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Phytochemical Constituents and Phytotoxic Potential Study of Morinda Citrifolia Grown In South West Nigeria

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ABSTRACT

Morinda citrifolia (Noni) is a tree in the coffee family, *Rubiaceae*. Its native range extends through South East Asia and Australia. The species is now cultivated throughout the tropics and widely naturalized. It is traditionally used to treat various ailments which include wound and injuries, arthritis, diabetes, hypertension, inflammation, cardiovascular diseases, cancer in some parts of Africa. This study evaluates the phytochemical constituents and phytotoxic potential of leaves and pulp of *Morinda citrifolia*. Standard method of analysis was used for the phytochemical screening and phytotoxic evaluation, while infrared spectroscopy was used for identification of functional groups. In the phytochemical studies of the pulp, phenols, diterpenes, alkaloid, terpenoid was present in all the tested extracts. Saponin, flavonoid, coumarin, phytosterol was found present in the distill water and ethanolic extracts while anthraquinones and anthocyanins were found only in ethanolic extract. In the phytochemical studies of the leaves, alkaloid, triterpene, phenol, tannins were present. Flavonoid, anthraquinone, glycosides were present only in distill water and ethanolic extracts. Coumarins was present in both ethanol and n- hexane extracts while terpenoid was present only in ethanolic extract. Infrared spectroscopy results showed the presence Carbonyl compound, sp^3 OH, sp^2 OH, nitroso compound, aliphatic alkanes, alkynes, amines and aldehydes groupings in pulp and leaves extracts. Phytotoxic evaluation revealed that the percentage growth inhibition was dependent on the concentration of the extract. The percentage growth inhibition of shoot at 2.0 % concentration for maize and soya bean seedlings ranged from 60% - 45% in the tested extracts, while that of the root at 2.0% ranged from 67% - 60% inhibition. This showed that the root was more inhibited than the shoot.

Keyword:*Morinda citrifolia*, Phytochemical, Phytotoxic.

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INTRODUCTION

The noni plant is actually a member of the coffee family, and the fruit presents itself as bumpy and yellowish-white in colour belonging to a tree in the coffee family *Rubiaceae*, noni goes by the scientific name *Morinda citrifolia* [1]. Like many other fruits, noni fruit is squeezed into a juice and sold as such, but you can also get it as a juice concentrate, a powder, and it can even be found in beverages blended with other ingredients and juices [1,2].Noni goes beyond the juice department,of course. It can be purchased as a fruit made from the dehydrated pulp and crushed leaves can be found in natural medicines and cosmetics [4]. Noni oil is produced from pressed seeds and used topically in many products, including shampoos. Noni products are sold all over the world, but they're most popular in North America, Mexico, Asia and Australia [5-6]. The traditionally, noni is used to treat ailments such as diabetes, cardiovascular diseases, arthritis, wounds and injuries [3,6]. This study evaluates the phytochemical constituents and phytotoxic potential of *Morinda citrifolia* extracts

Plate 1: *Morindacitrifolia*Plant

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MATERIALS AND METHODS

SAMPLE PREPARATION

Morinda citrifolia pulp were collected from home garden in Ibadan, Oyo state, South-West Nigeria. The sample was identified by Prof A.T.J Ogunkunle, a taxonomist in the Department of Pure and Applied Biology, LadokeAkintola University of Technology, Ogbomoso, Nigeria. The samples were washed with distilled water, cut into smaller sizes and air dried in the laboratory. The dried samples were pulverized into powder using attrition mill and stored in clean bottle.

EXTRACTION

One thousand kilogram (1 kg) each of the pulverized pulp and leaves were extracted with n-hexane, ethyl acetate and ethanol using separating funnel. The extracts were concentrated using rotary evaporator to dryness under vacuum. These were stored at room temperature for further analysis.

PHYTOCHEMICALSCREENING TEST

Phytochemical examination was carried out on the extracts using the standard methods described by Harborne [15, 14,17]

Test for Alkaloids: Extracts was dissolved in dilute Hydrochloric acid and filtered

Wagner's test: Filtrates was treated with Wagner's reagent (Iodine in potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Hager's test: filtrates was treated with Hager s reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate [10].

Test for Saponinns: Foam test: 5ml of exact was shaken with 2ml of water. If foam produced persist for ten minutes it indicates the presence of saponins.

Test of Phytosterol: LibermannBurchard's test: Extracts was treated with chloroform and filtered. The filtrate was treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols [9]

Test for Triterpenes: Salkowski's test: Extracts was treated with chloroform and filtered. The filtrate was treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

Test for Phenols

Ferric Chloride test: Extracts was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Test for Tannins: Gelatin test: To the extract, 1% gelatin solution containing sodium Chloride was added. Formation of

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white precipitate indicators the presence of tannins.

Test for Flavonoid: Lead acetate test: 2ml of Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids

Test for Diterpenes: Copper acetate test: Extracts was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates presence of diterpenes.

Test forAnthraquinones: Three milliliters of extract were treated with 3ml of benzene and 5ml of 10% ammonia was added to the mixture. Formations of pink, violet or red coloration indicate the present of anthraquinones.

Test for Anthocyanins: Two milliliters of extract were treated with 2ml of hydrochloric acid (HCl) and 2ml of ammonia was added to the mixture. Formations of pinkish red to bluish violet coloration indicate the present of anthocyanins.

Test for Coumarins: Two millilitersof extract were treated with 3ml of 10% sodium hydroxide (NaOH). Formations of yellow coloration indicate the present of coumarins.

Test for glycosides: 2ml of extract was treated with 2ml chloroform ($CHCl_3$) and 2ml of ethanoic acid (CH_3COOH) was added to the mixture. Formations of violet blue to green coloration indicate the present of Glycosides

Test for Terpenoids: Two milliliters of extract were treated with 2ml acetic anhydride [$(CH_3CO)_2$] and 2-3 drops of concentrated tetraoxosulphate IV acid was added to the mixture. Formations of deep red coloration indicate the present of terpenoids.

PREPARATION OF TEST SOLUTIONS

Test solutions were prepared by serial dilution of the extracts (n-hexane, ethyl acetate and ethanol extracts). Two grams (2 g) of each extract was dissolved in (10ml of DMSO+ 90ml of water) 100 ml of distilled water to get 2% solution [9,12]. One percent (1 %) of the test solution was prepared by taking 50 ml of 2 % solution into 100 ml of volumetric flask and making it up with the distilled water, 0.5% of the test solution was prepared by taking 50 ml of the 1% solution into 100 ml volumetric flask and making to mark with the distilled water, 0.25% of the test solution was prepared by taking 50 ml of the 0.5 % solution into 100 ml volumetric flask making it to mark with distilled water [18,23, 24].

Phytotoxic assay of test solutions: In order to study phytotoxic activities of the extract solution, different concentrations of the examined solutions were prepared and applied to check germination of both root and shoot of the germinating seeds. Seeds of maize (*Zea may*) and Pea (*Pisum sativum*) were collected from local market. The assay seeds were sorted for uniformity of size and all damaged seeds were discarded, Thereafter, these were washed with tap water and the surface sterilized using NaCl (10 % v/v) for 10 min, followed by washing in sterile distilled water. Bioassays were carried out using Petri dishes (90mm diameter) containing cotton wool as support. Test solutions (5 ml) was added to the cotton wool in the Petri dish and dried completely in vacuum

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at 40°C. Five selected seeds were placed on the cotton wool and incubated for 7 days at 25°C in the dark using prepared solutions of 0.25 %, 0.5%, 1%and 2 % respectively. The treated seeds of maize and pea were allowed to germinate on a moist cotton wool by addition of 5ml of distilled water daily for seven days. After seven days of incubation, the length of roots and shoots of germinated seeds were measured in centimeter (cm). Treated experimental sets were compared with the control. The experiment was repeated in triplicate. Percentage growth wascalculated using the formula;

Percentage Growth = GT/GC × 100. …… (1)

GT = Average growth length of Root and Shoot in the examined medium,
GC = Average growth length of root and shoot in the control index.

While the percentage growth inhibition was calculated using formula

Percentage Growth inhibition = 100 – (GT/GC ×100). …… (2)

GT = Average growth length of Root and Shoot in the examined medium,
GC = Average growth length of root and shoot in the control index.

Table 1: Phytochemical constituents of pulp extract of *Morinda citrifolia*.

Extracts	Alka-loids	Flavon oids	Anthraq uinones	Sapon-ins	Glycosides	Tannins	Phenols	Sterols	Triterpene s	Coumarins	Anthocy anins	Terp-enoids	Diterp enes
HE	+	-	-	-	+	-	-	-	+	-	-	-	+
EA	-	+	+	+	-	-	+	+	+	+	-	+	+
ET	-	+	+	+	-	-	+	+	+	+	-	-	+

Key: HE - n-Hexane, EA- Ethyl acetate, ET – Ethanol, + represent present, - represent not detected

Table 2: Phytochemical constituents of Leaves extract of *Morinda citrifolia*

Extracts	Alkalo-ids	Flavo noids	Anthraqu inones	Saponins	Glycosides	Tannin s	Phenols	Sterols	Triterpenes	Couma rins	Anthocyan ins	Terp-enoids	Diterp enes
HE	+	-	-	-	-	-	-	-	-	-	-	-	-
EA	+	+	-	+	-	+	+	+	+	+	-	+	-
ET	+	+	+	+	-	-	+	+	+	+	+	-	+

Key: HE - n-Hexane, EA- Ethyl acetate, ET – Ethanol, + represent present, - represent not detected

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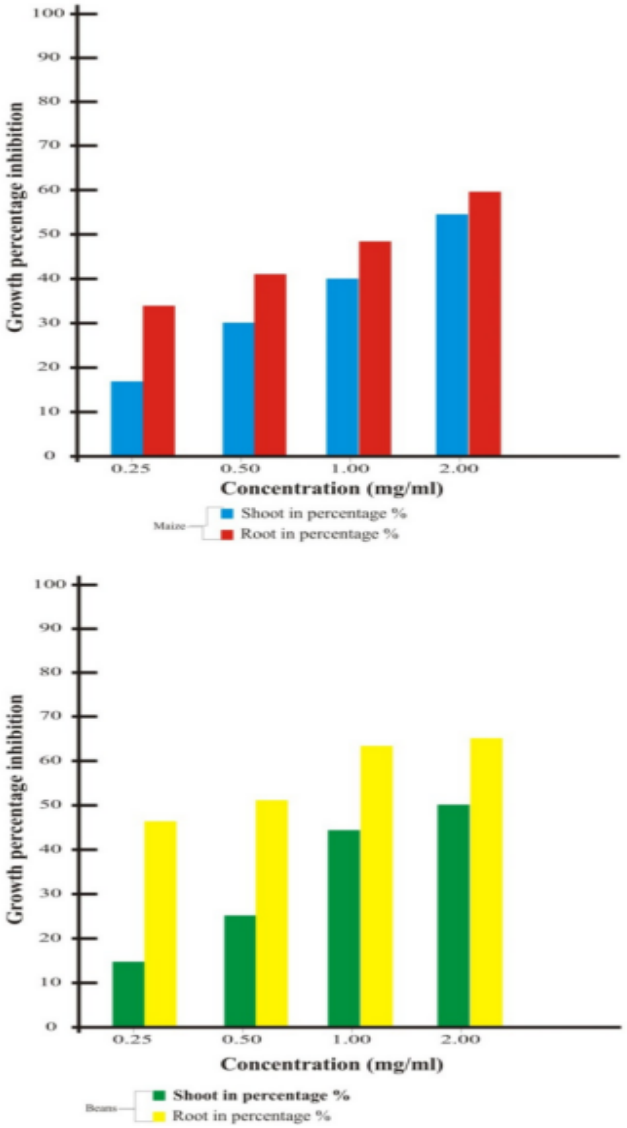

Figure 1: Growth percentage inhibition seed culture in n-hexane extract of *Morindacitrifolia*

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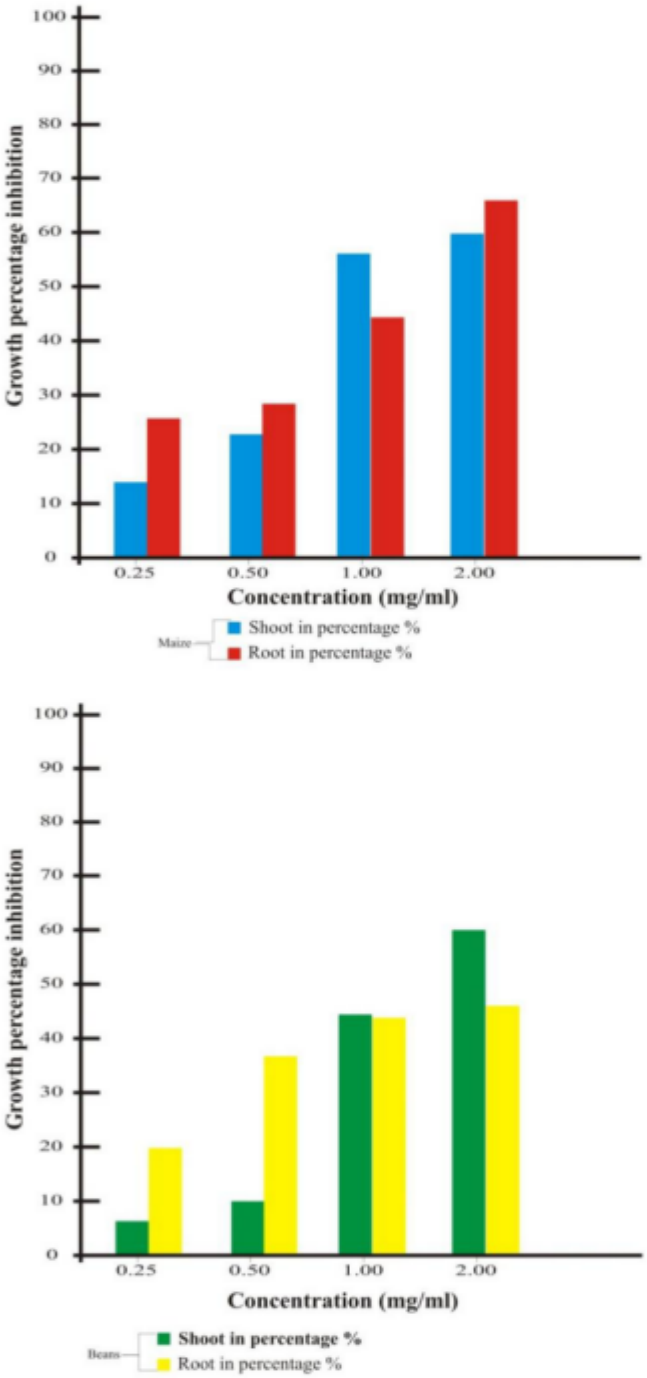

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Figure 2: Growth percentage inhibition seed culture in ethyl acetate extract of *Morindacitrifolia*

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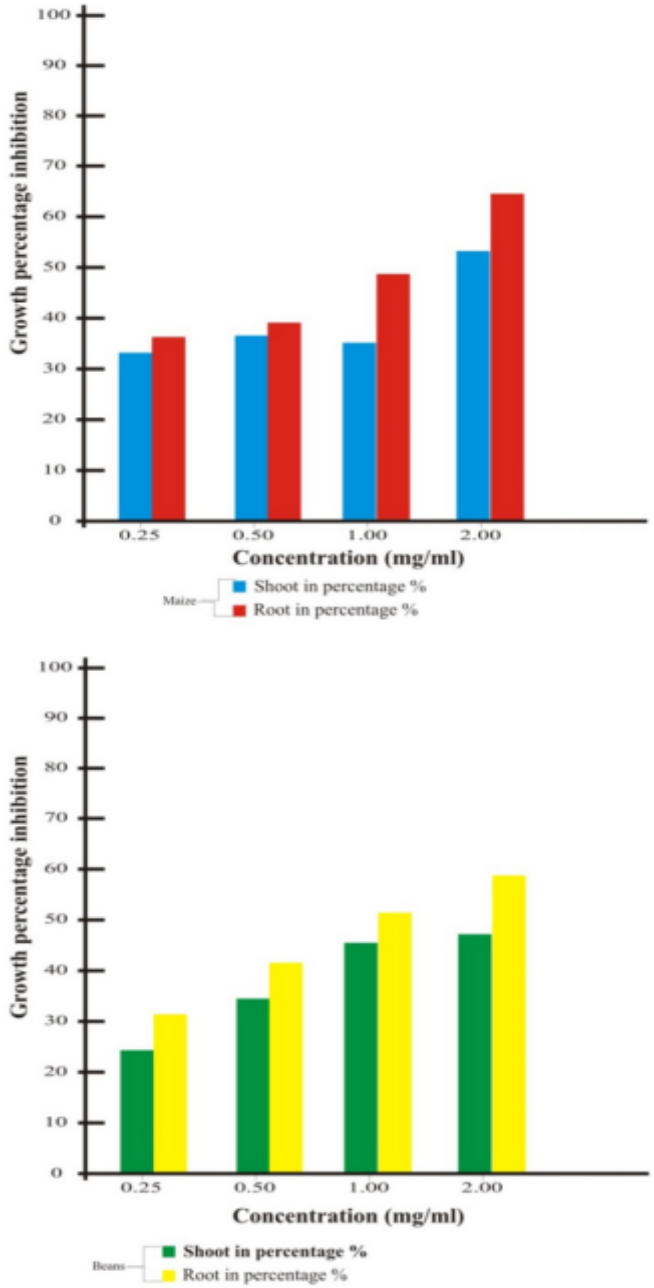

Figure 3: Growth percentage inhibition seed culture in ethanol extract of *Morindacitrifolia*

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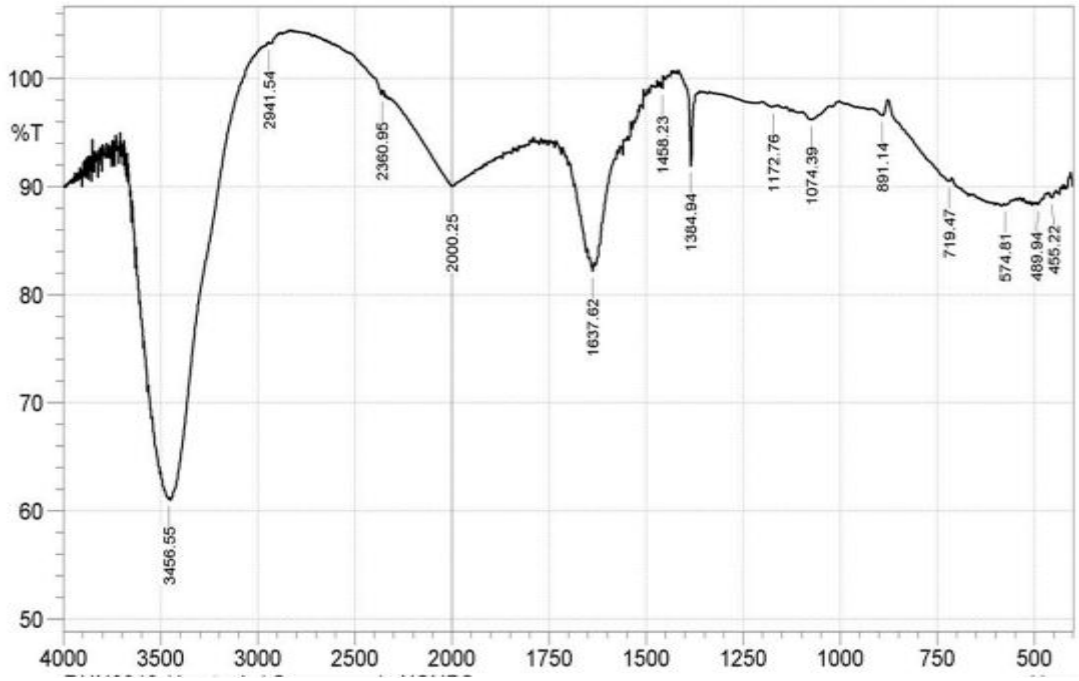

Figure 4 : Infrared spectrum data of pulp extract of *Morindacitrifolia*

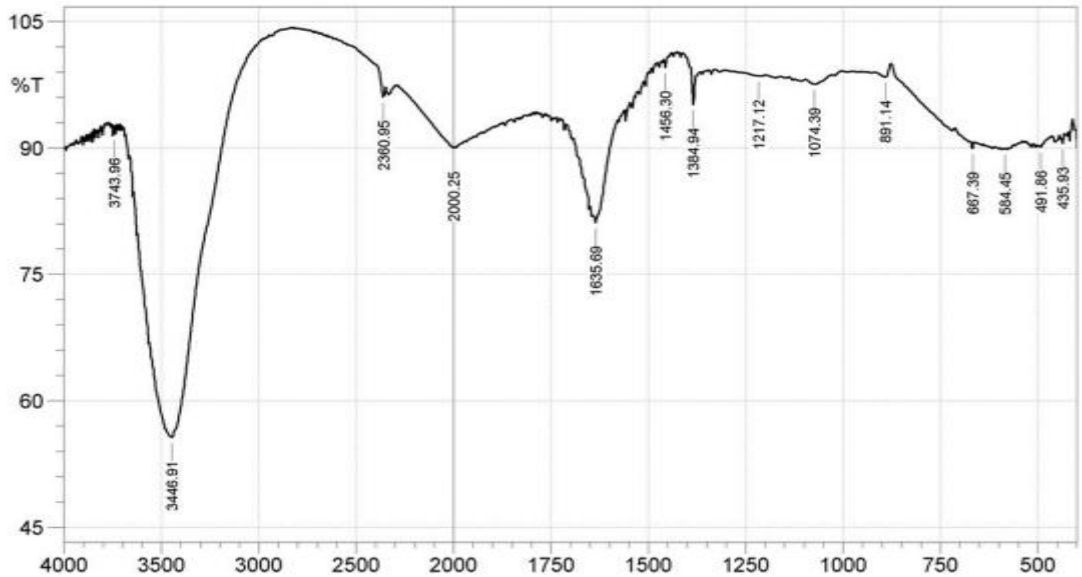

Figure 5 : Infrared spectrum data of leaves extract of *Morindacitrifolia*

I. RESULTS AND DISCUSSION

The extracts were screened for the presence of thirteen classes of phytochemical constituents as presented in tables 1 and 2.In table 1, the results revealed presenceof phenols and diterpenes in all the leaves extracts while, table 2 showed that alkaloids, phenols and diterpenes were all present in the pulp extracts.Figures 1,2and 3 showed the phytotoxic inhibitorypotential of the extracts. The results followed a dose dependent manner with concentration. The root is more inhibited than the shoot in all the tested extracts. The delay or inhibition of germination caused by phytotoxic plant extracts or substances were due to inhibition of the plant growth hormones such as gibberellin, auxins,[23]. Although germination bioassay is the most widely used method to inspect the phytotoxic activity early seedling growth is reported to be most sensitive parameter to test the phytotoxicity [16]. The bioassay results showed a significant reduction of shoot and root growth of the test species at 0.25 mg/lconcentration.

However, the sensitivity to the extracts varied to the tested maize and bean seeds. The higher sensitivity of seedling growth to phytotoxic activity of the extracts could be due to the presence of seed coat which acts as a barrier in between the embryo and its surrounding environment, the selective permeability of seed coats which may protect the inhibitory activity of phytotoxic extracts if they cannot pass through seed coats and the parameter that was used to measure germination (the protrusion of the root through the seed coat which does not necessarily mean growth by cell division, and so forth .

On the other hand, since roots are the first target tissue to confront with the phytotoxic substances, therefore inhibitory effects are more visible on roots rather than on shoots In summary, these results indicated that the extracts have phytotoxic properties and thus contain phytotoxic substances. The concentration dependent inhibitory activities of allelopathic plant extracts on germination and seedling growth were also reported by [23]. Therefore, the plant could be served as an important candidate for isolation and identification of allelopathic substances, which may promote the development of new natural herbicides. Besides this, the plant extracts or their residues could be directly used as bioherbicides [23, 24]. Figures 4 and 5 showed the infrared spectra data of the extracts with some significant peaks of alcohol, carbonyl, alkanes, amines functional groups.

II. CONCLUSION

In conclusion, the plant pulp and leaves extracts show phytotoxic activity because they contain phytochemicals The plant products which possess phytotoxins in inhibiting the growth of plants could serve as natural herbicides for weed management and control, as alternative to synthetic herbicides which are not biodegradable. However, Isolation and characterization of the phytotoxic substances will promote the development of plant products based natural herbicides from *Morindacitrifolia*.The result of phytochemical series show a very heavy presence of phenol, alcohol alkaloid diterpenes terpenoid in the three different extracts of ethyl acetate, ethanol and n-hexane for the pulp extract. Saponin, phytosterol, terpene, flavonoid and coumarin were present in the ethyl acetate and ethanol extract, antraquinones, anthrocyanin was present only in ethanol extract.It showed that ethanol was a very good extracting medium maybe due to the fact that we have alcohol group and carbonyl compound in the fruits of Morindacitrifolia. The leaves extract showed the presence of alkaloids, triterpenes, phenols, tannins, flavonoids, diterpenes were present in all three extracts. Anthraquinone, glycosides, coumarins was present but not in all the extracts.

Free hydroxyl of alcohol or phenol group, primary and secondary amine or amide were present. Alkyne, aromatic and aliphatic group, with aldehydes were functional group detected (Figures 4 and 5). Morindacitrifolia is indicated a range of health benefit which are already discovered the variant in Nigeria shows the presence of functional group that also exhibit the functional. It contains scopolein which inhibit the growth of Escherichia coli, which are responsible for intestinal infection Damnacanthal is reputed to inhibit the tyrosine kinase and starve tumors of glucose which they need for growth, it means it shows down cancer growth and can kill it. Phytosterols has the ability to decrease total and low-density lipoprotein (LDL) cholesterol, which is good for preventing clogging of the arteries and hypertension.

The concentration dependent inhibitory activities of Morindacitrifolia- allelopathic plant - extracts on germination and seedling growth, therefore, the plant could serve as an important candidate for isolation and identification of allelopathicsubstances, which may promote the development of new natural herbicides. Besides this, the plant extracts or their residues could be directly used as bioherbicides

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