PRINT ISSN 1119-8362
Electronic ISSN 2659-1502

Full-text Available Online at
https://www.ajol.info/index.php/jasem
https://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 27 (10) 2331-2335 October 2023

Evaluation of Phytochemical Constituents and *In-Vitro* Antioxidant Properties of *Ficus asperifolia* Leaf Extracts

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ABSTRACT: This study evaluated the phytochemical constituents and *in-vitro* antioxidant properties of *Ficus asperifolia* leaf extracts. The evaluation of the phytochemistry showed the presence of bioactive compounds of saponins, alkaloids, terpenes, tannins, steroids, phlobatannin, cardiac glycosides and carbohydrates were confirmed present in the extracts of *F. asperifolia*. Flavonoid concentration varied significantly ($p<0.05$) in the increasing order of aqueous > ethanol > methanol extract. The nitric oxide scavenging activities of the aqueous extracts of *F. asperifolia* were significantly ($p<0.05$) higher in comparison to the methanol and ethanol extracts respectively. The total antioxidant capacity of the methanol extract were significantly ($p<0.05$) higher when compared to both the aqueous and ethanol extracts respectively. The results of this study support the application of *F. asperifolia* in herbal medicine.

DOI: https://dx.doi.org/10.4314/jasem.v27i10.26

Cite this paper as: JOHNNY, D. I; KADIRI, H. E. (2023). Evaluation of Phytochemical Constituents and *In-Vitro* Antioxidant Properties of *Ficus asperifolia* Leaf Extracts. *J. Appl. Sci. Environ. Manage.* 27 (10) 2331-2335

Received: 27 August 2023; Revised: 25 September 2023; Accepted: 04 October 2023 Published: 30 October 2023

Keywords: Phytochemical; Antioxidants; Free radicals; *Ficus asperifolia*

The cell which the simplest unit of life is besiege with a lot of biological processes. Biological processes at the cellular level are basically characterized by two main activities of metabolism (anabolism and catabolism) (Koizumi *et al*., 2013). Metabolic activities at cellular level induces cellular aberrations, this condition are caused by the actions of free radical species resulting as products of side reactions of some key metabolic pathways (endogenous) and also from exogenous sources from xenobiotics (Jiang *et al*., 2015). The activities of these deleterious ionic species conversely up regulate and down the activities of key enzymes, metabolites, electrolytes, genetic molecules and information transfer and processing (Cevallos *et al*., 2019). It is well known that one of the underlying mechanism/theory of pathology (diabetes, obesity, asthma, cardiovascular disease, neurological disorders etc.) is link to the mechanism of oxidative stress, a condition in which the cellular concentration of the activities the reactive ionic species (oxygen and nitrogen) exceed the cellular threshold of the buffering capacity of the antioxidant defense of the cells (Gian *et al*., 2023). One important and abundance class of molecules with a host of these biological activities and other diverse functions are plant bioactive ingredients (Prakash *et al*., 2017; Zaini *et al*., 2022). Plants are known to host diverse of millions phytochemical compounds whose identification, characterization, function are still undergoing scientific investigation on daily basis. The biological activities (anti-oxidants, anti-inflammatory, anti-diabetic, anti-microbial, anti-tumor) of bioactive ingredients are mediated with less side effect in comparison to chemical synthesized drugs, coupled with the fact that these compounds are relative abundance, makes them cheaper and alternative source of drugs (Kyriakoudi *et al*., 2021).

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Ficus asperifolia (commonly called sandpaper plant) belongs to the *Moraceae* family, it is a small or average size tree, terrestrial or epiphyte which can reach 20m in height and it is found in several African countries including Nigeria. The leaves are enormous and displayed spirally, the limb is largely oval or has a form of ellipse and the roots are most often fibrous (Adjanohoun *et al*., 1996). The plant's leaf has been reported to possess antioxidant (Ojo and Akintayo 2014), hypolipidemic and hepatoprotective properties (Omoniwa *et al*., 2013), while it is used in traditional medicines in gastric ulcers and wound treatment as well as an anthelmintic and a purgative agent (Soforowa, 1996).

MATERIALS AND METHODS

Reagents and Chemicals: DPPH (1, 1-diphenyl-2-picryl-hydrazyl) were procured from Sigma-Adrich Co., St. Louis, USA. Other chemicals and reagents were of analytical grade.

Plant Materials and Extracts preparation: Leaves of *F. asperifolia* was collected from a bush at Eidenu - Irrua, Edo-state and authenticated at the Department of Botany. Preparation of the different leaf extracts (aqueous, methanol and ethanol) were done using the method described by Anigboro *et al.* (2019).

Phytochemical Analysis: The phytochemical screening of the leaf extracts of *F. asperifolia* were carried out as described by Njoku and Obi (2009) and Borokini and Omotayo (2012).The quantitative phytochemical analysis of the different extracts of *F. asperifolia* were evaluated using the method of Singleton and Rossi, (1965) for Phenols and Tannin using the method of Singleton and Rossi, (1965), while that of Flavonoids, Protein, reducing sugar, Alkaloid and Phytate were done according to procedures described by Jia *et al.* (1999), Lowry *et al.* (1957), Miller (1959), Shamsa *et al.* (2008) and Vaintraub and Lapteva (1988) respectively.

In-vitro Free Radical Scavenging and Antioxidant Activity Assay: The *in-vitro* scavenging activity of the various *F. asperifolia* extracts samples against and nitric oxide ($NO^{\bullet}$) radicals and total antioxidant capacity were carried out in accordance with the protocols of Marcocci *et al*. (1994) and Prieto *et al.* (1999) respectively.

Statistical Analysis: All data obtained were subjected to statistical analysis. Values were reported as Mean ± Standard deviation while one way ANOVA was used to test for differences between treatment groups. The results were considered significant at p-values of less than 0.05, that is, at 95% confidence level ($p<0.05$). Turkey post hoc will be used as basis for comparison for level of significance**.**

RESULTS AND DISCUSSION

Qualitative Phytochemical screening of leaf extracts of Ficus asperifolia: The result of the qualitative phytochemical screening of leaf extracts of *F. asperifolia* are presented in Table 1. The outcome showed the detection of bioactive compounds of saponin, Alkaloids, terpenes, tannins, steroids, phlobatannin, cardiac glycosides and carbohydrates were extracts of *F. asperifolia*.

Table 1: Qualitative phytochemical screening of extracts of *Ficus asperifolia*

Phytonutrients	Extracts		
	Aqueous	Methanol	Ethanol
Saponin	+++	_	+
Phlobatannin	-	-	-
Cardiac glycosides	-	-	-
Flavonoid	-	-	-
Tannin	+	_	+
Alkaloids	+++	_	_
Terpenes	++	+	+
Phenol	-	-	-
Steriods	_	+	_
Carbohydrates	_	_	_
Protein	-	-	-
Thiols	-	-	-

Key: + = present, ++ = moderately present, +++ = highly present, - = absent

Quantitative Phytochemical determination of leaf extracts of F. asperifolia: The outcome of the quantitative determination of leaf extracts of extracts *F. asperifolia* are depicted in Table 2. The result revealed that the concentration for flavonoid varied significantly ($p<0.05$) in the increasing order of aqueous > ethanol > methanol extract.

Table 2: Quantitative phytochemical evaluation of extracts of *Ficus asperifolia*

Bioactive ingredients	*Ficus asperifolia*		
	Aqueous	Methanol	Ethanol
Phenol (mg/gm GAE)	0.90 ± 0.02^{a}	1.13 ± 0.06^{b}	1.13 ± 0.02^{b}
Tannin (mg/gm TAE)	0.57 ± 0.01^{a}	0.75 ± 0.04^{b}	0.75 ± 0.01^{b}
Flavonoid (mg/gm CAE)	1.67 ± 0.10^{a}	2.28 ± 0.06^{b}	2.60 ± 0.09^{c}
Reducing sugar (mg/gm GluE)	3.60 ± 0.10^{a}	1.65 ± 0.05^{b}	2.27 ± 0.06^{c}
Phytate (mg/gm)	0.67 ± 0.20^{a}	0.60 ± 0.80^{a}	0.12 ± 0.07^{a}
Alkaloids (mg/gm ATE)	2.20 ± 0.05^{a}	1.90 ± 0.20^{b}	1.64 ± 0.05^{b}

Values are mean±standard deviations of triplicate determinations. Values not sharing common superscript on the same row differ significantly ($p<0.05$). GAE = Gallic acid equivalent, CAE = Catechin equivalent, TAE = Tannic acid equivalent, GluE = Glucose equivalent, ATE = Atropin equivalent.

Nitric oxide scavenging activities of leaf extracts of F. asperifolia: The findings of the inhibition of nitric oxide by leaf extracts of *F. asperifolia* are depicted in table 3. The activities of aqueous extract of *F. asperifolia* were observed to be significantly ($p<0.05$) higher in comparison to both the ethanol and methanol extracts.

Table 3: Nitric oxide scavenging activities of extracts of *Ficus asperifolia*

Conc. (mg/ml)	% Inhibition		
	Aqueous	Methanol	Ethanol
0.25	42.1±0.88^a	23.1±1.54^b	25.6±0.88^b
0.50	59.5±0.89^a	41.0±0.89^b	46.7±0.88^c
1.0	64.6±3.08^a	47.7±1.54^b	51.8±1.78^b
2.0	69.7±0.89^a	56.9±1.54^b	52.8±1.78^c
4.0	80.0±1.54^a	66.6±0.85^b	74.9±3.20^a

Values are mean ± standard deviations of triplicate determinations. Values not sharing common superscript on the same row differ significantly ($p<0.05$).

Total antioxidant capacity of leaf extracts of F. asperifolia: The outcome of total antioxidant capacity of leaf extracts of *F. asperifolia* are shown in table 4. It was observed that the activities of the methanol extract were significantly ($p<0.05$) higher when compared to both the aqueous and ethanol extracts respectively.

Table 4: Total antioxidant capacity of *Ficus asperifolia* extracts

Conc. (mg/ml)	695nm		
	Aqueous	Methanol	Ethanol
0.25	0.08±0.00^a	0.19±0.00^b	0.05±0.00^c
0.50	0.09±0.00^a	0.22±0.00^b	0.08±0.06^c
1.0	0.15±0.00^a	0.35±0.02^b	0.15±0.01^b
2.0	0.21±0.00^a	0.43±0.00^b	0.23±0.00^c
4.0	0.40±0.01^a	0.80±0.03^b	0.44±0.00^c

Values are mean± standard deviations of triplicate determinations. Values not sharing common superscript on the same row differ significantly ($p<0.05$).

Studies have shown that polyphenols are employed as targeted therapeutic and prophylaxis agents in managing health ailments. It is believed that these class of compounds are abundance in plants and show different variants regardless of their genus and species. Polyphenolic compounds are known to possess medicinal attributes such as antimicrobial, antioxidants, anti-viral, anti-tumor, anti-inflammatory, anti-diabetic, anti-obesity etc. (Ogunka-Nnoka *et al*., 2019). These attributes are linked to their activities causing modulatory actions on basic biomolecules, metabolites, enzymes and cause the scavenging of reactive oxygen and nitrogen species at cellular level (Anigboro *et al*., 2019). The findings of this study showed that the extracts of *F. asperifolia* contains bioactive ingredients of saponin, alkaloids, terpenes, tannins, steroids, phlobatannin, cardiac glycosides and carbohydrates. Polyphenols class of flavonoids, phenol and tannins have reported to cause the modulatory actions on the glycoprotein enzymes. These enzymes basically consist of two polypeptide chain folding of the N and C- terminal amino residues, both amino acids are residues are responsible for the catalytic activities of the enzymes (Sari *et al*., 2017). The biological activities of bioactive compounds also span to the free radical scavenging activities. These activities as play an influential role in regulatory activities of enzymes and the maintenance of the general wellbeing of the cell. The generation of reactive species (oxygen and nitrogen) is a co-existing process with the normal cellular metabolism (Claar *et al*., 2015; Kadiri and Ilondu, 2020; Kadiri *et al*., 2020). Whether it involves the catabolism or the anabolism process side reactions do occur in some key metabolic pathways which generate this reactive species. These side reactions are lead to the wearing and tearing of the biological cells (aging) which result in the depletion of the endogenous defense system of the cell, a physiological condition termed as oxidative stress (Ezzat *et al*., 2020). The presence of free radical in the cells beyond it physiological threshold (oxidative stress condition) is accompanied with a lot deleterious activities on basic biomolecules of protein, carbohydrates, nucleic acids. The attack on these biomolecules consequentially result in the un-coordinated influx or efflux materials in and out of the cell, signaling of metabolic pathways, biosynthesis and catabolism, denature protein (including enzymes), aberration in genetic information and processing thereby result in health challenging conditions (Abbas and Winks, 2014). The result of the *in-vitro* antioxidants of the extracts *F. asperifolia* showed the plant prowess in combating the activities of reactive species, this process is via free radical scavenging, quenching, breaking of chain reaction and also a combination of these mechanism. The extracts of *F. asperifolia* caused reduction in the colouration of the nitric oxide from dark pink to light and phosphomolybedate from lemon green to dark green. Nitric oxide is produced during immune response to pathogen and early response of inflammations onset (Fidrianny *et al*., 2015). These activities connote the plant relative abundance of phytonutrients, which was observed from the qualitative and quantitative phytochemistry of this study. Studies have confirmed the biological activities of some bioactive compounds such as flavonoids, alkaloids, phenols, tannins, glycosides, saponins, terpenes, etc. with activities such as anticancer, and antioxidants, anti-malarial, anti-tumour, anti-microbial, cardio-protective, antidiabetic etc.

Conclusion: The phytochemical composition of *F. asperifolia* are key to its biological activities. This study showed that relative abundance of the evaluated

phytochemicals correlate positively to the respective extracts *in-vitro* antioxidant activities.

REFERENCES

Abbas, S; Wink, M (2014). Green tea extract induces the resistance of Caenorhabditis elegans against oxidative stress. *Antioxidants*, *3*(1): 129-143.

Adjanohoun, JC; Aboubaker, N; Dramane, K; Ebot, ME; Ekpere, JA; Enow-Orock, EG (1996). Contribution to Ethnobotanical and Floristic Studies in Cameroon. *Trad. Med. Pharma*, 301 – 325.

Anigboro, AA; Avwioroko, OJ; Ohwokevwo, OA; Pessu, B (2019). Bioactive components of Ficus exasperata, Moringa oleifera and Jatropha tanjorensis leaf extracts and evaluation of their antioxidant properties. *EurAsian J. BioSci*. *13*(2).

Borokini, TI; Omotayo, FO (2012). Comparative phytochemical analysis of selected medicinal plants in Nigeria. *Int. J. Adv. Chem. Res.*, 1(1): 11 – 18.

Cevallos, SA; Lee, JY; Tiffany, CR; Byndloss, AJ; Johnston, L; Byndloss, MX; Bäumler, AJ (2019). Increased epithelial oxygenation links colitis to an expansion of tumorigenic bacteria. *MBio*, 10(5): 10-1128.

Claar, D; Hartert, TV; Peebles Jr, RS (2015). The role of prostaglandins in allergic lung inflammation and asthma. *Exp. Rev. Resp. Med.*, 9(1): 55-72

Ezzat, MI; Okba, MM; Ahmed, SH; El-Banna, HA; Prince, A; Mohamed, SO; Ezzat, SM (2020). In-depth hepatoprotective mechanistic study of Phyllanthus niruri: In vitro and in vivo studies and its chemical characterization. *PloS one*, 15(1): e0226185

Fidrianny, I; Rizkiya, A; Ruslan, K (2015). Antioxidant activities of various fruit extracts from three *Solanum* sp. using DPPH and ABTS method and correlation with phenolic, flavonoid and carotenoid content. *J. Chem. and Pharm. Res.*, 7(5): 666-672.

Gain, C; Song, S; Angtuaco, T; Satta, S; and Kelesidis, T (2023). The role of oxidative stress in the pathogenesis of infections with coronaviruses. *Front. Microbial*. 13: 1111930.

Jia, Z; Tang, M; Wu, J (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem*. 64: 555 – 559.

Jiang, T; Harder, B; De La Vega, MR; Wong, PK; Chapman, E; Zhang, DD (2015). p62 links autophagy and Nrf2 signaling. *Free Radical Biology and Medicine*, 88: 199-204.

Kadiri, HE; Okoro, IO; Ichipi-Ifukor, PC (2020). Tetrapleura tetraptera fruit protects against cyanide induced toxicity in rats. *Iraqi J. Sci.* 61(10): 2504-2514.

Kadiri, HE; Ilondu, EM (2020). Spectral characterization, antioxidant and anti-genotoxic properties of methanol and ethanol stem extracts of *Cissus araloides* hook. *EurAsian Journal of Biosciences*, 14(2): 4481-4491.

Koizumi, S; Ohsawa, K; Inoue, K; Kohsaka, S (2013). Purinergic receptors in microglia: functional modal shifts of microglia mediated by P2 and P1 receptors. *Glia*, *61*(1): 47-54.

Kyriakoudi, A; Spanidi, E; Mourtzinos, I; Gardikis, K (2021). Innovative delivery systems loaded with plant bioactive ingredients: Formulation approaches and applications. *Plants*, 10(6): 1238.

Lowry, OH; Rosebrough, NJ; Farr, AL; Randall, RJ (1951). Protein easurement with the Folin Phenol reagent. *J. Biol. & Chem.*, 193: 265-275.

Manzocco, L; Anese, M; Nicoli, M.C (1998). Antioxidant properties of tea extracts as affected by processing. *Food Sci. Technol.*, 31(7–8): 694 – 698.

Marcocci, I; Marguire, JJ; Droy-lefaiz, MT; Packer, L (1994). The nitric oxide scavenging properties of Ginkgo biloba extract. *Biochemical and Biophysical Research Communications*, 201:748 – 755.

Miller, GL (1959). Use of dinitrosalicylic acid for determination of reducing sugar. *Anal. Chem.*, 31(3): 426 – 428.

Njoku, OV; Obi, C (2009). Phytochemical constituents of some selected medicinal plants. *African J.f Pure and Applied Chemistry*, 3 (11): 228 – 233.

Ogunka-Nnoka, CU; Ohwokevwo, OA; Onyeike, EN (2019). Nutraceutical potentials of *Spilanthes filicualis*. *Global J. Sci. Front. Res.*, 19(1): 22-28.

Ojo, OA; Akintayo, CO (2014). Assessment of antioxidant activity of *Ficus asperifolia* Miq aqueous extract - *In vitro* studies. *J. Phytopharmacol*., 3(1): 16 – 21.

Omoniwa, BP; Luka, CD; Soji-Omoniwa, O (2013). Effect of Aqueous Leaf Extract of *Ficus asperifolia* on Cardiac enzymes and lipid profile in male albino rats. *J. M. Sci*., 13(5): 373 – 378.

Prakash, V; Jaiswal, NISHITA; Srivastava, MRINAL (2017). A review on medicinal properties of Centella asiatica. *Asian J. Pharm. Clin. Res*., 10(10): 69-74.

Prieto, P; Pineda, M; Aguilar, M (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal. Biochem.,* 269: 337 – 341.

Sari, AC; Elya, B (2017). Antioxidant activity and lipoxygenase enzyme inhibition assay with total flavonoid assay of Garcinia porrecta Laness. stem bark extracts. *Pharmacog. J.*, *9*(2).

Shamsa, F; Monsef, H; Ghamooshi, R; Verdian-rizi, M (2008). Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. *Thailand J. Pharmaceut. Sci.*, 32: 17 – 20.

Singleton, VL; Rossi, JA (1965) Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *Am. J. Enolog. Viticult*, 16: 144-158.

Soforowa, A (1996). *LeafesMédicinales et Médecine Traditionnelle d'Afrique*. Edition Karthala; Paris, France.

Vaintraub, IA; Lapteva, NA (1988). Colorimetric determination of phytate in unpurified extracts of seeds and products of their processing. *Anal. Biochem.* 175(1): 227 – 230.

Zaini, HM; Roslan, J; Saallah, S; Munsu, E; Sulaiman, NS; Pindi, W (2022). Banana peels as a bioactive ingredient and its potential application in the food industry. *J. Funct. Foods*, 92: 105054.