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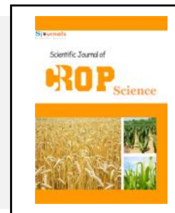

Original article

Phytochemical screening and physical constant evaluation of *Ficus Abutilifolia Miq.* (*Moraceae*) leaves, stembarks and roots for quality control

C.A. Ukwubile
Department of Basic and Applied Sciences/Science Laboratory Technology, Federal Polytechnic Bali Taraba State, Nigeria.

*Corresponding author; Department of Basic and Applied Sciences/Science Laboratory Technology, Federal Polytechnic Bali Taraba State, Nigeria.

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A B S T R A C T

Ficus abutilifolia has been used to treat various ailments in Nigeria especially in the Northern and Southern parts, where the plant's aqueous extracts has been use to treat typhoid fever, syphilis, and amoebic dysentery. Because of these uses, the plant's parts had been adulterated for commercial purposes. The study was aimed at providing accurate parametric data for quality control of the products. Preliminary phytochemical screening of the parts showed that leaf contained saponins, tannins, flavonoids, alkaloids and anthraquinones whereas alkaloids and anthraquinones were not noticed in stembarks ethanol extracts, flavonoids and tannins were the only phytochemicals present in the roots, as were confirmed using TLC plates and ultraviolent spectrometry apparatus. Percentage moisture content was 4.10±0.08 (leaf), 3.10±0.06 (stembarks) and 2.60±0.05 (root). Ash values were 12.47±0.20 (leaf), 6.22±0.14 (stem bark) and 3.04±0.09 (root). The acid insoluble ash value were 1.34±0.17 (leaf), 6.60±0.25 (stembarks) and 3.50±0.22 (root) whereas the water soluble ash value were 5.20±0.46 (leaf), 6.90±0.49 (stembarks) and 4.40±0.44 (root). Water extractive values were found to be greater than alcohol extractive values in the three parts; 6.32±0.50 (leaf), 1.70±0.06 (stembarks) and 0.50±0.12. The study showed that most of the data evaluated were in the acceptable range.

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1. Introduction

Ficus abutilifolia Miq. belongs to the family of Moraceae. The family is one of the largest families in the angiosperms. They are known for the sap which they produced from the stem, leaves as well as flower buds [1]. Most species in the family show various adaptations in their environment so as to survive various conditions [2]. Some are found growing very well on rocks, swamps [3]. Most of them are trees ranging from 21-50m high [4]. Their flowers are borne inside the plants in all the species.

The genus Ficus consists of about 850 species of woody trees, shrubs, epiphytes and semi epiphytes [4]. Collectively they are known as figs, and occur throughout the tropics with a few species extending into the semi – warm temperate zones.

Ficus abutilifolia Miq. is known by the common names large – leaves rock fig or rock wild fig. In Nigeria, it is called “wawan kurni (Hausa)”, “Agbulu (Igbo)” and Lapalapa (Yoruba)”. The conspicuous white-yellow roots and stems of the plant are impressive sight, well adapted to hugging rock faces and splitting rocks, hence giving this species a place among the true rock splitting figs. The species name abutilifolia stems from the resemblance of its leaves to those of abutilon – a popular ornamental genus [5].

F. abutilifolia is a small to medium sized, deciduous to semi-deciduous tree up to 50m high. The bark is whitish yellowish or white and smooth, powdery or somewhat flaking and it is this conspicuous bark that is immediately apparent. The trunk is usually twisted or contorted, the branchless, short and glabrous; lacking hairs and marked with leaf and stipular scars [6]. The leaves are broadly ovate and heart – shaped to almost round and are cordate at the base, ranging in size from 75 – 200 x 65 – 180mm . They are glabrous on both surfaces, occasionally with velvety hairs beneath, with 4-9 pairs of secondary veins, entire wavy margins and a petiole up to 120mm long. The fruits, which are 15-25mm in diameter, are borne singly or in pairs in the leaf axils on terminal branchlet, and are smooth to slightly hairy. They may be sessile or on short, stout stalks up to 15mm long and are green becoming yellow or red when ripe. The tree flowers between August and February, and occurring in the African continent in places such as Southern African provinces of Kwazulu – Natal, Mpumalunga, Gauteng, and the North west up into ,Mozambique, Zimbabwe, Botswana, Namibia, Malawi, Zambia, Ethiopia, Somalia and West to Guinea, Ivory coast, Nigeria and Eastern Sudan [6]. The plant can be grown by cutting, although propagation through the seeds is the best, it is a slow –growing plant [7].

The uses of the plant documented are somewhat limited and mostly medicinal in nature. Ficus abutilifolia Miq. has been used in traditional medicine in Nigeria and some African countries to treat various diseases. The aim of this study was to determine the phytochemical components as well physical constants of the plant’s parts so as to ensure quality control of its products, and therefore prevent adulterations.

1.1. Use of chemical constituents in ficus species

The use of chemotherapeutic agents in the treatment of diseases has been known from time immemorial. The ancient man discovered the therapeutic value of some herbs by trial and error [8]. The alternative use of folkloric medicinal plants detailed their alternative use in medicine in Jamaican society has been reported [9]. The effective inhibition of the growth of some Gram – positive and Gram – negative bacteria by ethanol extract of some figs has been reported [10].

Phytochemical screening of the leaves and stem bark of Ficus exasperata Vahl showed the presence of alkaloids, flavonoids, tannins, saponins and cyanogenic glycosides , and that the administration of ethanol extract of this plant at high doses could affect kidney function. These phytochemicals if checked properly could be found in most species of Ficus with only slight variation in the morphological parts of the plant. And these phytochemicals exhibit great potentials as antioxidant and antimicrobial in animals when used [11]. It had been reported that tannins have antibacterial property, and also saponins posses’ antifungal properly and thus interfering with the intake of microbial proteins by the bacteria [12]. Antimicrobial agents with low activity against an organism have a high MIC while a highly active antimicrobial agent gives a low MIC [13].

The toxic nature of some species of Ficus may be attributed to the presence of certain phytochemicals like cyanogenic glycosides and alkaloids in the leaves of the plant, and apart from this, some alkaloids even if they occur in trace quantity in the plant, may be very useful, as some correct renal disorder [14].

1.2. The genus ficus

The generic name Ficus is derived from a Latin word "fikus" which refers to both the fig tree and the fruits [15]. This genus had been found to be having their distribution in warm and temperate zones. The so-called common fig (Ficus carica) is a temperate species from the Middle East and Eastern Europe –mostly Ukraine, which had been widely cultivated from ancient times for its fruits. Figs occupy a wide variety of ecological niches. Ficus carica is a small deciduous tree whose fingered fig leaf is well known in art and iconography [16].

Figs with different plant habits have undergone adaptive variation in different biographic regions often leading to very high levels of alpha diversity. Although identifying many fig species can be difficult, figs as a group is one relatively very easy to recognize. Often the presence of aerial roots of the plant will give them away. Their fruits are also enclosed inflorescence, sometimes referred to as a syconium. Figs have a unique wasp, known as fig wasps that enter these closed inflorescences to both pollinate and lay their own eggs and has been a constant source of inspiration and wonder to biologist. All figs posses sap (latex), some in copious quantities [16].

1.3. Ficus abutilifolia miq. Description

Ficus abutilifolia Miq. can be classified taxonomically as follows:

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Rosidae
Order	:	Rosales
Family	:	Moraceae
Genus	:	Ficus
Species	:	abutilifolia

Distribution: African continent; South Africa, Mozambique, Nigeria, Sudan, Ivory Coast, Ethiopia, Guinea, and Somalia.

Habitat: Savannah woodlands, Bush veldt, on rocks, hard surface and in rain forest.

Leaves: broadly ovate; almost round at the tip.

Flowers: borne inside the plant and occurs between August and February.

Fruits: enclosed inflorescence (syconium) with very tiny seeds occurring on stems or leaf axils.

Stem: whitish in colour and twisted, up to 50m high.

Although F. abutilifolia is a fairly distinct species it does not resemble F. tettensis and F. glumosa which are readily distinguished by their smaller hairy leaves and hairy branch lets [16].

Figure I: Pictorial view of F. abutilifolia in its natural habitat

2. Materials and methods

2.1. Ethno medicinal survey of plant

Common plants used by the indigenous people of Ogurugu in the South Eastern Nigeria for medicinal purposes were catalogued base on collections during field trips and visits to traditional medicine practitioners. Various questions were posed to the herbalists and traditional medicine practitioners on how the drugs were used and recorded both in paper and tape recorder. Some of these plants grow wild while others were cultivated. The local names (Igbo) of these plants were collected and common (English) names were checked for easy identification. Ficus abutilifolia Miq was described as the preferred plant used for treating most common illnesses (such as typhoid fever, chronic dysentery, infections from sex, malaria and infertility) in the community. The over exploitation of the wild population of this plant, and also lack of conservation for the cultivated ones were major problems threatening the plant's survival.

2.2. Collection and preparation of plant materials

The whole plant was collected (leaves, stem barks and roots) fresh, from a savannah woodland at Dumbi village 12km from Zaria and identified at the herbarium unit of the department of Biological sciences, A.B.U Zaria. A voucher number of 900742 was deposited for the plant. Further confirmation was made at IITA, Ibadan. The plant was air dried for six days, and pounded using local mortar into fine powders.

2.3. Extraction of plant materials

About 200 gram of each of the powdered parts was weighed using electronic scale balance into three separating funnels respectively. 500 mls of absolute ethanol containing 300 mls of water was poured into the samples using cold maceration techniques. The set up were left to stand for 48 hours to enable the constituents dissolve thoroughly in ethanol. The filtrates were collected into a beaker and subsequently transferred into evaporating dish in order to concentrate them. The extracts were weighed and percentage yields for each extract was calculated and then stored in desiccators for further use.

Percentage yield = weight of extract x 100
Weight of ground plant

2.4. Physico-chemical evaluation of powdered drugs

Using the methods as described by [17], the following physical constants were determined: moisture content, Ash value, and acid insoluble ash value, water soluble ash value, and alcohol and water extractive values. Five readings were taken for each.

2.5. Moisture content determination by loss on drying method

3 grams of powdered plant was accurately weighed in tarred silica dish. It was then heated for one hour at 105 0C, cooled in desiccators and weighed. The procedure was repeated until there was no further loss in weight that is, until a constant weight was obtained. The percentage moisture content was calculated with reference to the initial weight of the powdered drug and value obtained was compared with the value in official books.

2.6. Determination of ash value

2 grams of the powdered drugs were weighed accurately into nickel crucibles for each part. The contents were heated gently until they were moisture free and the completely charred, at temperature, not more than 450 0C. The heating was continued until all the carbon was removed. The contents were cooled and weighed until constant weight was obtained for each drug. Total ash values were then calculated with reference to the initial mass of drugs.

2.7. Determination of acid insoluble ash value

The total ash produced from the above in the silica dish were transferred to beaker containing 25ml of dilute HCl and boiled for five minutes. The insoluble matters were collected on ash less filter papers. The contents in the beakers were washed in hot water, and the washings were passed through the filter paper. The washing was continued until the beaker was free from acid. The residue and the filter paper were dried gently and ignited in

tarred crucibles. The crucibles were cooled and weighed. Acid insoluble ash values were then calculated with reference to the initial weight of the drugs.

2.8. Determination of water soluble ash value of powdered drugs

The total ash produced was boiled in 25 ml of water for 5 min. The insoluble matters were collected as in section above. The same procedures above were repeated. The difference between the insoluble matter and the weight of the ash were taken as the water soluble ash value [18].

2.9. Determination of alcohol and water extractive values

2.9.1. Determination of alcohol extractive value

5 gram of powered drugs were shaken with 95% ethanol 50cm3 in 250ml Stoppard flasks for 6 hours using mechanical shaker and allowed to stand for 18 hours. They were filtered immediately. 20ml of the filtrates were transferred into tarred evaporating dish whose weight and that of the flat bottom were already known. Using hot plate, they were evaporated to dryness and dried to constant weight at 1050C in an oven and the final weight noted. The alcohol extractive values were calculated with reference to the initial weight of powdered drugs.

2.9.2. Determination of water extractive value

The above procedure in section was repeated using chloroform – water (0.25% V/V chloroform in pure water), in place of ethanol as the extracting solvent. Values were calculated with reference to the initial weight of drugs.

2.9.3. Phytochemical screening of ethanol extracts

Following standard procedures [19], [20], preliminary phytochemical screening of the extracts were done to test for the presence of saponins, flavonoids, alkaloids, cardiac glycosides, tannins and anthraquinones.

2.9.4. Determination of saponins

Two tests were carried out (frothing test and haemolysis test).

2.9.5. Frothing test

Small quantity of the extracts were each dissolved in 10ml of distilled water and shaken vigorously for thirty seconds and allowed to stand for about thirty minutes. Foam which persisted for more than thirty minutes indicate saponins.

2.9.6. Haemolysis test

The extracts were each put in two test tubes containing 2ml of 1.8% aqueous NaCl solution. 2ml of the extract was put into one test tube and other in 2ml of distilled water. 3 drops of mice blood was added to each test tube and observed for thirty minutes. Occurrence of haemolysis in the tube containing the extract indicates the presence of saponins.

2.10. Determination of flavonoids

2.10.1. Sodium hydroxide test (NAOH):

0.5 g of the extracts was added to 2 drops of aqueous NaOH solution. Yellow colouration was taken for presence of flavonoids.

2.10.2. Shinoda's test

This was carried out by heating the extract in 2ml of 50% methanol and then adding metallic magnesium plus four drops of concentrated HCl solution. Orange colour showed the presence of flavonoids.

2.11. Determination of tannins

2.11.1. Ferric chloride test

0.5g of the extracts were each stirred with 10ml of distilled water and then filtered. 2 drops of 5% $FeCl_3$ was then added. A green ppt. was taken for the presence of condensed tannins.

2.11.2. Lead acetate test:

0.5g of extract solutions were added to lead acetate solution, a brown colour was taken for the presence of tannins.

2.11.3. Goldbeater's skin test

A small piece of Goldbeater's skin was soaked in 2% HCl and rinsed with distilled water. It was then placed in the extract solutions for 5min and washed with distilled water. The skin was then transferred to a 1% solution of ferrous sulphate ($FeSO_4$). Presence of black colour on the skin was taken to confirm the presence of tannins [20].

2.12. Determination of alkaloids

2.12.1. Dragendorff's test

Few drops of Dragendorff's reagent were added to 0.5g of the extracts. A rose –red ppt. was taken to indicate the presence of alkaloids.

2.12.2. Meyer's test

Few drops of this reagent were added to sample extract in test tube, cloudy or creamy precipitate indicates alkaloids.

2.12.3. Wagner's test

Few drops of this reagent were added to sample extract in test tube, whitish precipitate indicates alkaloids.

2.12.4. Picric acid test

Few drops of 1% picric acid solution were added to the extract, yellow coloured solution indicates alkaloids.

2.12. 5. Tannic acid test

Few drops of tannic acid solution were added to the extract, black precipitate indicates alkaloids.

2.13. Determination of anthraquinones

2.13.1. Borntrager's test (for free anthracenes)

0.5g of the extracts was shaken with 10ml of chloroform and filtered. 5ml of 10% ammonia solution was added to the filtrate and stirred. The presence of pink-red colour was taken for the presence of free anthraquinones.

2.13.2. Modified borntrager's test (for combined anthracenes):

Combined anthracenes were also tested for by boiling the extracts with 5ml of 10% HCl solution for 3min, this will hydrolyse the glycosides to yield aglycones which are soluble in hot water only. The solution was filtered hot, and filtrates allowed cooling. The filtrates were the extracted with 5ml of benzene. The benzene layer was filtered off and shaken gently with half its volume with 10% ammonia solution. Cherry-red colour indicates combined anthracenes [20].

2.13.3. Chromatographic resolution for ethanol extracts of leaves, stembarks and roots of ficus abutilifolia using TLC techniques.

Chromatographic analysis of the ethanol extracts of leaves, stembarks and roots was done using standard method [21]. TLC plates 20cm x 20cm already prepared by the manufacturer were used. The solvent system was developed using various trials to obtain a good solvent to which is capable of moving the spots and separate the components very clearly. Methanol -chloroform (4:1) presented the best separation of the spots in one and half hour time. Extracts were spotted separately using a micropipette on the start-line, not too much to avoid tailing. Development of the plates was done by placing the plates in three chromatographic tanks for each extracts, containing the solvents methanol/chloroform (4:1) which represent the mobile phase. The set-up was allowed to

stand for an hour and 30 min for complete development. The plates were taken out of the tank and the solvent front14.80cm was marked using pencil. Visualization of the compounds was done in iodine tank. The spots on the TLC plates were circled to have a permanent record on how far the compounds moved on the plate. A sketch of the developed plates was drawn in laboratory note, and also photo graphic shots were taken. Analysis of the components was first done by calculating the retardation factor (Rf) values of the spots, and thereafter the spots were sprayed with various detecting reagents to know the class of compounds they contained. Rf values were calculated using the relationship:

Rf = Distance travelled by spot (no unit)
Distance of solvent front
Where,
Distance travelled by spot = distance from start line to centre of spot
Distance of solvent front = distance from start line to solvent front (14.8cm).

3. Results

3.1. Physical constants of the powdered drugs

Physical evaluation of the powdered drugs showed that the leaves had the highest percentage moisture constant 4.10±0.08 while the roots had the lowest 2.60±0.05.The highest percentage ash value was also shown in the leaves, and the root had the lowest 3.04±0.09. However, the acid insoluble ash value and water soluble ash value were 6.60±0.25 and 6.9±0.49 in the stem barks and the highest. Water extractive values were greater than alcohol extractive values with the leaves having the highest values of 6.3.2±0.50 and 2.50±0.15 respectively (Table 1).

Table 1
Physical Constants of Ficus abutilifolia Miq.

Parameters **Mean + SEM**	**Leaves**	**Stembarks**	**Roots**
Moisture content	4.10 ± 0.08	3.10 ± 0.06	2.60 ± 0.05
Ash value	12.47 ± 0.20	6.22 ± 0.14	3.04 ± 0.09
Acid insoluble ash	1.34 ± 0.17	6.60 ± 0.25	3.50 ± 0.22
Water soluble ash	5.20 ± 0.46	6.90 ± 0.49	4.40 ± 0.44
Alcohol extractive Value	2.50 ± 0.15	1.70 ± 0.06	0.50 ± 0.12
Water extractive Value	6.32 ± 0.50	2.91 ± 0.08	1.51 ± 0.06

Note: Results are means of + SE of duplicate estimations at n = 5.

3.2. Phytochemical screening and TLC resolution of extracts

Preliminary screening of the leaves extract which produced 21% (Table 2) yield revealed the presence of various classes of glycosides (saponins, flavonoids, and anthraquinones) as well as tannins and alkaloids (Table3.). Further confirmation using TLC techniques in methanol -chloroform (4:1) as solvent – system inferred this (Table 4. and Figure ii). The percentage yields of the stem and the root were 10.0 % and 4.0 % respectively (Table 2).

Table 2
Percentage Yields of Extracts of Ficus abutilifolia.

Extracts	**Yields (%)**
Leaves	21.0
Stembarks	10.0
Roots	4.0

Note: % Yield of extracts =Wt. of extract x 100
Wt. of ground plant.

Table 3
Phytochemical Screening of Ethanol Extracts of Ficus abutilifolia.

Constituent	Test	Observation	Inference		
			L	S	R
Saponins	Frothing test	Frothing occur more than 30 min	+	+	-
	Haemolysis test	Haemolysis in tube	+	+	-
	NaOH test	Yellow colour	+	+	-
Flavonoids	Shinoda's test	Orange colour	+	+	-
	Ferric chloride test	Green colour	+	+	+
Tannins	Lead acetate test	Brown ppt.	+	+	+
	Goldbeater's skin	Black colour	+	+	+
	Dragendorff's reagent	Rose-red ppt.	+	-	-
Alkaloids	Wagner's reagent	White ppt.	+	-	-
	Mayer's reagent	Cloudy ppt.	+	-	-
	Picric acid test	Yellow colour	+	-	-
	Tannic acid	Black cloudy ppt.	+	-	-
	Borntrager's test	Violet colour	+	-	-
Anthraquinones	Modified Borntrager's test	Cherry-red colour	+	-	-

Key: +=present, - = absent = leaf extract, S= stembarks extract, R= root extract.

Table 4
Phytochemical Screening and Chromatographic Resolution of Pure Ethanol Extracts.

Class of compounds	RF values of spots		
	Leaves	Stembarks	Roots
Alkaloids	0.53	-	-
Flavonoids	0.74	0.84	-
Saponins	0.81	0.65	-
Tannins	0.85	0.90	0.86
Anthraquinones	0.91	-	-

Note: - (not applicable), + (present), solvent system (methanol-chloroform; 4:1).

Spraying reagents used are: Alkaloids; Dragendorff's reagent, Flavonoids; aqueous NaOH solution, Saponins; Bial's reagent (0.9% $FeCl_3$ + Orcinol), Tannins; $FeCl_3$ solution, Anthraquinones; aqueous ammonia solution.

No alkaloids and anthraquinones were detected in the stem whereas only flavonoids and tannins (phenolic compounds) were noticed in the root extracts. However, chromatographic resolution (TLC) using the same solvent system separated only one spot in the root extract (Figure ii a and b). There were significance difference in the Rf values of spots calculated (0.53-0.91).

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a.

b.

Fig. ii. Photographic shots from chromatograms; a; final analysis of TLC plate by spraying with detecting reagents; Bial's reagent(saponins), Dragendorff's (alkaloids), aqueous ammonia(anthraquinones),NaOH(flavonoids),FeCl3(tannins),methanol-chloroform 4:1; b; TLC of leaves, stembarks and root extracts.

4. Discussions

Excess moisture in drug suggests not only that the buyer could be paying a high price for unwanted water but also that the drug has been prepared incorrectly or subsequent to preparation, has been wrongly stored. This can also lead to break down of important constituents due to enzyme activity and the other microbial attacks. The percentage moisture was found to be 4.10±0.08 w/w (for leaf), 3.10±0.06 w/w (for stem) and 2.60±0.05 w/w (for root). These values are within the range by Edward and Tyler [21] who suggested that the value should not exceed 10%w/w.

Values of physical constants evaluated fall within an acceptable range with water extractive values being greater than alcohol extractive values. This is due to the fact that water extracts more constituents than alcohol but ethanol had being reported to extract most of the plants active constituents [22].The fact that the water extractive value was greater than the water extractive value suggest the presence of highly polar constituents in the plant more than non polar and semi polar ones.

In the phytochemical screening of the plants, the leaves which have percentage yield 21% showed the presence of saponins, flavonoids, anthraquinones as well as tannins and alkaloids. Further confirmation using TLC techniques and methanol-chloroform (8:2) as solvent system also confirmed this when the spots were sprayed using appropriate reagents (Figure ii; a and b). However, only two constituents (flavonoids and tannins) were noticed in the root crude extracts. TLC resolution resolved only one spot in the root TLC plate which was later found to contain tannins and thus suggested that no glycosides may be present in the root extract. The stem extract did not show any presence of alkaloids and anthraquinones by TLC confirmation. These chemical constituents contributed to the biological activities of the plant. There are no doubts therefore, that these secondary metabolites identified in the plant material used in this study were responsible for the antibacterial activity exhibited by the leaf of the plant [23].

5. Conclusion

In the present era, plant and herb resources are abundant, but these resources are dwindling fast due to onward march to civilization [24]. Although a significant number of studies have been used to obtain purified plant chemicals, very few screening programmes have been initiated on crude plant materials. It has been widely observed and accepted that, the medicinal values of plants lie in the bioactive phytochemicals present in them[25] .Many researches had been carried out on malaria fever with little or none was done on typhoid fever yet, over millions of people died every year from typhoid fever disease. Therefore, the leaves extract of F. abutilifolia present an alternative source through which the non resistant drug for the treatment of the disease could be

discovered. The pure extracts of the plant serve as a quick therapy for the treatment of typhoid fever in endemic areas of Nigeria especially the Southern east and Northern Nigeria, as had been shown by some authors. The study showed that ethanol extracts of leaf of F. abutilifolia contains various phytochemicals which are responsible for the use of the plant in ethno medicinal prescriptions.

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