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Antioxidant, Toxicity and Phytochemical Screening of Extracts Obtained from Mariscus alterifolious Vahl

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Abstract
The plant *Marisus alternifolious* Vahl is a weed used in ethno medicine for the treatment of wounds, bacterial and gonorrheal infections. The dried whole plant was extracted with methanol (MMA) and partitioned using hexane (HMA), ethylacetate (EMA) and butanol (BMA). The fractions were subjected to phytochemical screening, antioxidant assay by the free radical scavenging effect on 2,2-diphenyl picryl hydrazyl radical and hydrogen peroxide while toxicity assay was by Brine shrimp lethality test. Phytochemical screening revealed the presence of alkaloids, flavonoids, cardiac glycosides, phenolics, steroids, saponins, tannins, terpenoids and reducing sugars as secondary metabolites. Brine shrimp lethality assay revealed that the hexane fraction was toxic with LC_{50} of 106.98 µg/ml while the ethylacetate, methanol and butanol fractions were non toxic with LC_{50} values >1000 µg/ml. HMA (76.22%), EMA (72.80%), MMA (76.04%), BMA (77.94%) at 0.0625 mg/ml scavenged free radicals better than ascorbic acid (AA) (44.0%) at the same concentration. In the hydrogen peroxide test, HMA (73.25%); EMA (79.55%); MMA (77.49%) and BMA (81.64%) scavenged hydroxyl radical at 0.25 mg/ml better than ascorbic (AA) (66.45%) even at its highest concentration (1.0 mg/ml). Phytochemicals like flavonoids, tannins and phenolics present in this plant may be responsible for its antioxidant activity and the wound-healing ability of the plant in ethno medicine.
Keywords: *Mariscus alterifolious,* phytochemicals, antioxidants, free radicals, toxicity

1. Introduction

Alkaloids, saponins, tannins, flavonoids, terpenoids, steroids and other chemical compounds which have preventive and curative properties are responsible for pharmacological activity of most plants. These plants which constitute one of man's most important natural resources, produce a diverse array of bioactive molecules, thereby making them a rich source of diverse type of medicines such as antimicrobial, anticancer, antiviral, antioxidant, hepatoprotective agents amongst others (Farnsworth and Bingel, 1977, Newmann, *et al*., 2000, Acharya and Shrivastava, 2008, Sofowora, 2008).

Antioxidant chemistry is a major research focus of many scientists in recent times due to the fact that antioxidants inhibit oxidation reactions; a chemical reaction that transfers electrons from a substance to an oxidizing agent producing free radicals. Free radicals start chain reactions that damage living cells, degrade minerals and spoil food. Oxidation has therefore been implicated in many diseases. Antioxidants can therefore limit the risk of life-threatening diseases such as cancer and heart diseases (Potterat, 1997, Sies, 1997, Vertuani *et al.,*, 2004, Duarte and Lunec, 2005, Thang *et al.*, 2011). Our group has been involved in the screening of many indigenous plants for inherent biological activity, isolation and characterization of secondary plant metabolites (Onocha et al., 2003, Onocha and Ali, 2010, Onocha and Olusanya, 2010, Oloyede et al., 2010, Oloyede and Farombi, 2010, Onocha et al., 2011a,b). *M. alternifolious* Vahl used in ethno medicine is one of such plants being investigated.

The plant *Mariscus alternifolius* Vahl found in the tropics of Africa is a specie of the genus *Mariscus* Vahl of the family Cyperaceae. The specie originated from South Africa and is popularly referred to as "royal flatsedge." *M. alternifolious* Vahl is a weed found in wet sites and damp grassy places throughout the South-Western region of Nigeria and is used generally in the treatment of wounds, bacterial and Gonorrheal infections. The swollen Culm-bases are edible when cooked (Burkill, 1985, Jstor, 2011, Soladoye et al, 2013). Adeniyi *et al*.(2013, 2014) reported the presence of reducing sugars and antimicrobial activities of some of the extracts of *M. alternifolius* Vahl and suggested that the sedge plants could further be exploited to determine their active secondary plant metabolites. It was further reported that the plant can also be employed in phytoremediation (Ogbo et al., 2009). The chemical composition, free radical scavenging and antimicrobial activities of the essential oil of *M. alternifolius* Vahl has been reported (Onocha et al., 2015) indicating that the plant is a rich source of phytochemicals of medicinal importance. In continuation of our studies on medicinal plants we now report the antioxidant, toxicity and phytochemical screening of extracts of *Mariscus alternifolius* Vahl.

2. Materials and Methods

2.1 Sample collection and preparation

Fresh whole plant of *Mariscus alternifolius* Vahl were collected from the compound of Sacred Heart Hospital, Lantoro, Abeokuta in March 2011 and identified by Mr. Esimenkhuai Donatus, of the Herbarium Laboratory

Department of Botany, University of Ibadan. It was confirmed by Messrs. Ekundayo A.A and Ugbogu O. A, both of the Forestry research institute of Nigeria (FRIN), Jericho, Ibadan where a herbarium specimen was deposited (FHI 108908). More samples were collected from the University of Ibadan botanical garden's environment in April, 2012. The plant samples (10.7 kg) were air-dried and crushed using Thomas-Willey milling machine at the wood extraction laboratory, Department of Chemistry, University of Ibadan, Ibadan, Nigeria.

2.2 Chemicals and Reagents

Methanol, n-Hexane, ethyl acetate, ethanol, chloroform, Fehling's solution A and B, 5% ferric chloride, Dragendoff's reagent, disodium hydrogen phosphate, dihydrogen potassium phosphate, hydrogen peroxide, iodine, sodium chloride, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), conc. sulphuric acid, α-tocopherol, butylated hydroxylanisole, ascorbic acid and 1% hydrochloric acid.

2.3 Equipment and Apparatus

Soxhlet apparatus, Weighing balances, Pre-coated TLC plates, UV-visible spectrophotometer (UNICO 1200), pH meter (3520 pH meter JENWAY), Fourier Transform Infra red spectrophotometer,

2.4 Extraction

The milled plant sample weighing 4.4 kg was extracted with 8.5 liters methanol using an improvised Soxhlet apparatus. The crude extract (methanol extract MMA) obtained was partitioned using n-hexane, ethylacetate and butanol to obtain the hexane (HMA), ethylacetate (EMA) and butanol (BHA) fractions respectively.

2.5 Phytochemical Screening

Phytochemical screenings as described by Harborne (1998) were carried out on the extracts to determine the presence of secondary metabolites such as alkaloids, cardiac glycosides, flavonoids, glycosides, phenolics, reducing sugars, saponins, steroids, tannins and terpenoids.

2.6 Brine shrimp lethality Test.

Toxicity to lower organisms was tested using the Brine shrimp lethality test. Brine shrimp eggs (50 mg) obtained from Artermia Incorporated, USA were hatched in sea water poured into a plastic having a divider which cut it into two unequal compartments (70/30) and was allowed to stay for 48 hours. The hatched larvae moved into the smaller compartment.

Stock solution of extracts were prepared by dissolving 20 mg in 1ml of DMSO and diluted with 1 ml of sea water to give a solution containing 1000 µg/ml as the stock. Other concentrations, 100 µg/ml and 10 µg/ml were prepared from the stock solution by serial dilution. Ten active-hatched shrimps were added into test tubes. The analysis was done in triplicate. Each test tube containing 0.2 ml of each concentration was diluted with 4 ml of sea water. 10 larvae of brine shrimps were introduced into the test tubes with the aid of a dropping pipette. A control experiment containing only the sea water, DMSO and the brine shrimp was carried out. The set-up was left for 24 hours after which the survived larvae brine shrimps were counted and recorded and the results averaged. The median lethality concentration, LC_{50}, was deduced for each extract by probit analysis using Finney computer programme (Vanhaecke, 1981, Sleet and Brendel, 1983, Karishnaraju, 2005).

2.7 Free radical scavenging activity of DPPH

The crude methanol extract, hexane, ethyl acetate and butanol fractions were screened for free radical scavenging activity using a stable radical 2, 2-diphenyl-1-picrylhydrazyl radical; 3.4 mg was dissolved in 100 ml of methanol to give a 100 µM solution and 0.5 ml each of samples taken from the stock solution of 1.0 mg of sample in 100 ml of methanol was added to 3.0 ml of methanol solution of DPPH. The decrease in absorption at 517 nm of DPPH was measured 10 min later. The actual decrease in absorbance induced by the test compound was calculated by difference between the absorbance of test compound and that of the control. The absorbance of the blank or control, methanol solution of DPPH was also taken. All absorbance measurements of samples were run in triplicates. Other concentrations (0.5, 0.25, 0.125 and 0.0625 mg/ml.) were prepared from the stock solution by serial dilution and analyzed. The same experiment was carried out on butylated hydroxyl anisole (BHA), ascorbic acid and α-Tocopherol which are antioxidant standards. The Radical scavenging activity (RSA) was calculated as the percentage inhibition of DPPH (Oloyede and Farombi, 2010, Onocha *et al.,* 2011a and 2011b).

2.8 Scavenging Effect on hydrogen peroxide (H_2O_2) radical

The extracts of *M. alternifolius* Vahl was tested for their hydroxyl radical scavenging activity and was determined spectrophotometrically at 285 nm. A solution of 2 mM hydrogen peroxide was prepared in phosphate buffered-saline (PBS - pH 7.4). Concentration ranging from 1.0-0.0625 mg/ml was added to the H_2O_2 solutions. The decrease in absorbance of H_2O_2 at 285 nm was determined 10 minutes later against a blank solution containing the

test extract in PBS without H_2O_2. All tests were run in triplicates. The results were presented as percentage inhibition and calculated as in the DPPH experiment (Oloyede and Farombi, 2010, Onocha *et al.,* 2011a and 2011b).

3. Results and Discussion

On phytochemical screening, the crude methanol extract of *M. alternifolius* Vahl was found to contain all the secondary metabolites screened namely: Phenolics, carbohydrates, tannins, alkaloids, glycoside, cardiac glycosides, flavoniods, reducing sugars, steroids, terpenoids and saponins. The other three fractions were found to contain alkaloids and terpenoids. The hexane and ethylacaetate fractions contained cardiac glycosides, saponins and steroids while tannins and reducing sugars were present in the ethylacetate fraction. Only the hexane, and butanol fractions were found to contain flavoniods. Phenolic compounds are synthezied in plants as secondary methabolites. They exhibit several biological activities such as antioxidation, anti-inflamatory, anti-aging as well as inhibition of angiogenesis and cell proliferation. Most of these biological activities have been associated with their intrinsic reducing capability towards pro-oxidants (Duarte and Lunec, 2005, Han *et al*; 2007)

The result of phytochemical screening obtained gave credence to the pharmacological application of *M. alternifolious* Vahl in ethnomedicine. Many of these phytochemicals are cytotoxic in nature and some have the ability to scavenge radical species or act against microorganisms.

3.1 Brine shrimp toxicity test

The hexane fraction had LC_{50} of 106.9819 µg/ml while the ethylacetate, methanol and butanol fractions had LC_{50} values of 2549.0740, 4299.8890 and 2892.800 µg/ml respectively (Table 1) indicating that the non-polar fraction of *M. alternifolious* Vahl contains toxic metabolites while the other fractions were non

Table 1: Brine shrimp toxicity test

s/n	FRACTION	SOLVENT	CONCENTRATION (µg/ml)	*No*	*Nos*	*NoD*	LC_{50} (µg/ml)
1	MMA	Sea water and DMSO	1000 100 10	30 30 30	20 25 27	10 5 3	4299.8890
2	HMA	Sea water and DMSO	1000 100 10	30 30 30	0 16 30	30 14 0	106.9819
3	EMA	Sea water and DMSO	1000 100 10	30 30 30	20 29 30	10 1 0	2549.074
4	BHA	Sea water and DMSO	1000 100 10	30 30 30	24 27 30	6 3 0	2892.800
5	Control	Sea water and DMSO	0	30	30	0	-

'DMSO'= dimethyl suphoxide. *'**No**'* is the initial number of active/living shrimps. *'**Nos**'* is the number of survival after 24 hours. *'No_D'* is the number of dead shrimps after 24 hours. *'LC_{50}'* is the lethality at 50% concentration. $LC_{50} \geq 1000$ = Non toxic. $LC \leq 1000$ = Toxic

toxic. The brine shrimp lethality test is an *in vitro* toxicity screening which is also predictive of cytotoxicity and pesticidal activity (Onocha *et al.,* 2011a and b). Toxicity of test samples to brine shrimps larvae is indicative of medicinal activity in inhibiting cell or tumor growth although Aiyelaabge *et al.* (2010) advised that their use at high doses should be monitored.

3.2 Scavenging effect on DPPH

Substances containing atoms with an unpaired electron in the outer orbit are free radicals while reactive oxygen species (ROS) are free radicals involving oxygen molecules. Generation of free radicals in excess and in an unregulated fashion causes serious life-threatening ailments in human and calls for balancing of generation and elimination of free radicals in human system (Potterat, 1997, Sies, 1997, Vertuani *et al.,*, 2004, Duarte and Lunec, 2005, Thang *et al.*, 2011).

Table 2: Scavenging effects of methanol, hexane, ethyl acetate and butanol extracts of *Mariscus alternifolius* Vahl on DPPH*

	Concentration (mg/ml).				
SAMPLE	1.0	0.5	0.25	0.125	0.0625
MMA	0.3613 ± 0.000	0.3687 ± 0.005	0.3130 ± 0.002	0.25357 ± 0.002	0.2235 ± 0.002
HMA	0.3960 ± 0.000	0.3858 ± 0.000	0.3755 ± 0.004	0.3098 ± 0.000	0.2219 ± 0.001
EMA	0.3706 ± 0.002	0.3653 ± 0.003	0.3338 ± 0.001	0.2753 ± 0.006	0.2538 ± 0.001
BMA	0.3242 ± 0.016	0.2881 ± 0.000	0.2757 ± 0.000	0.2589 ± 0.003	0.2058 ± 0.000
BHA	0.0370 ± 0.006	0.0480 ± 0.002	0.0490 ± 0.004	0.0650 ± 0.003	0.1600 ± 0.091
AA	0.0850 ± 0.010	0.2890 ± 0.028	0.2980 ± 0.024	0.3200 ± 0.082	0.5150± 0.015
α-Toc.	0.6800 ± 0.029	0.7040 ± 0.004	0.7050 ± 0.007	0.7070 ± 0.007	0.7210 ± 0.017

*HMA = Hexane extract, SB = Ethyl acetate extract, MMA = Methanol extract, BMA = butanol extract, BHA = Butylated hydroxyanisole, AA = Ascorbic acid, α-Toc = α-Tocopherol. Each value represents the mean ± standard deviation of triplicate analysis. Absorbance of DPPH at 517nm = 0.9330 ± 0.023.

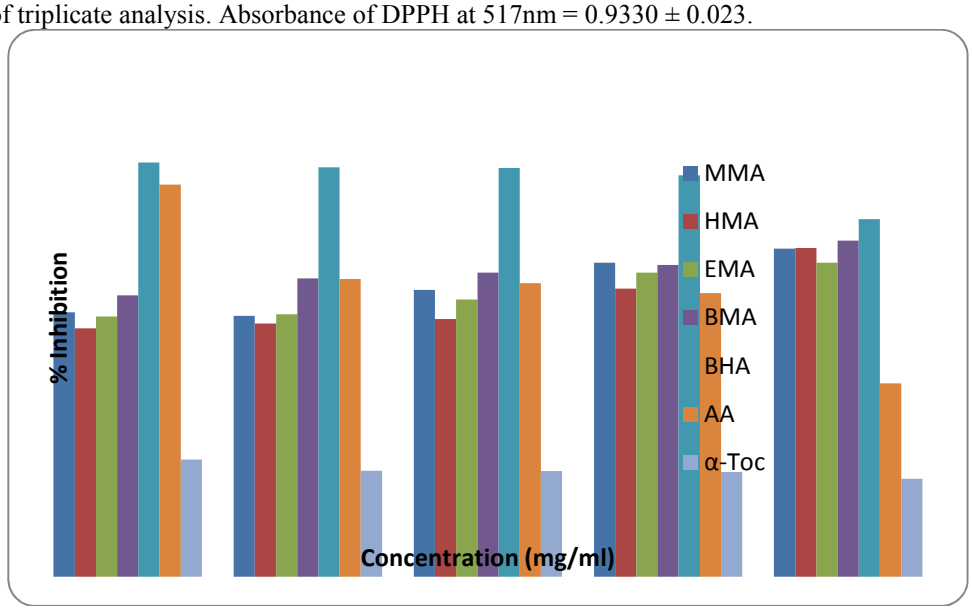

Fig 1: %inhibition for Samples, HMA = Hexane extract, EMA = Ethyl acetate extract, MMA = Methanol extract, BMA = butanol extract and reference standards; BHA = Butylated hydroxyanisole, AA = Ascorbic acid, α-Toc = α-Tocopherol in the DPPH screening.

Absorbance of samples at 517 nm on 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) showed that there was decrease in absorbance with decrease in concentrations while the standards showed increase in absorbance with decrease in concentrations (Table 2). Percentage inhibition of samples and the three standards used: Ascorbic acid (AA), Butylatedhydroxyanisole (BHA) and α-Tocopherol (Fig.1)showed that: HMA (76.22%), EMA (72.80%), MMA (76.04%) and BMA (77.94%) at 0.0625 mg/ml scavenged hydroxyl radical better than ascorbic acid and α-Tocopherol at the same concentration.

3.3 Scavenging effect on hydrogen peroxide

Reactive oxygen species (ROS) is produced in high amount at the site of wound as a defense mechanism against invading bacteria, but when the concentration of ROS is enormous, severe tissue damage can be induced and this can lead to neoplastic transformation that decreases the healing process by damaging cellular membrane, DNA, proteins and lipids (Stohs and Bagchi, (1995, Knight, 1998, Vertuani *et al.,* 2004). Antioxidants play significant role in the successful treatment and management of wounds (Houghton, 2005, Jorge, 2008). Scavenging of hydrogen peroxide by plant extracts may be attributed to the presence of important secondary metabolites such as phenolics, flavonoids and terpenoids (Oloyede and Farombi, 2010). Scavenging effects of extract and fractions of *M altern.ifolius* Vahl and standards on hydrogen peroxide (H_2O_2) radicals displayed a decrease in absorbance with decrease in concentrations while the standards exhibited increase in absorbance with decrease in concentrations at 285 nm (Table 3). The extract, fractions and standard exhibited a similar scavenging effect on hydrogen peroxide as was observed on DPPH.

Table 3: Scavenging effects of methanol, hexane, ethyl acetate and butanol extracts of *Mariscus alternifolius* Vahl on Hydrogen peroxide radicals.

	Concentration (mg/ml).				
SAMPLE	1.0	0.5	0.25	0.125	0.0625
MMA	1.1243 ± 0.000	1.0948 ± 0.001	0.9648 ± 0.008	0.8805 ± 0.000	0.8484 ± 0.008
HMA	1.1674 ± 0.070	1.0808 ± 0.003	1.053 ± 0.000	1.0091 ± 0.003	1.0118 ± 0.003
EMA	1.1714 ± 0.001	1.1169 ± 0.009	1.0657 ± 0.022	0.8648 ± 0.022	0.7708 ± 0.007
BMA	1.2205 ± 0.001	1.1921 ± 0.002	1.0994 ± 0.001	0.8930 ± 0.001	0.6921 ± 0.047
BHA	0.0413 ± 0.002	0.0617 ± 0.019	0.0740 ± 0.002	0.0947 ± 0.000	0.0174 ± 0.001
AA	0.1952 ± 0.000	0.2078 ± 0.012	1.2645 ± 0.001	2.7586 ± 0.005	2.9236 ± 0.021
α-Toc.	0.0321 ± 0.045	0.0633 ± 0.032	0.1552 ± 0.061	0.1807 ± 0.015	0.5150 ± 0.049

*HMA = Hexane extract, SB = Ethyl acetate extract, MMA = Methanol extract, BMA = butanol extract, BHA = Butylated hydroxyanisole, AA = Ascorbic acid, α-Toc = α-Tocopherol. Each value represents the mean ± standard deviation of triplicate analysis. Abosorbance of Hydrogen Peroxide at 285 nm = 3.7690 ± 0.004

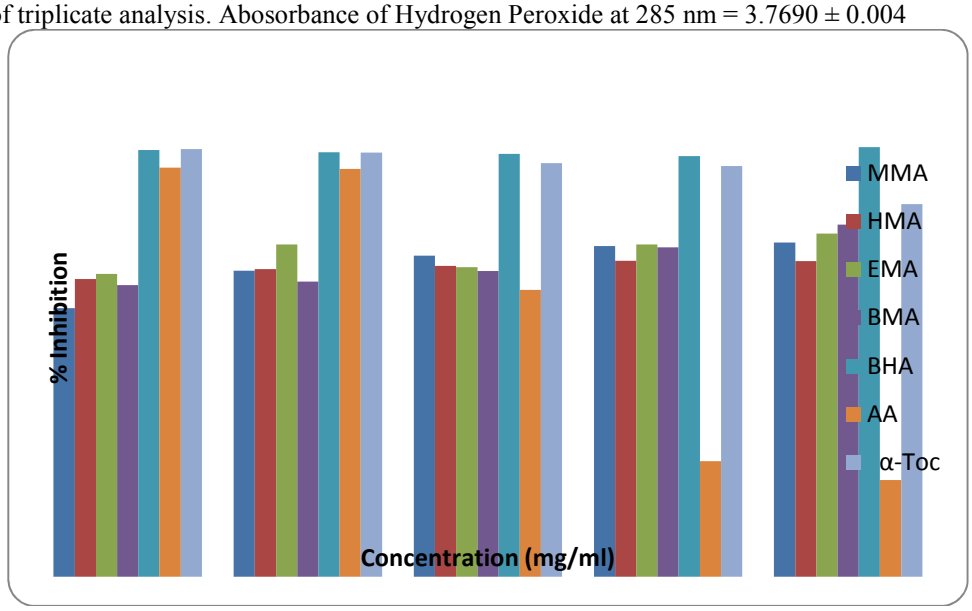

Fig 2: %inhibition for Samples, HMA = Hexane extract, SB = Ethyl acetate extract, MMA = Methanol extract, BMA = butanol extract and reference standards; BHA = Butylated hydroxyanisole, AA = Ascorbic acid, α-Toc = α-Tocopherol in the H_2O_2 screening.

Figure 2 showed that the extracts HMA (73.25%); EMA (79.55%); MMA (77.49%) and BMA (81.64%) scavenged hydroxyl radical at 0.25 mg/ml better than only AA (66.45%) even at its highest concentration (1.0 mg/ml). *M. alternifolious* Vahl's extracts were able to scavenge hydroxyl radicals generated from H_2O_2 in a concentration dependent manner with significant activity when compared with natural and synthetic antioxidants: α-tocopherol, ascorbic acid and butylated hydroxylanisole respectively. With this observed activity, it can be deduced that the extracts can also reduce ROS production at wound sites. It can therefore be inferred that the wound-healing ability of the plant may be as a result of the antioxidant activity (Miller and Britigan, 1997, Jorge, 2008, Adetutu, 2011, Ipek *et al*., 2012)

4. Conclusion

Mariscus alternifolius Vahl revealed interesting secondary metabolites which are of medicinal importance. Antioxidant screening of the plant using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical at 517 nm and hydrogen peroxide (H_2O_2) radical at 285 nm showed that the methanol extract, hexane, ethyl acetate and butanol fractions possess the ability to scavenge free radicals which cause damages to biological system. The brine shrimp lethality test carried out on the extracts showed that only the hexane extract was toxic having LC_{50} value lower than 1000 µg/ml. This study has therefore justified the ethno medicinal use of *M. alternifolious* Vahl for treating wounds.

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