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# EFFECT OF METHANOLIC EXTRACTS OF *TELFAIRIA OCCIDENTALIS* AERIAL PARTS ON *IN VITRO* DISSOLUTION OF FOLIC ACID TABLETS

**Musa, A. U.[1]*, Oloyede, R. B.[1], Haruna, A.[1], Salisu, A.[2]**

[1]Department of Pharmaceutical and Medicinal Chemistry, Kaduna State University, Kaduna, Nigeria

[2]Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria, Nigeria

*Corresponding author: abubakarmusausman1@gmail.com

## ABSTRACT

**Introduction**: There is a high prevalence in the use of herbs for the treatment of several diseases in developing countries due to their accessibility and affordability, thus making it important to evaluate the effect of herbs on conventional drugs.

**Aim**: The aim of this study was to evaluate the effect of methanolic extracts of *Telfairia occidentalis* aerial parts on *in vitro* dissolution of folic acid Tablets.

**Methods**: Two simple UV spectrometric methods were adopted and validated for the assay and dissolution tests of folic acid Tablets; taking wavelengths of maximum absorption of folic acid in phosphate buffer (pH 9) and distilled water as 282.5 nm and 377.2 nm respectively.

**Results**: The assay result of obtained for percentage recovery of folic acid Tablets was 78.86 %, which is far below the BP (2009) acceptable range of 90- 110 %. The control dissolution rate of folic acid in distilled water showed a steady increase in dissolution rate, with no decline within 15 minutes while the test dissolution rate of the drug in aqueous solution of the methanolic extract of *Telfairia occidentalis* showed a rapid increase and then sharp decline in dissolution rate within the same time frame.

**Conclusion and Recommendation:** The dissolution pattern of folic acid Tablets in the presence of *Telfairia occidentalis* was observed to be erratic and different from that of folic acid alone. Therefore, it is recommended that *in vivo* pharmacokinetic studies should be carried out in order to quantify and establish this drug-herb interaction.

**Keywords:** Folic acid, *Telfairia occidentalis*, dissolution rate, UV spectroscopy

## INTRODUCTION

Folic acid is the parent compound for a large group of compounds known as folates which humans do not synthesize, and as such require pre-formed folates as vitamin supplements [1]. It is a water-soluble vitamin that has many natural sources and human daily requirements can be achieved by consumption of a balanced diet [2]. It is used in the prophylaxis of megaloblastic anaemia, neural tube birth defects and used as an adjunct in the management of some cardiovascular disease, dementia, cognitive function alterations, osteoporosis and several types of cancer [3]. Anaemic episodes in humans are very common with about 24.8 % of cases reported yearly, and this could be as result of side effect of drugs or commonly due to symptoms of underlying diseases [4].

The leaves of *Telfairia occidentalis*, commonly known as fluted pumpkin leaves is an edible vegetable plant that belongs to the family Cucurbitaceae. It is a tropical vine grown mainly in West Africa for its vegetable because of its high nutritive value. It has a protein content of 21 % and is rich in calcium, phosphorus and Iron. It is known as Ubong

Archives of Pharmaceutical Sciences and Biotechnology Journal

Volume 1 Issue 1 June, 2021 Page 68 - 75 ISSN: 2971_611X

APSB-07

APSBJournal

by the Ibibio, Ugu by the Igbo and Iroko by the Yoruba in Nigeria [5]. It is widely consumed as food and has been reported to be used in the management of anaemia in ethno-medicine, and it can be inferred that its ability to combat anaemia is due its high mineral, vitamin and protein contents [6].

The use of herbal products as alternative and/or complementary medicine is globally popular especially in Africa despite the lack of sufficient information on the safety of herbal products due to affordability and accessibility [7]. However, the concurrent consumption of herbal products and conventional drugs is of serious concern because of herb-drug interaction (HDI)[8]. Most HDIs are well established through human studies, however, due to the rigorous and time-consuming nature of *in vivo* studies, most HDIs are initially demonstrated through *in vitro* studies [9]. Initial assessment of the *in vitro* interactions of HDIs has important implications for predicting the likelihood of natural product-drug interactions or a possible mechanism of interaction [10]. Due to the widely documented cases of HDIs there is a possibility of an interaction occurring between folic acid and *T. occidentalis*, hence the need for this study. The aim of this study is to determine the effect of methanolic extract of *T. occidentalis* on the *in vitro* dissolution of folic acid, which will serve as a preliminary study for further pharmacokinetic studies.

## MATERIALS AND METHODS

### Equipment

UV/VIS spectrophotometer (UV-752, PEC MEDICAL USA), Analytical balance (AR223CN, OHAUS Corporation Pine Brook, NJ, USA), Dissolution Test Apparatus (Hindustan Apparatus Management company, India), Rotary shaker M/C SR. No RS 693 (Ambala Cantt Ambala-Cantt Haryana, India), pH meter HI9313-5 (Hanna Instruments, U.S.) and Melting Point Apparatus (Sigma Scientific glass Company, India).

### Reagents

Methanol, distilled water and phosphate buffer 0.1M 1000 ml pH 9 ($Na_2HPO_4/NaH_2PHO_4$)

### Sampling of folic acid standard powder and Tablets

Folic acid standard powder was purchased from Leyan, China. Folic acid 5 mg Tablets were purchased from a repuTable pharmacy in Kaduna metropolis in November 2019 and information about the batch number, manufacturing date and expiry date for each brand was noted down.

### Collection of plant material

The aerial parts of *T. occidentalis* were collected in Kaduna metropolis in October 2019. The plant was authenticated and identified (voucher number: 1751) in the Department of Biological Science, Kaduna State University, Kaduna.

### Herbal preparation

The aerial parts of fresh *T. occidentalis* were air dried under room temperature in the research laboratory of the Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Kaduna State University and pulverized into coarse powder. The pulverized plant material (200 g) was extracted with 50 % methanol for 72 hours by maceration.

Archives of Pharmaceutical Sciences and Biotechnology Journal

Volume 1 Issue 1 June, 2021 Page 68 - 75 ISSN: 2971_611X
APSB-07

APSBJournal

**Melting point determination of folic acid reference powder**

Little quantity of folic acid reference powder was taken using capillary tube and introduced into the melting point apparatus and the temperature at which it melted was taken.

**Infrared (FTIR) analysis of folic acid reference powder and Tablets**

The FTIR spectrometer was allowed to warm up and cleaned with acetone. Small amount of folic acid reference standard powder was placed under the probe and the probe was twisted until it locked into place, the IR spectrum of standard powder was recorded. The same procedure was followed for the commercial folic acid Tablet after it was crushed in to fine powder.

**Preparation of phosphate buffer (0.1 mol/L)**

Disodium phosphate, 20.21 g was weighed and transferred into 800ml of distilled water and stirred, and 3.39 g of sodium dihydrogen phosphate was added to the solution and stirred. The solution was adjusted to pH 9 with 0.1 M sodium hydroxide using pH meter to monitor the pH changes. The solution was made up to 1000 ml with distilled water.

**Preparation of stock standard solution**

The folic acid stock standard solution was prepared by measuring 10 mg of folic acid reference powder, which was transferred into a conical flask and made up to 20 ml with 0.1 mol/L phosphate buffer. It was stirred with the rotary shaker to obtain the stock solution of 500 µg/ml.

**Assay of folic acid Tablets**

Matias *et al*. [11] method for assay of the folic acid Tablets was adopted.

**Construction of calibration curve for assay of folic acid Tablets**

Aliquot of 1.8 ml stock standard solution of folic acid (500 µg/ml) was taken and made up to 20 ml mark of the volumetric flask with phosphate buffer (pH 9) to give a concentration of 17.5 µg/ml. Subsequently, serial dilution was carried out to give concentration of 15, 12.5, 10.0, 5.0, 2.5 and 1.0 µg/ml. The absorbance of each was determined in a UV spectrophotometer at 282.5 nm using the phosphate buffer (pH 9) as blank solution.

**Assay of folic acid Tablets**

The average weight of 20 Tablets was taken and then finely powdered, a portion of the powder equivalent to 50 mg of folic acid was accurately weighed and dissolved in 100 ml of phosphate buffer at pH 9 and shaken for 20 minutes with rotary shaker. The solution was filtered through a membrane filter. From this, 0.9 ml of the solution was taken and diluted to 25 ml (to obtain 17.5 µg/ml) with phosphate buffer at pH 9 using graduated flask and were analyzed by UV spectrophotometer at 282.5 nm and all the absorbance were taken in triplicate.

**Dissolution test of folic acid Tablets**

The method for dissolution of the folic acid developed by Matias *et al*. [11] was adopted.

**Construction of Calibration Curve for Dissolution Test**

Using the electrical weighing balance, 10 mg of folic acid reference powder was weighed

Archives of Pharmaceutical Sciences and Biotechnology Journal

Volume 1 Issue 1 June, 2021 Page 68 - 75 ISSN: 2971_611X

APSB-07

APSBJournal

and transferred into beaker, 20 ml of the distilled water was transferred into the beaker to obtained the stock solution 500 µg/ml. Aliquot of 1.8 ml stock standard solution of folic acid (500 µg/ml) was taken and made up to 20 ml mark of the volumetric flask with water to give a concentration of 17.5 µg/ml. Subsequently, serial dilution was carried out to give concentration of 15, 12.5, 10.0, 5.0, 2.5 and 1.0 µg/ml in labeled test tubes. The absorbance of each was determined in a UV spectrophotometer at 377.2 nm using the distilled water as blank solution.

**Dissolution test of folic acid Tablets alone**

The dissolution medium was heated to 37 ± 0.5 $^0$C, one Tablet of folic acid was placed in the vessel and the dissolution vessel (which contains 500 ml of distilled water) was immediately operated at 50 rpm for 15 minutes. Five sampling points (1, 4, 10, 12 and 15 minutes) were defined to monitor the dissolution. A 3 ml sample was collected using syringe between the surface of the dissolution medium and the top of the rotating paddle at each sampling point. An equivalent of the dissolution medium was not replaced and this was taken into cognizance during calculation of the concentration of folic acid at each sampling point. The temperature of the mixture was periodically verified and the vessel was kept covered over the entire duration of the dissolution test. The samples were filtered with a membrane filter prior to the analysis by UV spectrophotometer at 377.2 nm and the absorbance of each sample was taken.

**Dissolution test of Folic Acid Tablets in the Presence of *T. occidentalis***

The dissolution medium was heated to 37 ± 0.5 $^0$C and 2 g of *T. occidentalis* extract was added to dissolution vessel (containing 500 ml of distilled water) to form a solution and stirred with a glass rod. One Tablet of folic acid was placed in the vessel and the dissolution vessel was immediately operated at 50 rpm for 15 minutes. Five sampling points (1, 4, 10, 12 and 15 minutes) were defined to monitor the dissolution. A 3 ml sample was collected using syringe between the surface of the dissolution medium and the top of the rotating paddle at each sampling point. An equivalent of the dissolution medium was not replaced and this was taken into cognizance during calculation of the concentration of folic acid at each sampling point. The mixture temperature was periodically verified and the vessel was kept covered over the entire duration of the dissolution test. The samples were filtered with a membrane filter prior to the analysis by UV spectrophotometry at 377.2 nm and the absorbance of each sample was taken in triplicate.

## RESULTS AND DISCUSSION

The crude methanolic extract (CME) of *T. occidentalis* obtained was 20.8 g, which is equivalent to a percentage yield of 10.4 % w/w. The folic acid reference powder was identified and the purity determined by melting point determination and elucidating the IR spectra. The melting point of folic acid reference standard used in the research work met the specified requirement for folic acid purity at 250°C, as stipulated in USP specifications [12]. Figures 1 and 2 shows the FTIR spectra of folic acid reference powder and folic acid Tablet respectively. The presence of folic acid in the Tablet formulation was also confirmed with IR spectroscopy. The IR spectra of the reference powder and Tablet were compared with a

Archives of Pharmaceutical Sciences and Biotechnology Journal

Volume 1 Issue 1 June, 2021 Page 68 - 75 ISSN: 2971_611X

APSB-07

APSBJournal

standard reference spectrum of folic acid and the peaks were found to be superimposable. Figure 3 shows the calibration curve used to determine the concentration of folic acid in the Tablets used for the *in vitro* dissolution studies.

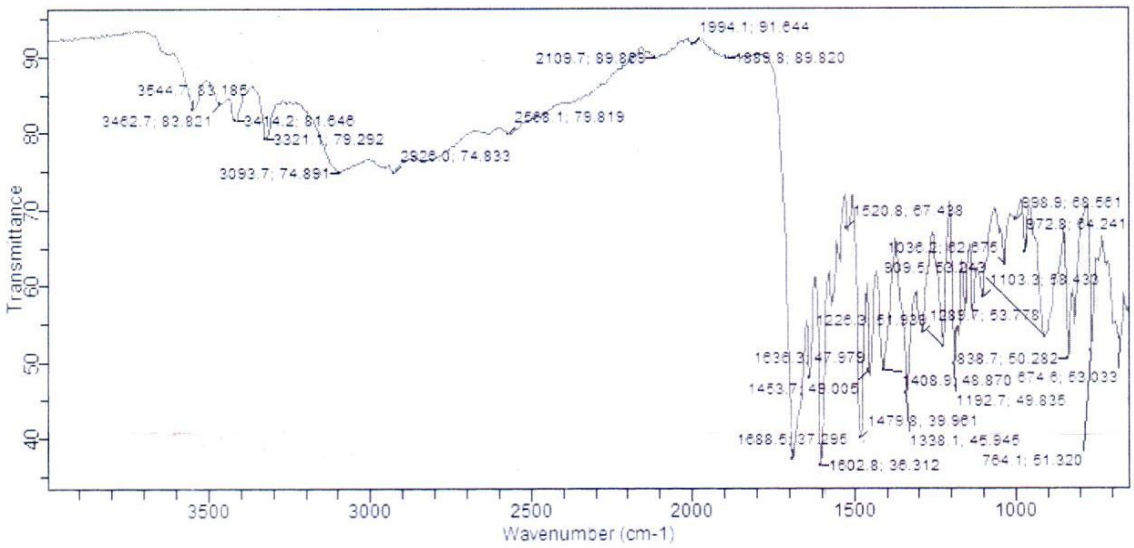


**Figure 1: FTIR Spectra of Folic acid Reference Powder**

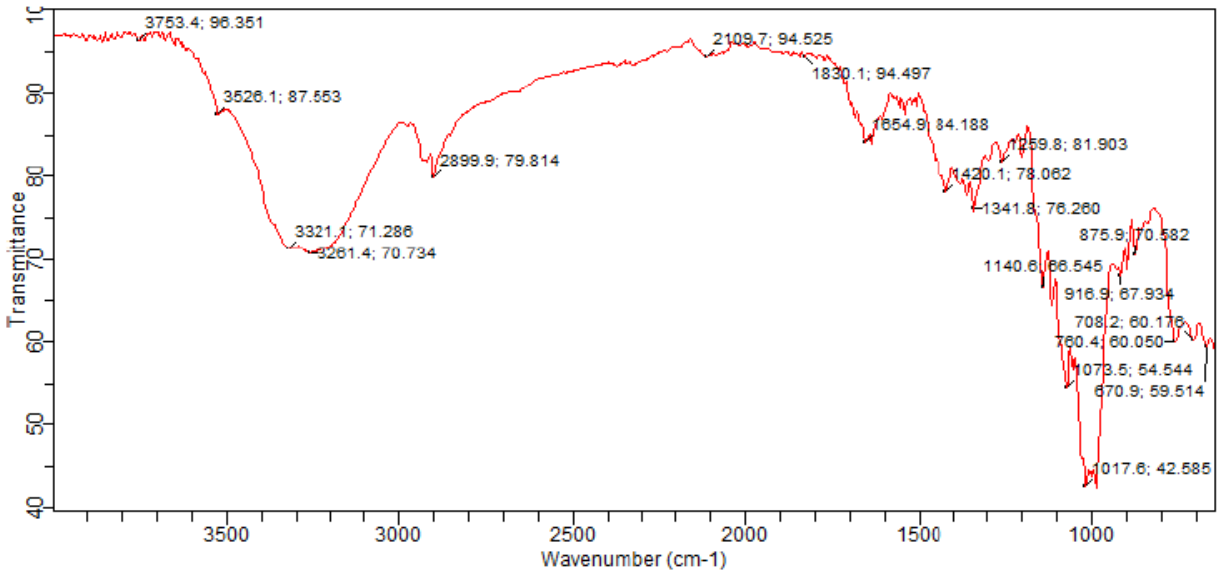


**Figure 2: FTIR Spectra of Folic Acid Tablet**

Archives of Pharmaceutical Sciences and Biotechnology Journal

Volume 1 Issue 1 June, 2021 Page 68 - 75 ISSN: 2971_611X

APSB-07

APSBJournal

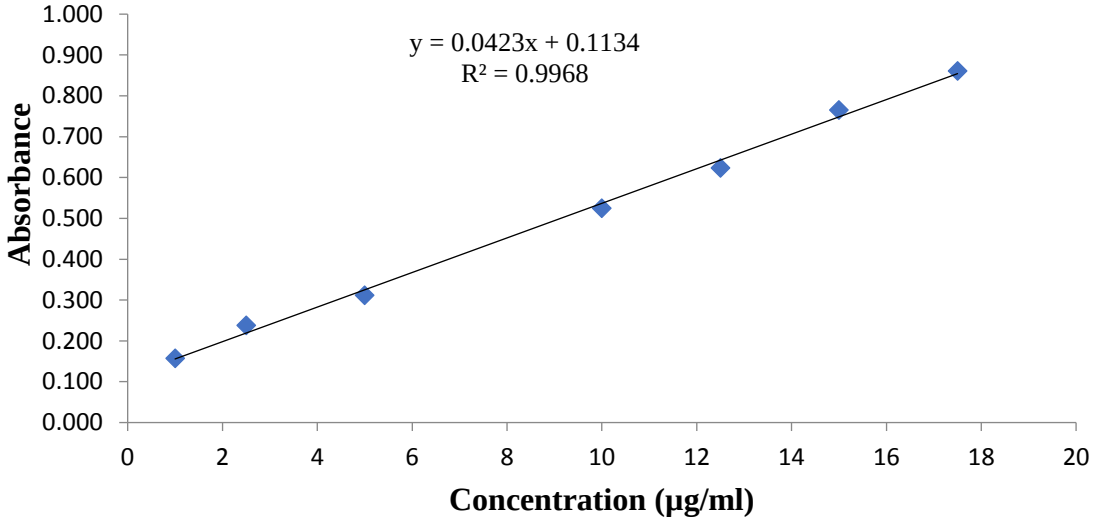


**Figure 3: Calibration curve for the assay of folic acid in Tablet formulations**

The percentage recovery folic acid Tablets used for the *in-vitro* dissolution studies of 78.86 %, as seen in Table 1 shows that this batch of folic acid does not meet the British Pharmacopeia specification of 90 to 110 % of stated content [13]. This further strengthens the argument of the importance of routine quality assessment of drugs in the market and the need to carry out assay of drugs before carrying out their dissolution studies.

**Table 1: Assay of folic acid tablets used for the *in-vitro* dissolution studies**

| Expected concentration of folic acid in tablet solution (µg/ml) | Actual concentration of folic acid in tablet solution (µg/ml) | Percentage recovery (%) |
|---|---|---|
| 17.50 | 13.80 | 78.86 |

The calibration curve used for *in vitro* dissolution studies of folic acid is shown in Figure 4. The dissolution studies as depicted in figure 5, showed that there was rapid dissolution of folic acid within the first minute (almost 80 %), and then a gradual increase in the dissolution until it reached a peaked at 150 %. However, in the presence of *T. occidentalis* there was no dissolution before the first minute, but a sharp increase in dissolution was observed after the first minute, peaking at about 250 %, and then a sharp decline in dissolution was observed. A previous study [14] on the interaction of metformin and a herbal product reported an alteration in the pharmacokinetics of the drug, thus also suggesting the potential effect of herbal medicines on the pharmacokinetics of drugs.

Archives of Pharmaceutical Sciences and Biotechnology Journal
Volume 1 Issue 1 June, 2021 Page 68 - 75 ISSN: 2971_611X
APSB-07
APSBJournal

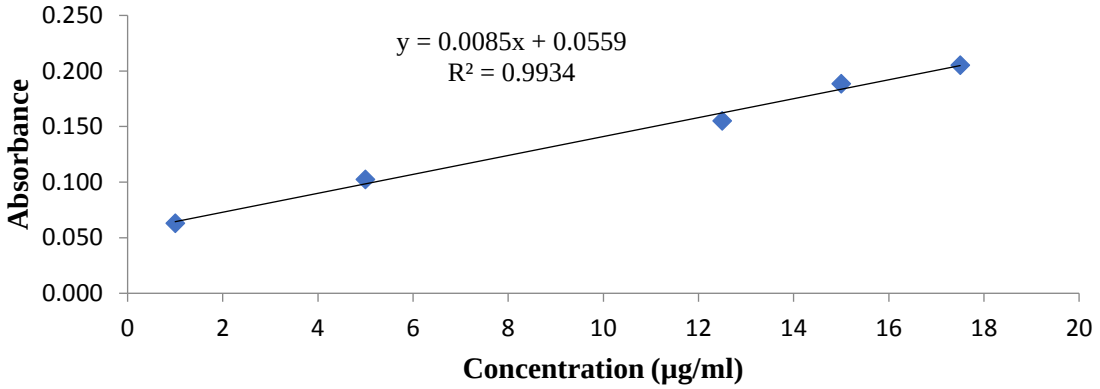


**Figure 4: Calibration Curve for the Dissolution Test of Folic Acid Tablet**

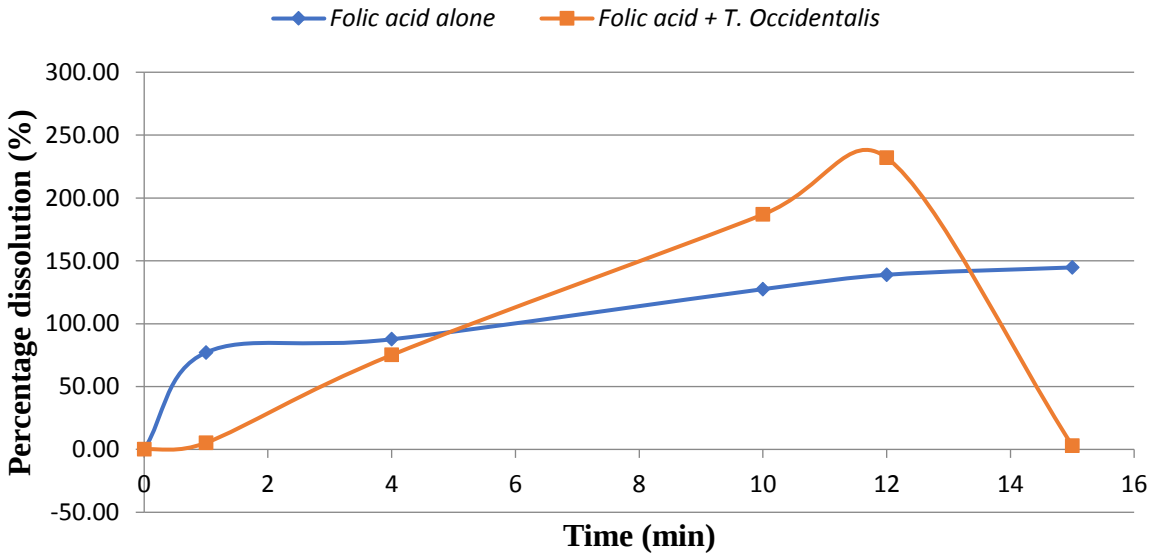


**Figure 5: Dissolution Profile of Folic Acid alone and in the Presence of *T. occidentalis***

## CONCLUSION

The result of this study suggests that the *in vitro* dissolution rate of folic acid tablets is altered in the presence of *T. occidentalis*. This can be interpreted as an indication of the existence of potential HDI, which should be further established by further *in vitro* and *in vivo* pharmacokinetic studies.

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