3, 0.4 and 0.5mL (equivalent to 0.02, 0.04, 0.06, 0.08 and 0.10mg of diosgenin respectively) each of the standard solution was transferred to a dry test tubes. Vanillin-acetate solution (0.20mL of 5% (*w/v*)) was added, and followed by the addition of 0.80mL sodium perchlorate solution. The mixture was shaken and heated for 15mins in a water bath at 60^{o}C. After cooling, 5mL of glacial acetic acid was added to each test tubes and the absorbance of the mixtures determined against the blank using a UV/Visible spectrophotometer at 548 nm.

Determination of Total Flavonoids Content (TFC)

Aluminium-chloride colourimetric assay was used to determine the total flavonoids content in the extracts as adopted by Agbo *et al*.[10] The extracts (1mg/mL) were prepared following the same procedure in preparing quercetin as reported above. The absorbance of the extracts was measured against the reagent blank at 510nm with a UV/Visible spectrophotometer. The total flavonoids content was determined from the calibration curve and expressed as milligram of quercetin equivalent per gram of extracts (mgQE/g).

Determination of Total Glycosides Content (TGC)

Balet's reagent colourimetric method was used for the determination of the total glycosides content of the extracts as previously reported with slight modification by Tofighi *et al*.[12] The extract (1mg/mL) was dissolved with 6mL of distilled water and 1mL of 12.5% (*w/v*) lead acetate solution was added. The mixture was made up to 10mL with distilled water and filtered. The filtrate (5mL) was transferred to a volumetric flask, 1mL of 4.77% (*w/v)* Na_2HPO_4 solution added, and made up to 10mL with distilled water and filtered. Baljet's reagent (10mL) was added to 1mL of the clear filtrate and the mixture allowed to stand for 1h and diluted with 20mL of distilled water. The absorbance of the mixture was read against the blank at 495nm with a UV/Visible spectrophotometer. The total glycosides content was determined from the calibration curve and expressed as milligram of digitoxin equivalent per gram of extracts (mgDE/g).

Determination of Total Saponins Contents (TSC)

Vanillin-acetate colourimetric method was used for the determination of the total saponins content of the extracts as previously reported by Benyong *et al*.[13] with slight modification. A freshly prepared 5% (*w/v*) vanillin-acetate solution (0.20mL) was added to 0.10mL of the extract (1mg/mL) in a test tube followed by the addition of 0.80mL (*w/v*) sodium perchlorate solution. The mixture was shaken and heated for 15mins in a water bath at 60^{o}C and cooled. 5mL of glacial acetic acid was added to the mixture and the absorbance of the mixture read against the blank at 548nm using a UV/Visible spectrophotometer. The total flavonoids content was determined from the calibration curve and expressed as milligram of diosgenin equivalent per gram of extracts (mgDE/g).

Data analysis

The entire quantitative tests were carried out in triplicates and data collected were for the host and parasite were subjected to Analysis of Variance (ANOVA) using IBM SPSS ver. 20.

Abbreviations

TGC - Total glycoside content

TSC - Total saponins content

$AlCl_3.6H_2O$ - Aluminium chloride hexahydrate

NaOH - Sodium Hydroxide

ANOVA - Analysis of Variance

Declarations

- Ethics approval and consent to participate: Not applicable
- Consent for publication: Not applicable
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- Authors' contributions: AGC and OPC managed the analyses of the study, edited the draft of the manuscript. NRC and OEO managed the literature searches, performed the statistical analysis and wrote the first draft of the manuscript. AGC and NAE designed the study, wrote the protocol and edited the draft of the manuscript. All authors read and approved the final manuscript.
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- Availability of Data and Materials: Not applicable

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Figures

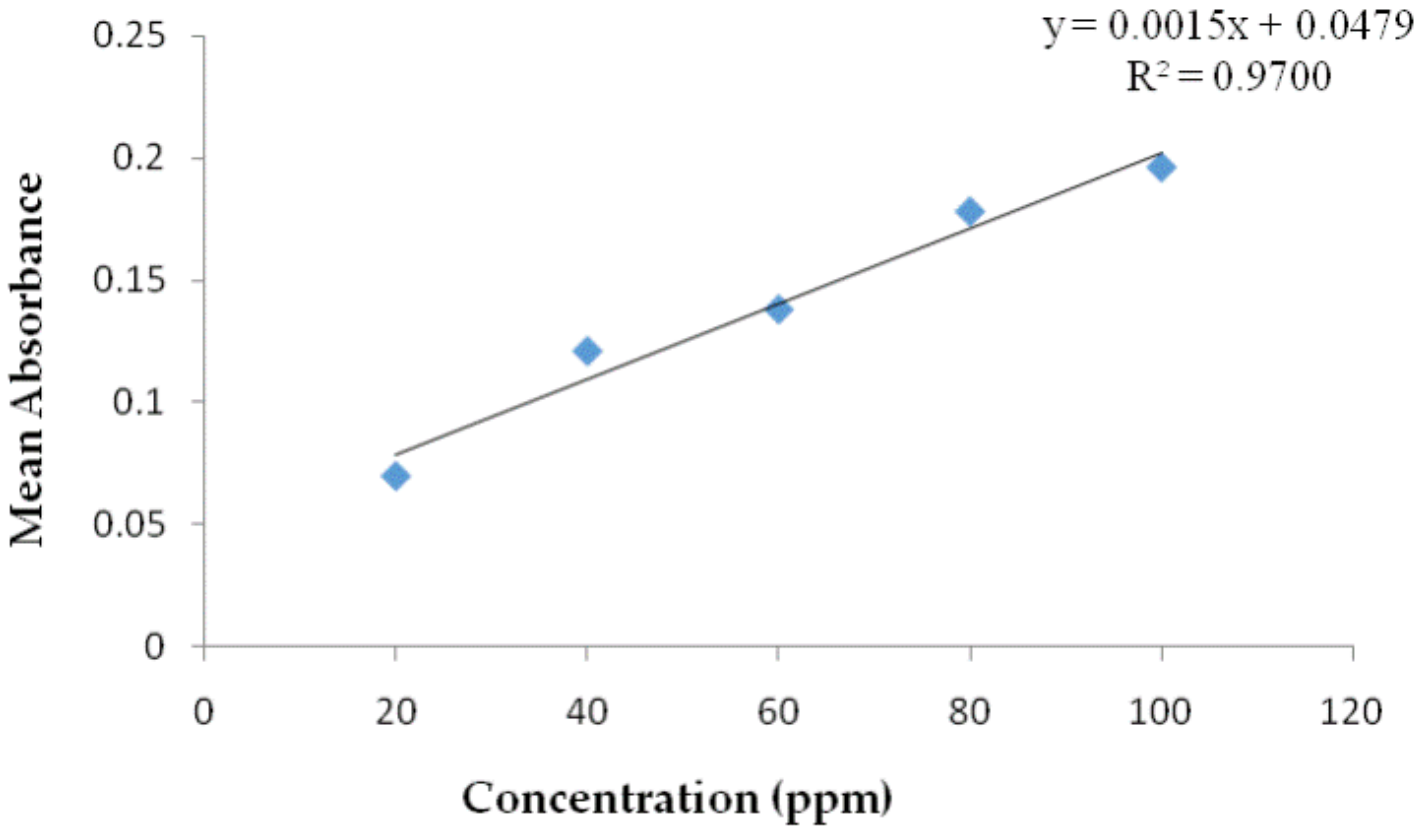

Figure 1

Calibration Curve for Quercetin Standards

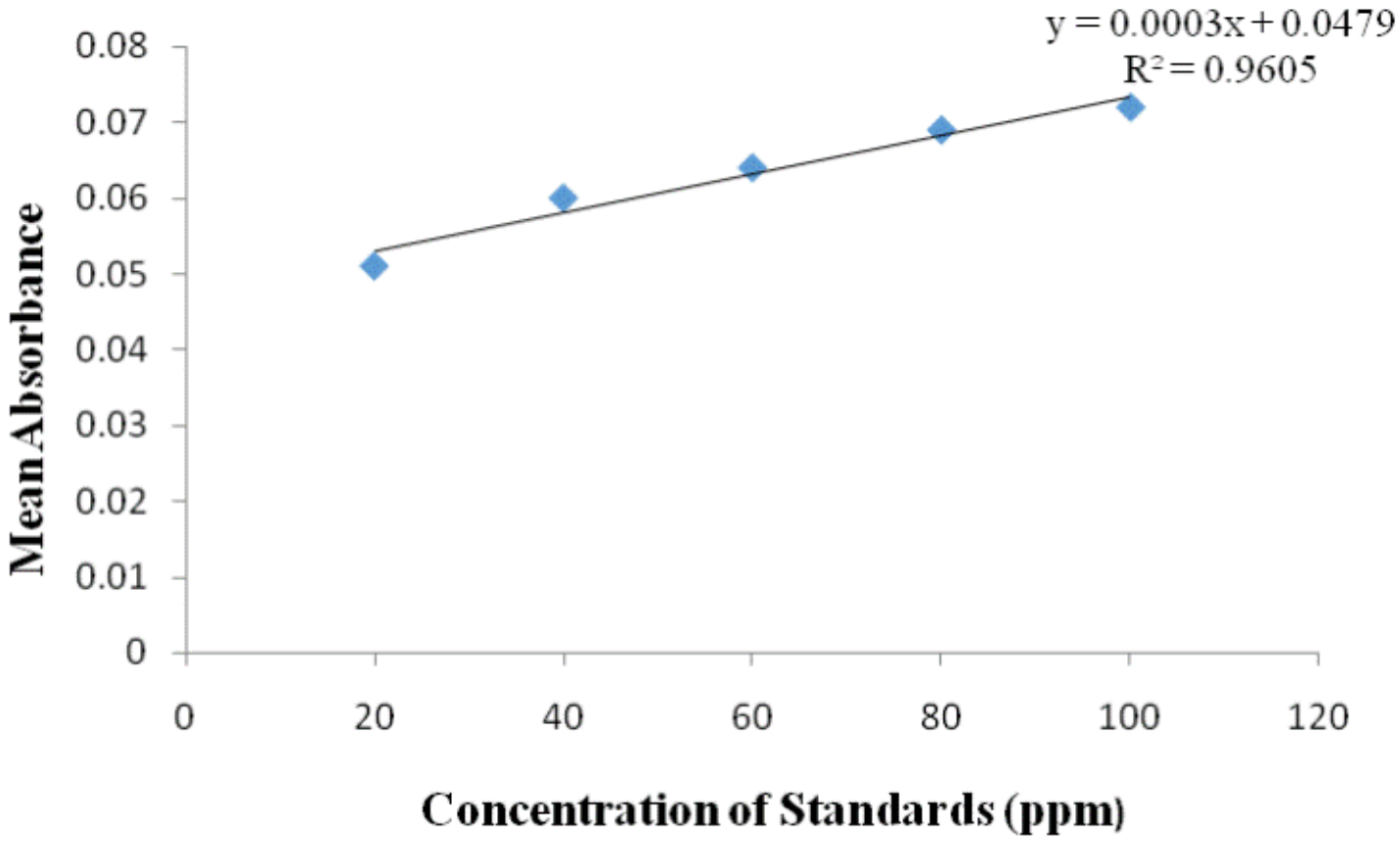

Figure 2

Calibration Curve for Digitoxin Standards

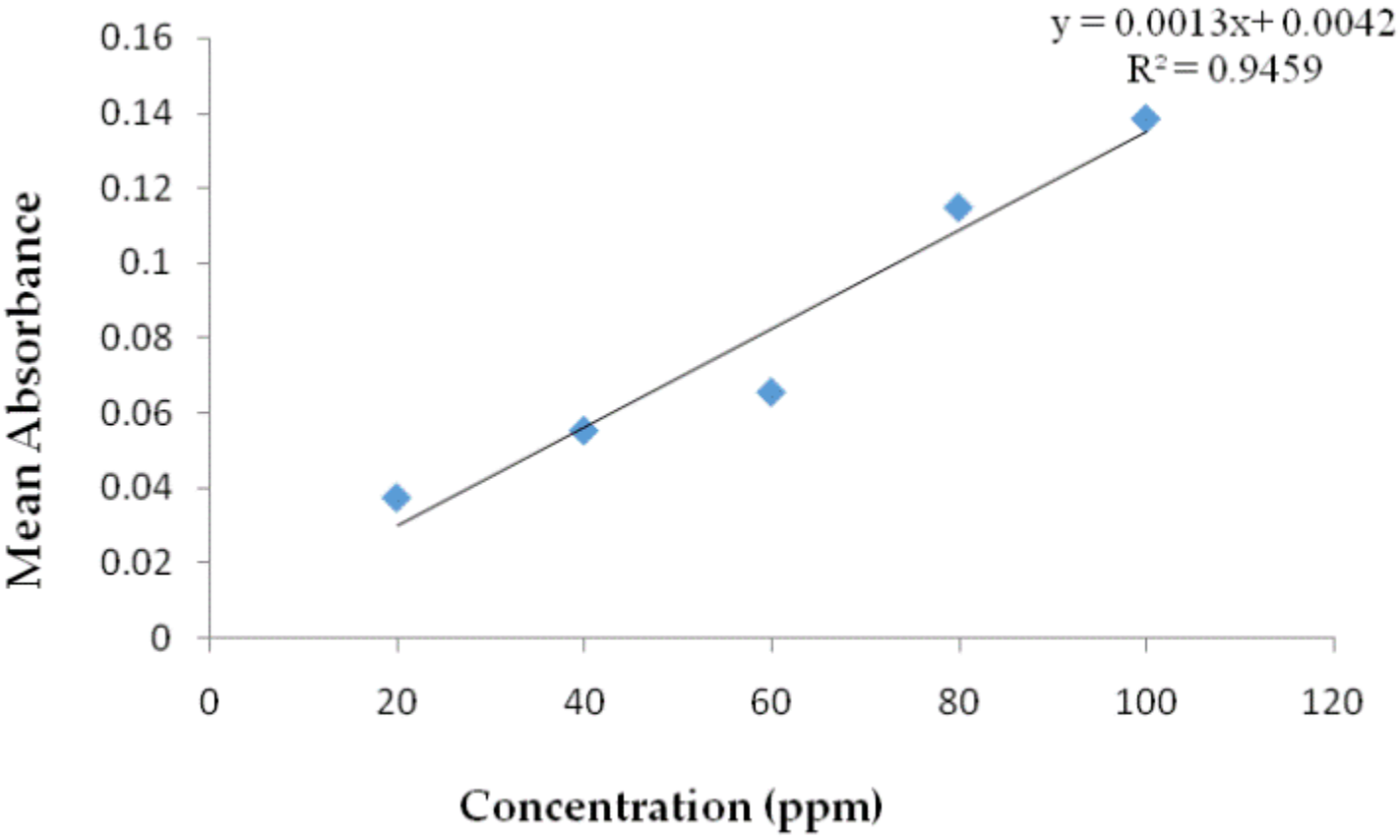

Figure 3

Calibration Curve for Diosgenin Standards