International Journal of Innovative Research and Advanced Studies (IJIRAS)
Volume 5 Issue 5, May 2018

ISSN: 2394-4404

Phytochemical And Antioxidant Study Of Different Solvents Extract Of Monechma Ciliatum (Acanthaceae): An In Vitro Study

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Abstract: ***Traditionally, Monechma ciliatum is used to treat different diseases such as body pain, liver, cold, diarrhoea and sterility in women. This study is aimed at investigating the phytochemical analysis and in vitro antioxidant activity of n-hexane, ethyl acetate and methanol leaves extract of M. ciliatum. The phytochemical analysis was performed using standard methods while the in vitro antioxidant activity was evaluated by the DPPH free radical scavenging ability. Sequential extraction with solvents of increasing polarity was carried out to obtain the n-hexane, ethyl acetate and methanol extracts. Phytochemical screening revealed the presence of alkaloids, steroids, phenols, anthraquinones, flavonoids, tannins, saponins and cardiac glycosides. The antioxidant activities of the plant were tested quantitatively and were found to be active with IC_{50} of 1.34, 1.39 and 1.44 µg/mL for the methanol, ethyl acetate and n-hexane respectively. Ascorbic acid the synthetic antioxidant employed possess a near 100% free radical inhibitory activity with an IC_{50} value of 1.04 µg/mL. The research shows lay credence to the traditional application of the plant. Further research is recommended on the isolation and characterization of the antioxidant compounds from this plant.***

Keywords: Antioxidant, ROS, free radical, DPPH, phytochemical

I. INTRODUCTION

The use of medicinal plants for the treatment of diverse diseases is widespread, and has been proficient for many years (Afaf *et al*., 2015). Phytochemical components in medicinal plants are of great importance in the manufacture of drugs (Kitaz, 2017) and the affectivity of many available drugs is studied by many workers to validate the folkloric claims of medicinal plants for several pharmacological activities (Firuzi *et al*., 2005).

Antioxidants are group of substances that when present in low concentrations compared to those of an oxidisable substrate significantly delays or prevents oxidation of that substance while preferentially being oxidized themselves (Auudy *et al*., 2003) They protect cells against the destructive effects of reactive oxygen species (ROS), such as superoxide

anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (HO•) formed by the partial reduction of oxygen (Abdul *et al*., 2017). An imbalance between antioxidants and ROS results in oxidative stress in cell and leading to cellular spoil (Vaz *et al*., 2011). This situation often leads to physiological disorders viz., cancer, ageing, atherosclerosis, inflammation, ischemic injury and neural degeneration (Kitaz, 2017; Abdul *et al*., 2017). Accumulated evidence suggests that reactive oxygen species (ROS) can be scavenged through chemoprevention utilizing natural antioxidant compound present in foods and medicinal plants (Nabilah *et al.*, 2011).

Monechma ciliatum (Jacquin) locally known as "Damfarkami" in the Hausa language belong to the family Acanthaceae. The plant (Figure 1) is a small herb that grows a few inches above the ground. Its leaves are simple, measuring about 4–7 x 1–2 cm (Murtada and Abdelkarim, 2013). The leaves are linear or narrowly linear-lanceolate growing up to 10 cm long and 1.25 cm broad (Taha and Mustapha, 2015). The leaves of the plant has been reported to be used as remedy for general body pain, diarrhea, sterility in women and has been demonstrated to possess oxytocic property *in vivo* and *in vitro* (Mariod *et al*., 2010). This research is aimed at investigating the qualitative phytochemical constituent and the *in vitro* antioxidant activity of the crude extracts in an attempt to validate or otherwise its tradomedicinal uses.

Figure 1: M. ciliatum in its natural habitat

II. MATERIALS AND METHODS

A. PLANT MATERIALS COLLECTION, PREPARATION AND IDENTIFICATION

Fresh and healthy leaves of *M. ciliatum* were collected from the premises of Sokoto State University Sokoto, North Western Nigeria in June, 2017. They were washed with clean running water to remove earthy impurities, identified and authenticated at the Botany Unit, Department of Biological Science, Usmanu Danfodiyo University, Sokoto where a herbarium specimen was deposited and a voucher number UDOH/ANS/0191 issued. They were then air dried for three weeks, powdered with the aid of a clean mortar and pestle before being stored in an air tight glass container until use.

B. CHEMICALS

Methanol (Sigma Aldrich), 1,1-Diphenyl-2-picrylhydrazyl (Merck India), acetone (Merck India), ethyl acetate (Sigma Aldrich), n - hexane (Sigma Aldrich) and other chemicals and reagents were all of analytical grade.

C. EXTRACTION

100 g of the powdered leaves was macerated with 500 mL analytical grade n-hexane for 48 hours. Stirring was performed intermittently to enhance the extraction. The n-hexane solvent with its extracted components was decanted and filtered with Whatman filter paper. The filtrate was concentrated under reduced pressure at 45°C in a rotator evaporator and dried at room temperature to constant weight. This was the n-hexane leaves extract (NHE). The same procedure was employed using ethyl acetate and methanol to obtain the EAE and ME respectively. All the extracts were concentrated in the rotary evaporator at 45^{o}C and stored at 4^{o}C till use.

D. PHYTOCHEMICAL ANALYSIS

Simple chemical tests to detect the presence of carbohydrates, proteins and secondary metabolites in the NHE, EAE and ME fractions were done in accordance with standard methods (Stahl, 1973; Sofowora, 1982; Trease and Evans, 1978).

E. ANTIOXIDANT ASSAY USING 2, 2-DIPHENYL-1-PICRYLHYDRAZYL (DPPH)

The DPPH assay has been largely used as a quick, reliable and reproducible parameters to search for the in-vitro antioxidant parameter of pure compounds as well as plant extracts. The scavenging effect of extracts on DPPH radical was estimated with method described by Ayoola *et al*., 2008. The concentrations 6.25, 12.5, 25.0 and 50.0 µg/mL in methanol of the extracts. A blank solution was prepared to contain the same amount of methanol and DPPH but lacks the extract(s) and the standard antioxidant. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 minutes. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid with the same concentrations was used as reference standard. The ability to scavenge DPPH radical was calculated by the following equation:

$$\text{DPPH RSA}(\%) = \frac{A0-A1}{A0} \text{ X } 100$$

Where RSA = radical scavenging activity; A_0 was the absorbance of DPPH radical + methanol, A_1 was the absorbance of DPPH radical + sample extract /standard. The values are presented as the means of triplicate analysis. The IC_{50} of the various extracts were also calculated by plotting a graph of the percentage inhibition against the logarithm to base 10 of the concentration and then extrapolated from the 50 % inhibition.

III. RESULTS AND DISCUSSION

A. PERCENTAGE YIELDS OF THE EXTRACT

The percentage yield of the n-hexane, ethyl acetate and methanol are 1.61%, 2.03% and 5.31% respectively. The result obtained is as a result of the variation in their polarity. The result is in agreement with the report of Halilu *et al*., 2013

who stated that polar solvents is a better extractor of phytochemicals than non polar solvents.

B. PHYTOCHEMICAL ANALYSIS

The preliminary phytochemical screening of extracts of *M. ciliatum* showed the presence of important secondary metabolites which is presented in Table 1.

Phytochemicals	Tests	Extracts		
		NHE	EAE	ME
Alkaloids	Mayer's	ND	ND	+
	Dragendroff's	ND	ND	+
	Wagner s	ND	ND	+
	Hagers	ND	ND	+
Steroid/Triterpenes	Salkowski	+	+	+
	Liebermann-Burchard	+	+	+
Phenolic compounds	Ferric chloride	+	+	+
	FolinCiocalteu's	+	+	+
Anthraquinone	Borntrager's	ND	ND	+
	Ferric chloride	ND	ND	+
Flavonoids	Shinado Test	-	+	+
	Ferric chloride	-	+	+
	Lead acetate	-	+	+
Tannins	Ferric chloride	-	-	+
	Gelatin	-	-	+
Saponins	Frothing	-	+	+
Cardiac glycosides	Keller Kiliani's	+	+	+

Key: (+) = presence of phytochemical; (-) = absence of phytochemical; ND = not detected

Table 1: Preliminary phytochemical screening of M. ciliatum Leaves Extracts

The preliminary phytochemical screening of the extracts of *M. ciliatum* showed the presence of important secondary metabolites presented in Table 1. Phytochemical are secondary metabolites that enable plants to overcome temporary or continuous threats integral to their environment, which is beneficial to the human in term of medical treatment (Molyneux *et al*., 2007). The result shows that methanol being a better solvent in terms of its percentage yield extraction; also contain the highest number of active phytochemicals which is in agreement with the findings of Halilu *et al*., 2013. The presence of these phytochemicals is responsible for the diverse tradomedical application of the plant.

C. DPPH RADICAL SCAVENGING ASSAY

The DPPH free radical scavenging property of the extracts of *M. ciliatum* is shown in Figure 1

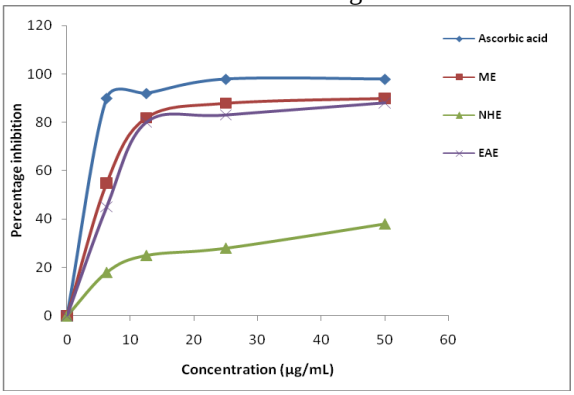

Figure 1: DPPH radical scavenging activity of the extracts of M. ciliatum and the synthetic antioxidant (ascorbic acid) at different concentrations. Each point is a mean from triplicate measurement

Ideally wide ranges of antioxidant defense mechanism protect the body against the harmful effect of free radicals which are continuously produced as a result of normal metabolic processes in the body (Halliwell *et al*., 1989). Oxidative stress on the other hand is a consequence of an imbalance between the production of reactive oxygen species (ROS) and antioxidants defense system of human body (Abuashwashi and Palomino, 2016). Antioxidants both enzymatic and non enzymatic antioxidants defence systems helps to protect the body from the damages caused by ROS like superoxide, hydroxyl, hydrogen peroxide and nitric oxide (Salem *et al*., 2015).

Several researchers have reported that medicinal plants due to the presence of phenolics compounds, carotenoids, vitamins and terpenoids help to suppress the production of oxidative stress by increasing the antioxidants systems (Tohda *et al*., 2006). The potency of these compounds lies in their potency to scavenging free radical and reduces the development of oxidative stress synonymous with many chronic diseases.

The extracts were able to capable of scavenging DPPH free radical in a concentration dependent manner in the order ME > EAE > NHE (Fig 1). The IC_{50} of ascorbic acid, ME, EAE and NHE were 1.04 µg/mL, 1.34 µg/mL, 1.39 µg/mL and 1.44 µg/mL respectively. This is in agreement with the findings from the phytochemical screening which shows the presence of phenolics, tannins and flavonoids in the ME, phenolics and flavonoid in the EAE while only phenolics was found in the NHE. This is in agreement with the report of Sheel *et al*., 2014; Gokhan *et al*., 2016 and Kitaz, 2017 who stated that there is an encouraging association between the phenolic content and radical-scavenging activity. The result also showed that the standard antioxidant (ascorbic acid) had stronger activity than the tested extracts, probably because the former contain more purified compounds than the latter.

IV. CONCLUSION

The present study summarizes that *M. ciliatum* is a good source of various metabolites like steroids, flavonoids, phenolics, alkaloids among others. The extracts of the plant showed promising radical scavenging activity comparable to standard ascorbic acid hence the leaves of the plant could be a potential source of natural antioxidant. These results encourage researchers to do further *in vitro* and *in vivo* research that will explore the role of bioactive constituents responsible for these activities as well as carry out studies at molecular level.

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