



Bioactive components of *Ficus exasperata*, *Moringa oleifera* and *Jatropha tanjorensis* leaf extracts and evaluation of their antioxidant properties

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Abstract

Purpose: This study investigated the phytochemistry and *in vitro* free radical scavenging activities of aqueous leaf extract of *Ficus exasperata* (FE), *Moringa oleifera* (MO) and *Jatropha tanjorensis* (JT).

Methods: Spectrophotometric evaluation was employed in the determination of the total phenols, total flavonoids, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical inhibition, nitric oxide (NO^{*}) radical inhibition, ferric reducing antioxidant power (FRAP) and total antioxidant capacity (TAOC).

Results: Qualitative screening of bioactive compounds confirmed the presence of terpenes, flavonoids and phenols in all extracts amidst other phytochemicals. Total phenols and total flavonoids estimation were highest in *Ficus exasperata* (96.10 ± 2.70 mg gallic acid equivalent g⁻¹ and 28.45 ± 1.80 mg catechin equivalent g⁻¹, respectively). *Moringa oleifera* leaf extract exhibited the highest radical scavenging activity against DPPH and NO^{*} radicals, while *Jatropha tanjorensis* exhibited the highest FRAP and TAOC. Nitric oxide radical inhibition by *M. oleifera* extract was significantly higher (p<0.05) at 7.0 mg mL⁻¹ (64.7 ± 0.32%). The 50% inhibitory concentration (IC₅₀) values of the plant leaves for FRAP and TAOC were in the order JT < MO < FE, whereas for DPPH and NO^{*} scavenging activities the IC₅₀ values were in the order MO < JT < FE.

Conclusions: The findings of this study clearly showed that the antioxidant properties of *M. oleifera* and *J. tanjorensis* leaves were higher than those of *F. exasperata*. The knowledge of the different bioactive components and antioxidant prowess of these medicinal plants could be used as a guide in making the choice of medicinal plant to use for treatment or prophylaxis against cellular aberrations in which oxidative stress is implicated.

Keywords: antioxidant, *Ficus exasperata*, free radicals, *Jatropha tanjorensis*, *Moringa oleifera*

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INTRODUCTION

Side reactions are commonly associated with energy metabolic pathways and are sometimes responsible for cellular degeneration and death (Selamoglu et al. 2017). The production of free radicals from metabolic side reactions and exogenous sources are known to mediate pathological conditions (Atanu et al. 2018, Pavithra and Vadivukkarassi 2015). Health challenging conditions have been bemoaned to activities of ROS (reactive oxygen species) and RNS (reactive nitrogen species) resulting in distortion of normal metabolic activities. Generally, radicals implicative role in disease pathology are attributed to loss of cell membrane stability, consequently resulting in interactions with basic molecules of proteins, carbohydrates, nucleic acids and lipids (Rahman et al. 2015). Oxidative products such as DNA adducts, carbonyl formation, ketoamines and

ketoaldehydes, aldehydes and malonyl aldehydes, 4-hydroxy-2-nonenal are deleterious to the cell, which are marked by oxidative stress (Fajer et al. 2018, Kunwar and Priyadarsini 2011, Noori 2012). Since several pathological conditions are attributed to oxidative stress, modern approach towards finding cure to disease conditions are being viewed from the perspective of preventing oxidative stress mediated process. This is possibly achieved via free radicals scavenging and radicals quenching (Karadag et al. 2009, Meles et al. 2019). Based on the fact that the endogenous antioxidants defense system depletes with time, supplementary requirements towards enhancing the endogenous system become essential in achieving

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better health (Farhan et al. 2019, Tonukari et al. 2013). Polyphenolic compounds and other related plant pigment are widely studied and used in clinical trial for prophylaxis and chemotherapy; this is due to their relative abundance in plants and their reduced side effects, as they are known to be rich sources of exogenous antioxidant (Al-samarrai et al. 2019, Ogunka-Nnoka et al. 2019).

Ficus exasperata, *Moringa oleifera* and *Jatropha tanjorensis* are shrubby plant predominantly found in the tropics and subtropics. They belong to the families of *Moraceae*, *Moringaceae* and *Euphorbiaceae*, respectively. In folklore medicine, *Ficus exasperata* is used for treatment of skin infections, cough and hemorrhoids, peptic ulcers, anti-inflammatory, antipyretic and antinociceptive properties as well as in enhancing proper functioning of uterine smooth muscles and hypertensive effect (Ughachukwu et al. 2012). *Moringa oleifera* is used in the treatment of inflammation and infectious diseases along with cardiovascular, gastrointestinal, hematological and hepato-renal disorder (Gopalakrishnan et al. 2016, Singh et al. 2013); while *Jatropha tanjorensis* are employed in the treatment of anaemia, diabetes and cardiovascular diseases (Chigozie et al. 2018). In this present study, we investigated the phytochemistry and *in vitro* free radical scavenging activities of aqueous leaf extract of *Ficus exasperata*, *Moringa oleifera* and *Jatropha tanjorensis*. The knowledge of their different bioactive components and antioxidant prowess could be used as a guide in choosing the best medicinal plant leaf for treatment or prophylaxis against cellular aberrations in which oxidative stress is implicated.

MATERIALS AND METHODS

Collection of Plant Material and Identification

Ficus exasperata, *Moringa oleifera* and *Jatropha tanjorensis* were harvested from an open space field at Ovu Town, Delta State, Nigeria in the Month of February, 2019. The plants specimen were identified and authenticated at the Department of Botany, University of Benin, Nigeria.

Extract Preparation

The Fresh leaves of *Ficus exasperata*, *Moringa oleifera* and *Jatropha tanjorensis* were washed with distilled water, debris were removed and air dried for two weeks and then reduced to coarse powder using a manual grinder. 100 g of coarsely powdered leaves were extracted with 400 mL of distilled water using cold maceration for 24 h. The extract was then filtered through cheese cloth with fine pore, and the filtrate was filtered for the second time using Whatman No. 1 filter paper. The resulting extract was then concentrated at 50°C in a rotary evaporator for 2hr and it was then transferred to a water bath maintained at 50°C and evaporated to dryness to yield a dark brown mass. The

obtained extract was put in a glass container and stored at 4°C until when required for use.

Qualitative Phytochemical Screening

Phytochemical screening of aqueous leaf extracts of *Ficus exasperata*, *Moringa oleifera* and *Jatropha tanjorensis* was carried out using standard methods described by Jamil et al. (2012) and Njoku and Obi (2009).

Determination of Total Phenol and Flavonoid Contents

Quantitative phytochemical determination of total phenol and flavonoids was carried out by the methods of Singleton and Rossi (1965) and Jia et al. (1999). The total phenol and flavonoid contents were expressed in milligramme gallic acid equivalent per gramme (mg GAE g⁻¹) and milligramme catechin equivalent per gramme (mg CAE g⁻¹), respectively.

Determination of Antioxidant Activity and Free Radical Scavenging Potential

DPPH radical scavenging activity assay

The scavenging activity of the leaf extracts on the free stable 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) was carried out as described by Manzocco et al. (1998). Briefly, 0.2 ml of different concentrations of sample was added to 2 ml of DPPH solution (0.3 mM) freshly prepared with methanol. After 30 min of incubation in the dark, the absorbance was measured at 517 nm. The percentage inhibition of DPPH radical was estimated as follow:

$$\% \text{ Inhibition of DPPH radical} = ([A_0 - A_1] / A_0) \times 100$$

Where A_0 is the absorbance of the control (blank, without extract) and A_1 is the absorbance in the presence of the extract.

Nitric oxide (NO[•]) free radical scavenging activity

The method of Marcocci et al. (1994) was followed. Two millilitres of 10 mM sodium nitroprusside dissolved in 0.5 ml of 10 mM phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of different concentrations of samples. The mixture was then incubated at 25°C. After 150 min of incubation, 0.5 ml of the incubated solution was withdrawn and mixed with 0.5 ml of Griess reagent [(1.0 ml sulfanilic acid reagent (0.33% in 20% glacial acetic acid at room temperature for 5 min with 1 ml of naphthylethylenediamine dichloride (0.1% w v⁻¹)). The mixture was then incubated at room temperature for 30 min and its absorbance was measured at 546 nm against blank. The percentage inhibition of NO radical was calculated as follow:

$$\% \text{ Inhibition of NO radical} = ([A_0 - A_1] / A_0) \times 100$$

Where A_0 is the absorbance of the control (blank, without extract) and A_1 is the absorbance in the presence of the extract.

Table 1. Phytochemical constituents of aqueous leaf extract of *Ficus exasperata*, *Moringa oliefera* and *Jatropha tanjorensis*

Phytochemicals	<i>Ficus exasperata</i>	<i>Moringa oliefera</i>	<i>Jatropha tanjorensis</i>
Saponins	+	-	+
Phlobatannins	-	-	-
Cardiac glycosides	-	-	+
Flavonoids	+	+	+
Tannins	++	-	+
Phenols	+	+	+
Steroids	-	-	-
Terpenes	++	+	+
Carbohydrate	-	+	-
Protein	-	-	-
Amino acid	-	-	-
Gum and mucilage	-	+	-

Key: + = Present, - = Absent.

Ferric reducing antioxidant power assay (FRAP)

Two and half millilitres of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of $K_3Fe(CN)_6$ (1% w v⁻¹) were added to 1.0 ml of different concentrations of samples (Oyaizu 1986). The resulting mixture is incubated at 50°C for 20 min, followed by the addition of 2.5 ml of Trichloroacetic acid (10% w v⁻¹). The mixture was centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution (2.5 ml), mixed with distilled water (2.5 ml) and 0.5 mL of $FeCl_3$ (0.1%, w v⁻¹). The absorbance was then measured at 700 nm against blank sample (that contained distilled water and sodium phosphate buffer).

Determination of total antioxidant capacity (TAOC)

The reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) of 1.0 mL was taken in screw capped tubes to which 0.1 mL samples were added and dissolved (Prieto et al. 1999). The tubes were capped and incubated in a thermal block at 95°C for 90 min. After cooling to room temperature, the absorbance of the aqueous solution in each tube was measured at 695 nm against a blank. Gallic acid was used as standard and the total antioxidant capacity was expressed as equivalents of gallic acid (GAE).

Statistical Analysis

Data obtained were subjected to statistical analysis. Values were reported as Mean ± Standard deviation. For comparisons between samples, data was analyzed by ANOVA and Tukey's multiple comparison test (SPSS, version 17). The results were considered significant at p-values of less than 0.05 (p<0.05).

RESULTS

Phytochemical Constituents of Aqueous Leaf Extract of *Ficus exasperata*, *Moringa oliefera* and *Jatropha tanjorensis*

The results of the qualitative phytochemical screening as presented in **Table 1** showed bioactive ingredients of saponins and tannins were detected in

Table 2. Total phenolic and flavonoid contents of aqueous extract of leaf *Ficus exasperata*, *Moringa oliefera* and *Jatropha tanjorensis*

Phytochemicals	<i>Ficus exasperata</i>	<i>Moringa oliefera</i>	<i>Jatropha tanjorensis</i>
Total phenols (mg GAE g ⁻¹)	96.10 ± 2.70 ^a	85.15 ± 0.90 ^b	95.03 ± 0.13 ^a
Total flavonoids (mg CAE g ⁻¹)	28.45 ± 1.80 ^a	7.20 ± 0.73 ^b	10.52 ± 0.24 ^c

Values are means ± standard deviations of triplicate determinations. Superscripts bearing different values in the same row differ significantly (p<0.05). GAE = Gallic acid equivalent; CAE = Catechin equivalent

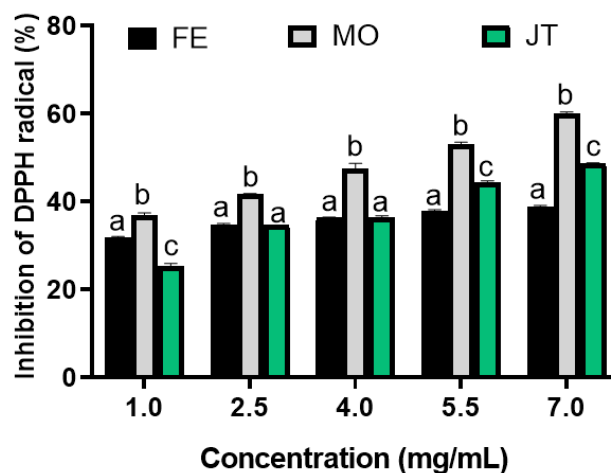


Fig. 1. Inhibition of DPPH radical (%) by *Ficus exasperata*, *Moringa oliefera* and *Jatropha tanjorensis* leaf extracts. FE = *F. exasperata*; MO = *M. oliefera*; JT = *J. tanjorensis*. Values are means ± standard deviations of triplicate determinations. Bars bearing different letters at the same concentration differ significantly (p<0.05)

both *Ficus exasperata* and *Jatropha tanjorensis*, whereas terpenes, flavonoids and phenols were confirmed present in all three (3) extracts.

Total Phenolic and Flavonoid Contents

The results of the quantitative determination of total phenols and flavonoids are as presented in **Table 2**. The results revealed that total flavonoids values were significantly (p<0.05) highest in *Ficus exasperata* extract when compared to both *Moringa oliefera* and *Jatropha tanjorensis* extracts (*Ficus exasperata*>*Moringa oliefera*>*Jatropha tanjorensis*). Although total phenol content was also significantly higher (p<0.05) in *Ficus exasperata* leaf extract than that of *Moringa oliefera*, it was not significantly different (p>0.05) from that of *Jatropha tanjorensis* leaf extract (**Table 2**).

Inhibition of DPPH Radical (%) by Aqueous Leaf Extracts of *Ficus exasperata*, *Moringa oliefera* and *Jatropha tanjorensis*

The results of the *in vitro* antioxidant and free radical scavenging activities of aqueous leaf extract of *Ficus exasperata* (FE), *Moringa oliefera* (MO) and *Jatropha tanjorensis* (JT) are presented in **Figs. 1 to 4**. The results in **Fig. 2** showed that DPPH (2, 2-diphenyl-1-picrylhydrazyl) scavenging activities of *Moringa oliefera* leaf extract were significantly higher (p<0.05) with

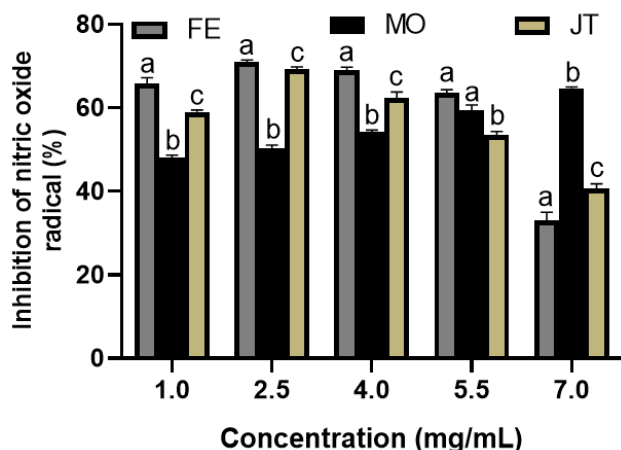


Fig. 2. Inhibition of nitric oxide radical (%) by *Ficus exasperata*, *Moringa oleifera* and *Jatropha tanjorensis* leaf extracts. FE = *F. exasperata*; MO = *M. oleifera*; JT = *J. tanjorensis*. Values are means \pm standard deviations of triplicate determinations. Bars bearing different letters at the same concentration differ significantly ($p < 0.05$)

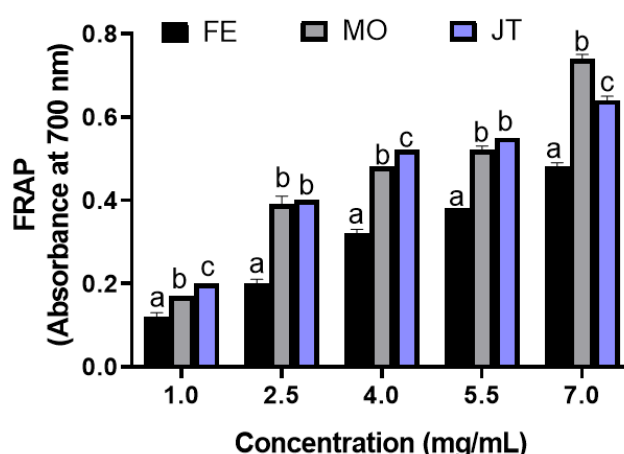


Fig. 3. Ferric reducing antioxidant power (FRAP) of *Ficus exasperata*, *Moringa oleifera* and *Jatropha tanjorensis* leaf extracts. FE = *F. exasperata*; MO = *M. oleifera*; JT = *J. tanjorensis*. Values are means \pm standard deviations of triplicate determinations. Bars bearing different letters at the same concentration differ significantly ($p < 0.05$)

Table 3. IC₅₀ for various antioxidant properties of *Ficus exasperata*, *Moringa oleifera* and *Jatropha tanjorensis* leaf extracts

Antioxidant activity	<i>Ficus exasperata</i>		<i>Moringa oleifera</i>		<i>Jatropha tanjorensis</i>	
	IC ₅₀ (mg mL ⁻¹)	R ²	IC ₅₀ (mg mL ⁻¹)	R ²	IC ₅₀ (mg mL ⁻¹)	R ²
DPPH	16.34	0.9650	4.55	0.9956	7.220	0.9678
NO•	6.241	0.8074	2.09	0.9750	6.237	0.7540
FRAP	5.160	0.9841	3.19	0.9488	3.160	0.9060
TAOC	4.420	0.8987	4.38	0.9422	3.810	0.9212

IC₅₀ = Minimum concentration required to exhibit 50% inhibition. R² = Coefficient of linear regression

increasing concentration when compared to both *Ficus exasperata* and *Jatropha tanjorensis* extracts. The minimum concentration (IC₅₀) of each plant leaf extract required to inhibit DPPH radical by 50% were 16.34, 4.55, and 7.22 mg mL⁻¹ for *F. exasperata*, *M. oleifera* and *J. tanjorensis*, respectively (Table 3). The coefficients of the linear regressions (R²) of the DPPH inhibition by the medicinal plants were 0.9650, 0.9956 and 0.9678 for *F. exasperata*, *M. oleifera* and *J. tanjorensis*, respectively. The order of the IC₅₀ values for DPPH radical inhibition was as follow: MO < JT < FE (Table 3), which affirmed that *Moringa oleifera* leaf extract was the highest scavenger of DPPH radical.

Inhibition of Nitric Oxide Free Radical

The results of the inhibition of nitric oxide radical by aqueous leaf extract of *Ficus exasperata*, *Moringa oleifera* and *Jatropha tanjorensis* are presented in Fig. 2. Nitric oxide scavenging activities were observed to be concentration dependent in both extracts of *Ficus exasperata* and *Jatropha tanjorensis* until a concentration value of 4 mg mL⁻¹ where a slight decrease was observed. *Moringa oleifera* leaf extract, however, inhibited NO• radical in a concentration dependent manner although. At a concentration of 7.0

mg mL⁻¹, the nitric oxide radical scavenging activity was significantly higher ($p < 0.05$) in *Moringa oleifera* leaf extract (64.7 \pm 0.32%) than *Jatropha tanjorensis* (40.7 \pm 1.1%) and *Ficus exasperata* (33.09 \pm 1.92%). Values of IC₅₀ of *F. exasperata*, *M. oleifera* and *J. tanjorensis* leaf extract for inhibition of nitric oxide radical were 6.241 mg mL⁻¹ (with coefficient of linear regression, R² = 0.8074), 2.09 mg mL⁻¹ (R² = 0.9750) and 6.237 mg mL⁻¹ (R² = 0.7540), respectively (Table 3). Similar to DPPH inhibition, the IC₅₀ values for inhibition of nitric oxide radical were also in the order MO < JT < FE (Table 3), which indicated that *Moringa oleifera* leaf extract was the highest scavenger of nitric oxide radical.

Ferric Reducing Antioxidant Power of Aqueous Leaf Extract of *Ficus exasperata*, *Moringa oleifera* and *Jatropha tanjorensis*

The results of ferric reducing antioxidant power of aqueous leaf extracts of *Ficus exasperata*, *Moringa oleifera* and *Jatropha tanjorensis* are presented in Fig. 3. Ferric reducing antioxidant potentials of *Jatropha tanjorensis* leaf extract were significantly higher ($p < 0.05$) from 1 mg mL⁻¹ to 5.5 mg mL⁻¹ when compared to those of both *Moringa oleifera* and *Ficus exasperata* (*Jatropha tanjorensis* > *Moringa oleifera* > *Ficus exasperata*). *Moringa oleifera* leaf extract, however, recorded the highest values at 7 mg mL⁻¹ (0.74 \pm 0.01) compared ($p < 0.05$) to both *Ficus exasperata* (0.48 \pm 0.01) and *Jatropha tanjorensis* (0.64 \pm 0.01) extracts. The IC₅₀ values of *F. exasperata*, *M. oleifera* and *J. tanjorensis* leaf extract for ferric reducing antioxidant power (FRAP) were 5.16 mg mL⁻¹ (R² = 0.9841), 3.19 mg mL⁻¹ (R² = 0.9488) and 3.16 mg mL⁻¹ (R² = 0.9060), respectively (Table 3). The IC₅₀ values for FRAP were in the order JT < MO < FE (Table 3), which indicated that *J. tanjorensis* leaf extract exhibited the highest ferric reducing power.

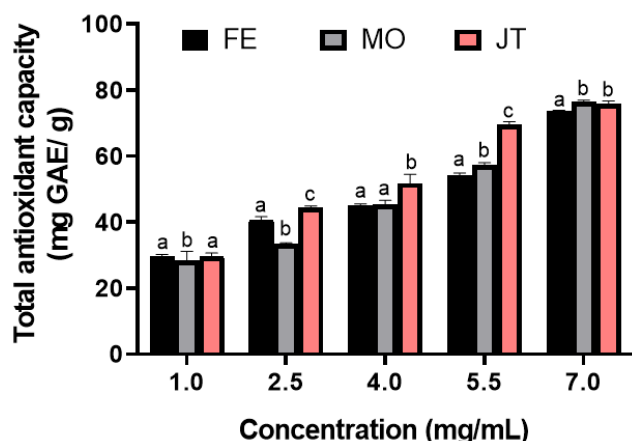


Fig. 4. Total antioxidant capacity (TAOC) of *Ficus exasperata*, *Moringa oliefera* and *Jatropha tanjorensis* leaf extracts. FE = *F. exasperata*; MO = *M. oliefera*; JT = *J. tanjorensis*. Values are means \pm standard deviations of triplicate determinations. Bars bearing different letters at the same concentration differ significantly ($p < 0.05$). GAE = Gallic acid equivalent

Total Antioxidant Capacity of the Extracts

The results of the total antioxidant capacity of aqueous leaf extracts of *Ficus exasperata*, *Moringa oliefera* and *Jatropha tanjorensis* are as shown in **Fig. 4**. The total antioxidant capacities of *J. tanjorensis* extract were seen to be significantly higher from 2.5 mg mL⁻¹ to 5.5 mg mL⁻¹ when compared to both *Ficus exasperata* and *Moringa oliefera*. However, *Moringa oliefera* leaf extract exhibited the highest values at 7 mg mL⁻¹ (76.29 \pm 0.64 mg GAE g⁻¹) compared ($p < 0.05$) to both *Ficus exasperata* (73.50 \pm 0.38 mg GAE g⁻¹) and *Jatropha tanjorensis* (75.83 \pm 0.82 mg GAE g⁻¹) extracts. **Table 3** shows the minimum concentration (IC₅₀) of the three medicinal plant leaf extracts required to exhibit 50% total antioxidant capacity (TAOC). The values of IC₅₀ for TAOC were 4.42 mg mL⁻¹ ($R^2 = 0.8987$), 4.38 mg mL⁻¹ ($R^2 = 0.9422$) and 3.81 mg mL⁻¹ ($R^2 = 0.9212$), for *F. exasperata*, *M. oliefera* and *J. tanjorensis*, respectively (**Table 3**). Similar to FRAP results, the IC₅₀ values for exhibition of 50% TAOC were also in the order JP < MO < FE (**Table 3**), which also indicated that *Jatropha tanjorensis* leaf extract exhibited the highest total antioxidant capacity (TAOC).

DISCUSSION

Basically, *in vitro* studies across all fields of biological sciences are meant to elucidate the basis by which biological molecules carry out their functions within a cell. In this study, estimation of the antioxidant capacities of the various medicinal plants' leaf extracts was done using spectrophotometric methods that are usually associated with colour reduction changes in most of the assays, as seen in DPPH from purple to yellow and FRAP (ferric reducing antioxidant power) from yellow to

prussian blue etc (Pavithra and Vadivukkarassi 2015, Rahman et al. 2015, Tonukari et al. 2015) were ascertained. Antioxidant mechanisms against the effect of radical *in vitro* species are via free electron donation, thereby countering ROS and RNS activities (Anigboro et al. 2018, Moukette et al. 2015). These actions are reflected in the various colour changes of these various assay. In some cases, certain molecules attributed to antioxidant activities like in case of ferric (III) reduction by reductones (Ogunka-Nnoka et al. 2019). The carbohydrate derivate mediates quenching of free radical chain via the donation of hydrogen atom (Singh et al. 2013, Farhan et al. 2019). NO is a unique radical essential in innate immune response towards microorganisms and tumor cells, the consequential effect of uncontrolled production results in initiation of inflammatory cascade in inflammatory site via vascular permeability and extravasation (Qaiz and Molvi 2018). The evaluation of the total antioxidant capacity (TAOC) employed in this study was to assess the cumulative antioxidants' properties of the three medicinal plants, *F. exasperata*, *M. oliefera* and *J. tanjorensis* (Lee et al. 2015).

The clamour for antioxidant compounds and properties exhibited are solely down to plant rich pigments termed phytonutrients. These groups of phytonutrients, especially the phenolics and flavonoids class, are believed and reported over time to exhibit radical scavenging activities which involve several mechanism of actions both *in vitro* and *in vivo* (Farhan et al. 2019). Plants are known to house a large group of phytochemical compounds, with more class being identified from time to time. Studies have showed that these compounds exhibit various medicinal functions (Anigboro et al. 2014). Flavonoids antioxidant properties are linked to their modulatory actions on autoxidation of lipids in cardiovascular disease and carcinogenesis (Atanu et al. 2018, Hezeena Begum and Muthukumaran 2014). Antimalarial and antibacterial properties are attributed to alkaloids, glucose and cholesterol regulations are link with saponins pharmacological activities (Cheenpracha et al. 2019, Chigozie et al. 2018, Stepanenko 2018), cardiac glycoside enhances muscular contraction of the heart.

The general performance of plant extracts in carrying out the pharmacological activities maximally is based on the presence of the bioactive ingredients of plants (Phytochemicals) that are also readily available within disease affected cells (Chigozie et al. 2018), with anti-nutrients serving as a major factor in medicinal and nutritional properties of plants.

The overall better performance exhibited by *Moringa oliefera* leaf extract, especially with respect to DPPH and nitric oxide radicals scavenging activities, could be singled out to its low levels of anti-nutrient, thereby ensuring that the bioactive molecules available are readily released for their functions. Prominently, the

ferric reducing power activity was well correlated with the detection of carbohydrates in the extract.

CONCLUSION

In this current study, *Moringa oleifera* and *Jatropha tanjorensis* leaf extracts had better antioxidants performance than *Ficus exasperata* extract, with *M. oleifera* and *J. tanjorensis* being almost equivalent among the three plant extracts investigated. The result of the phytochemical screening pin point more

abundance of phytochemicals in *Jatropha tanjorensis* extract, while the quantitative estimation of total phenol and total flavonoids were observed in highest amounts in *Ficus exasperata* extract. The current trend in this result buttresses the fact that despite relative abundance observed in terms of phytonutrients, ready availability and release are more essential toward exhibition of pharmacological activities by plants, considering the fact also that anti-nutrients are key regulators toward plant nutritional benefits and medicinal properties.

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