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QUALITATIVE AND QUANTITATIVE ANALYSIS OF TWO ANTI-TYPHOID PLANTS

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Abstract:

This work describes methods for performing qualitative and quantitative analysis onEntadaabbysinica and Ficusthonningii. The plants are used traditionally in the treatment of thypoid fever. Air-dried and powdered plant materials were tested for phytochemicals (phlobatannins, flavonoids, alkaloids, saponins, tannins, terpenoids, steroids, glycoside, and anthraquinones). Result of the phytochemical screening showed that Flavonoids and Steroids are present in both the plants. Terpenoids is present in Ficusthonningii, Tannins is present in Entadaabbysinica, Anthraquinones is present in Entadaabbysinica. Saponnins is present inFicusthonningii. While Glycosides, Phlobatannin, Alkaloid and Anthraquinones were absent in both the plants. The importance of the distribution of these chemical constituents were discussed. This study provides scientific methods to investigate the active components of the above mentioned plants.

1.0 Introduction

In a continued attempt to improve the quality of life, man has used plants as source of food, clothing, medicine, and for seeking relief from hardship of life. Some plants are known as medicine because they contain active substances. that cause certain reaction from relenting to the care of disease on the humans (Junior *et al.*, 1994). Traditionally, plants are used as source of treatment of diseases in different parts of the world (Eisenberg *et al.*, 1993) and their use contributes significantly to primary health care delivery (Holetz*et al.*, 2002). Plants contain compounds which have been demonstrated to be bioactive. Many of these compounds are secondary metabolites, which includes, Alkaloids, Tannins, Saponnins, Flavanoids, Glycosides, Essential oil, components etc. The qualitative and quantitative analysis of plants for bioactive principle may include the following; extraction of the plants

materials, separation and isolation of constituents of the biosynthetic pathway to a particular compound (Trease*et al*, 1992). In this study, the plants: *Ficusthonningi*and *Entadaabbysinica*, were investigated for their medicinal properties and the secondary metabolite they contain.

1.1 TYPOID FEVER

Thypoid fever also known simply as typhoid (Medline Plus Encyclopedia typhoid fever), is a common worldwide bacterial disease transmitted by the ingestion of food or water contaminated with the faece of an infected person, which contain the bacterium *Salmonella enteric*.

The disease has received various names, such as gastric fever, abdominal typhus, infantile remittent fever, slow fever, nervous fever and phytogenic fever. The name *typhoid* means "resembling typhus" and comes from the neuropsychiatric symptoms common to typhoid and typhus. (Oxford English Dictionary 2011) Despite this similarity of their name typhoid fever and typhus are distinct diseases and are caused by different species of bacteria (Cunha, 2014).

1.1.1 TYPHOID FEVER FACT

- Typhoid fever usually is caused by *Salmonella typhi* bacterium
- Typhoid fever is contracted by the ingestion of contaminated food or water
- Diagnosis of typhoid fever is made when the Salmonella bacterium is detected with a stool culture
- Typhoid fever is treated with antibiotics
- Approximately 3-5% of patients become carriers of the bacteria after the acute illness.

1.1.2 SIGNS AND SYMPTOMS OF TYPHOID FEVER

- Poor appetite
- Abdominal pain
- Headaches

- Aches and pains
- Fever often up to 40^{o}C (104^{o}F)
- Lethargy(usually if untreated)
- Intestinal bleeding or perforation (after two to three weeks of the disease)
- Diarrhea or constipation.

1.1.3 RESISTANCE OF TYPHOID FEVER TO ANTIBIOTICS

Resistance to antibiotics like ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, and streptomycin is now common, and these agents have not been used as first–line treatment for almost twenty years Typhoid that is resistant to these agents is known as multidrug-resistant typhoid (MDR typhoid).

Ciprofloxacin resistance is an increasing problem, especially in the Indian Subcontinent and Southeast Asia. Many countries are therefore moving away from using ciprofloxacin as the first line for treating suspected typhoid originating in South America, India, Pakistan, Bangladesh, Thailand, or Vietnam. For these patients, the recommended first line treatment is ceftriaxone. It has also been suggested that azithromycin is better at treating typhoid in resistant populations than both fluoroquinolone drugs and ceftriaxone. (Effa*et al.*, 2011)

1.2 Entadaabyssinica

Species identity

Taxonomy

Current name: *Entadaabyssinica*

Family: FabaceaeMimosoideae

Common names

(English): tree entanda

(Hausa): “tawatsa”

(Igbo): angaramiri

(Yoruba): gbengbe

1.2.1 ECOLOGY AND DISTRIBUTION

Natural Habitat

Entadaabyssinica is an understorey forest species found in association with *Albiziazygia*. It extends from Guinea to Cameroon and is also widespread in central and eastern tropical Africa. It is usually found in a savannah habitat.

Geographic distribution

Native : Angola, Benin, Cameroon, Congo, Cote d'Ivoire, Democratic Republic of Congo, Equatorial Guinea, Eritrea, Ethiopia, Gabon, Ghana, Kenya, Liberia, Mozambique, Nigeria, Sierra Leone, Sudan, Tanzania, Togo, Uganda, Zambia

Biophysical limits

Altitude: 1300-2050 m Soil type: *Entadaabyssinica* prefers sandy loam soils.

1.3 *FicusThonningii*

Taxonomy

Current name: *Ficusthonningii*

Family: Moraceae

Common names

(Africans): gewonewurgvy

(English): bark-cloth fig, common wild fig, strangler fig

(Fula): bikeshi

(Hausa): chediya

(Yoruba): odan

1.3.1 ECOLOGY AND DISTRIBUTION

Natural Habitat

The species is widely distributed in upland forest, open grassland, riverine androcky areas and sometimes in savannah. It occurs naturally from the Democratic Republic of Congo and Tanzania in the north to the Eastern Cape in South Africa. Trees are relatively drought resistant.

Geographic distribution

Native : Angola, Benin, Botswana, Burkina Faso, Cameroon, Central African Republic, Chad, Congo, Cote d'Ivoire, Democratic Republic of Congo, Djibouti, Eritrea, Ethiopia, Ghana, Guinea-Bissau, Kenya, Madagascar, Malawi, Mozambique, Namibia, Nigeria, Rwanda, Senegal, Sierra Leone, South Africa, Sudan, Swaziland, Tanzania, Togo, Uganda, Zambia, Zimbabwe

Biophysical limits

Altitude: 1000-2500 m, Mean annual rainfall: 750-2000 mm, Soil type: Occurs on a wide variety of soils but favors light, deep and well-drained soils with neutral to acidic reaction and humus-rich or deep loamy soil.

1.4 JUSTIFICATION FOR RESEARCH

Investigation is constantly going on for bioactive principles in plants to obtain drugs that will cure problems associated with microorganisms that are resistant to drugs, produce drugs with low side effects and toxicity and cheaper to produce. In northern Nigeria and even in some tropical Africa (*Ficusthoningii,* and *Entadaabbysinica*) are used for the treatments of many ailments in traditional medicine. There is need for a scientific study to justify some of the curative claims associated with its use in traditional medicines.

2.0 MATERIAL AND METHODS

2.1 COLLECTION AND IDENTIFICATION OF PLANTS

The plants, *Entidaabbysinica*, *Ficusthonningii*were collected from Matazu local government area of Katsina State, Nigeria. The samples were submitted and identified by Herbarium Curator, Department of Botany Bayero University Kano. The leaves were thoroughly washed with water and air-dried at room temperature. It was then ground into fine powdered form and was used for further investigation.

2.2.0 QUALITATIVE ANLYSIS

2.2.1 TEST FOR TANNINS

0.5 g of the crude powder was stirred with 10ml of distilled water. This was filtered and ferric chloride reagent was added to the filtrate, a blue-black precipitate was taken as evidence for the presence of tannin (Harborne, 1973).

2.2.2 TEST FOR TERPENOIDS

0.5 g of crude powder was dissolve in 5ml of methanol.2 ml of the extract was treated with 1 ml of 2,4-dinitrophenyl hydrazine dissolve in 100 ml of 2M HCL. A yellow-orange coloration was observed as an indication of terpenoids (Kolawale*et al.*, 2006).

2.2.3 TEST FOR SAPONNINS

0.5 g of crude powder was shaken with water in a test tube and it was warmed in a water bath and the persistent of froth indicates the presence of saponnins (Smolenski *et al.*, 1974)

2.2.4 TEST FOR FLAVONOIDS

A portion of crude powder was heated with 10ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution and observed a yellow coloration (Edeoga *et al.*, 2005).

2.2.5 TEST FOR ANTHRAQUINONES

0.5 g of crude powder was shaken with 10 ml of benzene and was filtered 0.5 ml of 10% ammonia solution was added to the filtrate and the mixture was shaken well and the presence

of the violet color in the layer phase indicated the presence of the anthraquinones (Trease *et al.,* 1996).

2.2.6 TEST FOR STEROIDS

0.5 g of crude powder was dissolved in 5 ml of methanol. 1 ml of the extract was treated with 0.5 ml of acetic acid anhydride and cooled in ice. This was mixed with 0.5 ml of chloroform and 1 ml of concentrated sulphuric acid was then added carefully by means of pippete (Kolawale *et al.,* 2006). At the seperation level of the two liquids, a reddish-brown ring was formed, as indication of the presence of steroids.

2.2.7 TEST FOR GLYCOSIDES

0.5 g of crude powder was dissolved in 5 ml of methanol. 10 ml of 50% HCl was added to 2 ml of methanolic extract in a test tube. The mixture was heated in a boiling water bath for 30min. 5 ml of Fehling`s solution was added and the mixture was boiled for 5 min to observe a brick red precipitate as an indication for the presence of glycosides (Harborne, 1973).

2.2.8 TEST FOR ALKALOIDS

0.5 g of crude powder was defatted with 5% ethyl ether for 15min. The defatted sample was extracted for 20min with 5ml of aqueous HCl on boiling water bath. The resulting mixture was centrifuged for 10min at 3000rmp 1ml of the filtrate was treated with few drops of Meyers reagent and the turbidity was observed (Harborne *et al.,* 1973).

2.2.9 TEST FOR PHLOBATANNINS

An aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid (HCl) to observe the deposition of red precipitate (Edeoga *et al.,* 2005).

2.3.0 QUANTITATIVE ANALYSIS

2.3.1 FLAVONOID DETERMINATION

10 g of each crude powder was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper no. 42 (125 m). The filtrate was later transfer into crucible and evaporated into dryness and weighed to a constant weight (Boham *et al.,* 1974)

2.3.2 TERPENOID EXTRACTION FOR THIN LAYER CHROMATOGRAPHIC (TLC) ANALYSIS.

50 g of the powdered leaves were extracted with solvent combination of methanol and water (4:1) at room temperature for 24h.The solution was filtered using whatman filter paper no. 1 and the filtrate was then evaporated to 1/10 volume at 40^{o}C. The evaporated filtrate was then acidified with 2M sulphuric acid (pH 0.89) followed by chloroform extraction (three times the volume). Stirred and allowed to stand in a separating funnel. Out of two layers formed, the non-aqueous layer was taken and evaporated to dryness. The dried extract contained components like terpenoids which were further used for thin layer chromatography analysis (Harborne*et al.*, 1984).

2.3.3 THIN LAYER CHROMATOGRAPHY

Glass plates (20x20 cm) were coated (0.5 mm) with silica gel (Qualigen fine chemicals) and sample applied correspond to approximately 5mg/ml of each extract dissolved in methanol. Chromatography was performed in 100% chloroform according to the method described by Harborne *et al.*, (1984). The spot were visualized using concentrated sulphuric acid as a spray reagent followed by heating of plate at 100^{o}C for 10min. The spot were identified base on the color produced on reacting with a spray reagent.

3.0 RESULT AND DISCUSSION

3.1 QUALITATIVE ANALYSIS OF THE TWO ANTI-TYPHOID PLANTS

Present study carried out on the plant samples revealed the present of secondary metabolites (Flavonoids and Steroids) in both plants. Terpenoids is present in *Ficusthonningii* but absent in *Entadaabbysinica*, Tannins is present in *Entadaabbysinica but absent in Ficusthonningii*,Anthraquinones, Glycosides, Phlobotannins, Alkaloides were absent in both the plants. *S*aponnins is present in *Ficusthonningii*but absent in*Entadaabbysinica.*

Table1. RESULT OF QUALITATIVE ANALYSIS OF TWO ANTI-TYPHOID PLANTS

TEST	*E. abbysinica*	*F. thonningii*
Phloba tannins	-	-
Flavonoids	+	+
Alkaloids	-	-
Saponins	-	+
Tannins	+	-
Terpenoids	-	+
Glycosides	-	-
Anthraqquinones	-	-
Steroids	+	+

Key: The positive sign (+) indicates the presence of constituents and the negative sign (-) indicate the absence of constituents. *E. abbysinica is Entidaabbysinica and F. thonningii is Ficusthonningii.*

3.2 QUANTITATIVE ANALYSIS OF TWO ANTI-TYPOID PLANTS

Quantitative estimation of the constituents in the studied medicinal plants is summarized in Table 2. *Ficusthonningii* contained higher contents of Flavonoids, (2.897g).

3.3 THIN LAYER CHROMATOGRAPHY OF EXTRACTS OF THE TWO PLANTS

Thin layer chromatography technique was used to separate different monoterpenoids. With chloroform (100%), it revealed spot in *Ficusthonningii and Entadaabbysinica*.

Table 2: RESULT OF QUANTITATIVE ANALYSIS OF TWO ANTI-TYPOID PLANTS

Plants	Initial wt of crucible in gram(g)	Final wt of crucible in gram(g)	Flavonoids contents in gram(g)
F. thonningii	22.783	25.680	2.897
E. abbysinica	22.783	23.941	1.158

Key: *E. abbysinica is Entidaabbysinica and F. thonningii is Ficusthonningii.*

3.4 DISCUSSIONS

In this paper, the results of the investigations showed that the two plant materials possessed almost all the important secondry metabolites.*Ficusthunningii*showed positive result for all the constituents analyzed, except for five i.e, Phlobatannin, alkaloids, Tannins, glycocides, anthraquinones as shown in (Table.1). Saponnins and Flavonoids have been linked or suggested to be involved with antibacterial and anti-viral activity while Flavonoids are thought to be responsible for antidiarrheal activity (Enzo, 2007). Investigation of the mode action indicate that Flavonoids increase colonic water and electrolyte re-absorption and other phytochemicals act by inhabiting intestinal mobility, while some components have been shown to inhabite particular enteropathogens (Enzo, 2007). Steriods in modern clinical studies have supported their role as anti-inflammatory and analgesic agents. These could explain the role of *Ficusthunningii* as an anti-microbial agent (Pithayanukul*et al.*, 2007).

In the method of separation by TLC of monoterpenes from *Ficusthonningii* using chloroform (100%) revealed that spot 1 was yellow suggesting that spot 1 could be limonene or alpha-Pinene (Harborne, 1984). Monoterpenes are typically found and widely distributed in almost all the plants.

4.0 CONCLUSION

In conclusion, the plants studied can be a potential source of useful medicinally active constituent. The procedure is simple and the resources required for the experiment are inexpensive. The experimental protocol, are straightforward, includes procedures that must be performed carefully to obtain good results.

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