

EVALUATION OF PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTIOXIDANT ACTIVITY OF ETHANOLIC AND HYDROETHANOLIC EXTRACTS OF THE FRUITS AND TWIGS OF *FICUS OVATA* VAHL (MORACEAE)

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ABSTRACT

Introduction: The use of medicinal plant is quasi-general throughout the continent; however some of the plants reputed in the indigenous system of medicine are not scientifically established for their activities. The study such as ethno medicine keenly represents one of the best avenues in searching new economic plants for medicine. The current study examined the phytochemicals, in vitro antioxidant activities of the Hydroethanolic and ethanolic extracts of twigs and fruits of *Ficus ovata*.

Materials and methods: These extracts were prepared by maceration of the twigs and fruits separately in ethanol or hydroethanolic solvent for 48hours. Then used for the phytochemical analysis and antioxidant potential screening in vitro which include polyphenol content test and the DPPH antiradical test.

Results: The Phytochemical screening revealed the presence of alkaloids, tannins, saponins, glycosides, phenols and flavanoids in all extracts except phlobatannin that was absent in the fruit extracts. Fruits extracts had the highest polyphenol content (EF= 718.142 ± 12.910mg CatEq vs HF= 486.876 ± 8.606 mg CatEq; P< 0.05) and the best DPPH antiradical scavenging effect (IC₅₀; EF= 2.7mg/ml, HF=0.70mg/ml) compared to twigs (P< 0.05).

Conclusion: our findings indicate that *Ficus ovata* content most bioactive phytochemicals use in traditional medicine and scavenging antiradical effects which could be essential for the improvement of cardiovascular related diseases.

RESUME

Introduction

L'usage de plante médicinale est quasi-général dans tout le continent ; malheureusement certaines plantes reconnues dans le system indigène de médecine ne sont

pas scientifiquement reconnus pour leur activités. L'étude comme ethnomédecine

représente l'un de meilleure voie de découvert de nouvelle plantes économique en médecine. Cette etude examine la phytochimie, activite antioxidant in vitro de l'effet hypoglycémiant et hypolipémiant des extraits de fruits et de tiges de *Ficus ovata*.

Matériels et Méthodes

Les extraits éthanolique et hydroethanologique de *Ficus ovata* ont été préparés et le criblage phytochimique a été fait. Le potentiel antioxydant a été évalué en mesurant la teneur en polyphénol et les effets inhibiteurs des extraits sur l'activité des radicaux libres de DPPH.

Resultat

Le criblage phytochimique a elucidé la présence de alcaloïde, tannins, saponines, glycosides, phénols et les flavonoïdes dans toutes les extraits sauf phlobatannin qui était absent dans l'extrait des fruites. L'extrait des fruites avait le taux elevee de polyphenol (EF= 718.142 ± 12.910mg CatEq vs HF= 486.876 ± 8.606 mg CatEq; P< 0.05) et la meilleure DPPH de effet anti radical (IC₅₀; EF= 2.7mg/ml, HF=0.70mg/ml) compare au tige (P< 0.05).

Conclusion: Cette étude a montre que *Ficus ovata* contient la plupart de phytochimie bioactive utilisé and médecine traditionnelle et les effets anti radical qui peut être essentiel pour l'amélioration des maladies cardiovasculaires.

INTRODUCTION

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases (**Poongothai, 2011**). There are many phytochemicals in fruits and herbs and each works differently. The therapeutic benefit of medicinal plants is often attributed to their antioxidant properties (**Ahmed and Urooj, 2009**). The adoption of crude extracts of plants, such as infusions, for self medication by the general public has arisen in the possibility that the impact of several diseases may be either ameliorated or prevented by improving the dietary intake of natural nutrients with antioxidant properties, such as vitamin E, vitamin C, B-carotene and plant phenolics such as tannins and flavonoids (**Abu et al., 2011**). *Ficus ovata*, a plant of the *Ficus* specie found in the savanna woodland, forest edges, river side forest and secondary forest, up to an altitude of 2100 m is distributed in the subtropical Africa including Cameroon (**Kuete et al., 2009**). The decoction of leaves of *Ficus ovata Vahl* is used to treat infectious diseases and facilitate childbirth. The decoction of the bark stems is used in the treatment of gastrointestinal infections, diarrhoea and as antipoison. In Benin, the leaves of *Ficus ovata* are used against external hemorrhoids, sprains and jaundice (yellowing) (**Kuete et al., 2009**) and its leaves are used in Ivory Coast against the psychoneuroses (**Assi et al., 1990**). Fruits are used to stimulate milk production in cows and stem bark use as food for mastication (**Hanelt et al., 2001**). The present study was aimed to investigate the phytochemical screening and antioxidant potential on extracts of fruits and twigs of *Ficus ovata*.

MATERIALS AND METHODS

Collection and identification of plant: The fruits and twigs of *Ficus ovata* were collected from Mount Kala, Centre region of Cameroon. The plant was identified at the Cameroon National Herbarium, Yaounde, where a voucher specimen was conserved under the reference number 26996SRF/Cam. The collected plant parts were separated from undesirable materials. They were dried under the shade separately. The plant parts were ground into tiny debris with the help of a suitable grinder, kept in a cool and dry place until analysis commenced.

Preparation of extracts: We weight 125g of the fruits and the twigs of *Ficus ovata* and macerated separately in 1L of ethanol 95% or 1L of ethanol: water solution in the ratio 1:1 and all for 48 hours. The whole mixture was successively filtered through a piece of clean, white cotton material. The filtrates obtained were evaporated to dryness in a drying room. We obtained four extracts which were hydroethanolic fruits; hydroethanolic twigs, ethanol fruits and ethanolic twigs

Phytochemical screening procedure:

1. **Test for Phenol** was done by dissolving 250 mg of each extracts in 4 ml of distilled water and the content heated for 15 minutes. After cooling and filtration 2 drops of freshly prepared ferric cyanate solution (1 ml FeCl_3 1% and 1ml $\text{KFe}(\text{CN})_6$) was added to 1 ml of each filtrate. The presence of a greenish-blue coloration indicated the presence of polyphenols
2. **Test for Alkaloids** (Mayer test) was done by heating 100 mg of the each extracts in 2 ml of H_2SO_4 2% for 2 minutes after which the content was filtered. Few drops of the Mayer reagent (1.358g $\text{HgCl}_2 + 500$ ml H_2O and 0.8g $\text{KI} + 200$ ml H_2O) were added in 1ml of the filtrate and the presence of a white precipitate or turbid solution was an indicator of the presence of alkaloids.
3. **Test for Saponines** was done by adding 250 mg of the extract in 5 ml of distilled water. After vigorous homogenisation the mixture was heated to boil, the appearance of foam that persisted 20 minutes after cooling was an indicative of the presence of saponines.
4. **Test for Tannins** was done by adding 100 mg of each extracts in 2 ml of distilled water followed by heating in a water bath and then filtering. Few drops of 3% ferric chloride were added in 1 ml of filtrate and the observation of a blue-black or greenish-dark coloration indicated the presence of tannins.
5. **Test for phlobatannins** was done by dissolving 100 mg of each extract in 2 ml of distilled water. After filtration, to 0.5 ml of each filtrate was added 1 ml of hydrochloric acid 1% and the deposit of a red precipitate was an indicator of the presence of phlobatannins.
6. **Test for glycosides** was done by dissolving 100mg of each extract in 5 ml of HCl then neutralised by 5 ml of 5% caustic soda (soude). Drops of Fehling's solution [A (40 g of $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ per litre) + B (160 g of tartrate double appearance of a red precipitate was an indicator of the presence of glycosides).
7. **Test for flavonoids** was done by dissolving 250 mg of each extract in 5 ml of sodium hydroxide 1N. The observation of a yellow solution is a preliminary evidence of the presence of flavonoids and the decolourisation of the yellow colour observe on the addition of a few drops of concentrated HCl confirms its presence.

Preparation of variable concentration of extracts

The mother solution (5 mg/ml) was prepared by dissolving 100 mg of the pure extract with 20 ml of water. After vigorous homogenising, the following daughter solutions were prepared. It is from this working solution that the test below was carried on.

Table V: Preparation of working solution of our extract

Tubes	1	2	3	4	5	6	7	8	9
Concentration (mg/ml)	5	2.5	1.5	0.75	0.5	0.25	0.05	0.025	0.012
Volume of extract (ml)	5	2.5	1.5	0.75	0.5	0.25	0.05	0.025	0.012
Volume of solvent (ml)	0	2.5	3.5	4.25	4.5	4.75	4.95	4.975	0.980
Final volume (ml)	5	5	5	5	5	5	5	5	5

Determination of the antioxidant potential of the plant extracts

polyphenol content using Folin ciocalteu method

Principle: This method is based on the reduction of a phosphomolibdic-tungstic chromogene by an antioxidant and a change of colour with the absorbance measured at 750nm using a spectrophotometer. This reagent consists of a mixture of tungstic and phosphomolybdic acids. In an alkali medium (sodium carbonate), it developed a blue colouration of which the absorbance is measured at 750nm. Ethanol (0.3 ml) in the place of extract is used as the blank. The polyphenolic concentration of the extracts was determined using folin-ciocalteu reagent (sigma chemical Co St Louis, MO) diluted 10 times before use. To determine the total polyphenol concentration, 10 µl of the hydrolyse extract was added in 1ml of Folin solution diluted 10 times, after 30 minutes of incubation the absorbance was measured at 750 nm using the spectrophotometer. Catechine was used as a standard. The antioxidant activity is expressed as the number of equivalents of catechine.

DPPH (1, 1-diphenyl-2-picrylhydrazyl) antiradical activity

Principle: DPPH is a relatively stable nitrogen centered free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH antioxidant assay is based on the ability of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for a visible deep purple colour. When DPPH accepts an electron donated by an antioxidant, the DPPH is decolorized, and can be quantitatively measured due to the changes in absorbance. A volume of 20 µl of a non-hydrolysed aqueous extract was introduced in 2 ml of a methanolic solution of DPPH (0.3 mM). After 30 minutes of incubation in the dark, the absorbance was measured with a spectrophotometer at 517nm. A control was also made (DPPH with water only). The percentage of inhibition of the DPPH radical by the specimen was calculated using the formula of **Yen and Duh, (1994)** as follows:

$$\% \text{ of inhibition} = \frac{A_c - A_e}{A_c} \times 100$$

Where A_c is absorbance at time = 0 min and A_e is the absorbance after 30 minutes of incubation.

RESULTS

Yield of extraction and phytochemical screening

The results of the extraction and phytochemical screening are represented on the tables 1 and 2 below.

Table 1: Yield of extraction

	Fruit	Fruit	Twigs	Twigs
solvent	Ethanol	Ethanol: water(1:1)	Ethanol	Ethanol: water(1:1)
% yield	7.28	5.66	2.78	7.21

Two solvent system was use for the extraction of the fruits and twigs of *F. ovata* which where ethanolic and hydroethanolic solvent systems. The above results show that the hydroethanolic solvent gave higher yield than the ethanolic solvent for extraction of the fruits and twigs.

Table 2: The phytochemical screening results

FOEF= Ficus ovata ethanolic fruits; FOET= Ficus ovata ethanolic twigs; FOHF= Ficus ovata hydroethanolic fruits;

	Alkaloids	Saponins	Flavonoids	Tannins	Phlobatannins	Glycosides	Phenols
FOEF	+	+	+	+	-	+	+
FOET	-	+	+	+	+	+	+
FOHF	+	+	+	+	-	+	+
FOHT	+	+	+	+	+	+	+

FOHT= Ficus ovata hydroethanolic twigs; +=presence; -=absence

Generally extracts of fruits and twigs of *Ficus ovata* contain groups of bioactive compounds such as alkaloids, glycosides, saponins, and polyphenols such as flavonoids, tannins and phenols. Phlobatannins were absent in the fruit extracts of *Ficus ovata* and alkaloids where absent in ethanolic fruits.

Result of the antioxidant potential of our plant extracts

Polyphenol content of extracts

Polyphenols content of extracts is represented on table 3 below

Table 3: Polyphenols content results

	FOEF	FOHF	FOET	FOHT
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Table 3 above showed that all extracts had polyphenols with FOEF having a higher content (718.142mg eq catechine). We also noted a significant difference ($P < 0.05$) in polyphenols content for all the extracts.

DPPH (1, 1-Diphenyl-2-Picrilhydrazyl) antiradical activity of extracts

Figure 1 below represent the inhibition percentages obtain after an evaluation of antiradical activity of the ethanolic and hydroethanolic fruits and twigs extracts respectively.

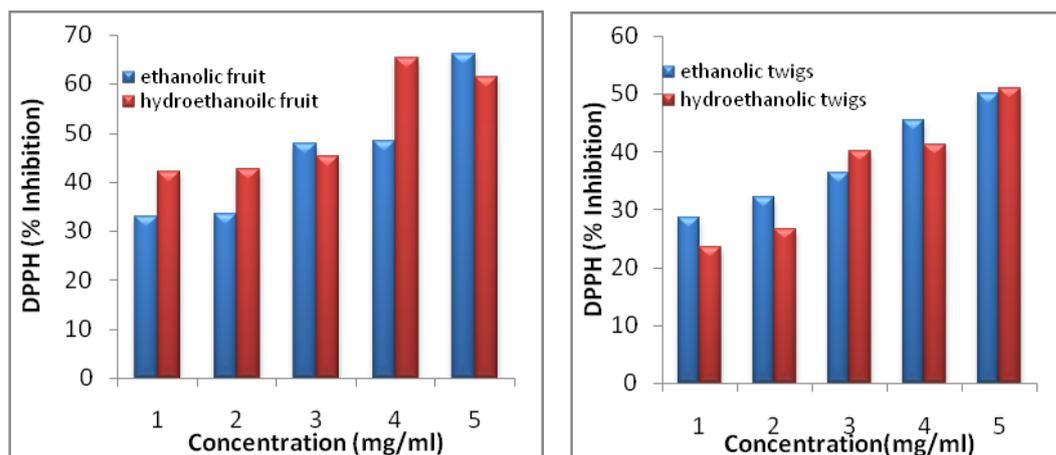


Figure 1(a and b): Antiradical activity of extracts using DPPH method

Figure 1 above show that for both plant parts, the hydroethanolic solvent was the best system with a high antiradical activity compared to ethanolic solvent system ($p < 0.05$) and the scavenging activity increases as concentration increases. Moreover the inhibition profile of the hydroethanolic fruits was the best with an IC₅₀ of 0.701 mg/ml as compared to the others and this IC₅₀ was significantly different ($p < 0.05$) from all the other extracts.

DISCUSSION

Preliminary, all four extracts screened for phytochemical, revealed the presence of groups of bioactive compounds such as alkaloids, glycosides, saponins, and polyphenolic compounds such as flavonoids, tannins and phenols. Phlobatannins were absent in the fruit extracts of *F. ovata*. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. For example, glycosides, saponins, flavonoids, tannins and alkaloids have hypoglycemic activities; anti-inflammatory activities. Previous studies show that saponins possess hypocholesterolemic and antidiabetic properties.

Generally the polyphenolic content test and the DPPH antiradical activity test showed that hydroethanolic extracts of fruits and twigs had the best solvent system as compared to the ethanolic extracts for each plant part. When comparing the total water soluble phenolic concentration with the DPPH radical scavenging antioxidant activity of the fruits and twigs extracts (table 3 and figure 1), no positive correlation was observed. This goes to support the hypothesis of *Brand Williams et al. (1995)* that the DPPH kinetic is proportional to the amount of OH group present on the phenolic compound (*Claudia et al., 2008*). Thus, the hydroethanolic extracts may be rich in phenolic compounds that have many OH groups leading to it high DPPH scavenging activity. These compounds act as hydrogen donors to free radicals by stopping lipid peroxidation at the stage of initiation (*Claudia et al., 2008*).

CONCLUSION

Our findings indicate that *Ficus ovata* content most bioactive phytochemicals use in traditional medicine. In general, *Ficus ovata* showed considerable antioxidant activity in DPPH free radical scavenging activity and total antioxidant capacity when compared to standard as ascorbic acid. However in vitro antioxidant activity plant extracts should be evaluated by other antioxidant methods e.g. nitric oxide scavenging activity, reducing power, lipid peroxidation assay.

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