Original Research Article

Activity Guided Fractionation of *Anchomanes difformis* (Blume) *Engl.* (ARACEAE) Stem Ethanol Extract: in Search of Free Radical Scavenging Agents

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Abstract

Antioxidants have been found to terminate the attack of reactive oxygen species which have been implicated in the pathophysiology of many diseases. This study is reporting the antioxidant activity and the preliminary phytochemistry of the ethanol extract from the stem of *Anchomanes difformis*. The ethanol extract (ADES) was obtained by cold maceration and partitioned into: n-hexane (ADHS), dichloromethane (ADDS) and aqueous (ADAS) fractions. Free radical scavenging activity was done using the standard diphenylpicrylhydrazine (DPPH) spectrophotometric method with ascorbic acid as the standard for comparison. Phytochemical screening was done using standard phytochemical screening reagents. Chromatography (Column and thin layer) techniques was used for the separation of antioxidant compounds from the bioactive fractions with antioxidant activity identified after spraying the developed thin layer chromatography plates with DPPH. Infra-red (IR) spectroscopy was used for functional group characterization of the isolated antioxidant compounds. The significant {$p<0.05$} trend in free radical scavenging activity (IC_{50} mg/ml) was: ADHS (>10.0) < ADES (> 10.0) < ADAS (4.0) < ADDS (1.8) < ascorbic acid (<0.3125). Saponins, sugar derivatives and triterpenoids were present as class of phytochemicals with alkaloids, anthraquinones, cardiac glycosides and phenolics absent. From IR spectroscopy analysis, antioxidant component coded ADD1 isolated from the ADDS fraction was partially characterized to be an aromatic (but not phenolic) compound having either a hydroxylated aliphatic or glycosylated side chain while the antioxidant component ADA1 isolated from the ADAS fraction was partially characterized to be a glycoside derivative with a saturated ketone aglycone moiety. The ADES was relatively safe ($LD_{50}>5000$ mg/kgbw). This result is suggestive that the ADDS and ADAS fractions are containing constituents that could act as good free radical scavengers and probably have the ability to inhibit the progression of tissue damage due to oxidative stress.
Keywords: *Anchomanes difformis,* stem, antioxidant, triterpenoids, glycosides.

INTRODUCTION

Free radicals includes the reactive oxygen species (ROS) and reactive nitrogen species (RNS). They have been implicated in some disorders as cancer, diabetes, inflammatory disease, asthma, cardiovascular diseases, neurodegenerative diseases and premature aging, as they act as precursors for oxidative damage to biomolecules [1]. The etiology of neurodegenerative disorders like Alzheimer, Parkinson and Huntington diseases is being associated closely with ROS [2]. ROS production by mitochondria can lead to oxidative damage to mitochondrial proteins, membranes and DNA thereby impairing the ability of mitochondria to synthesize ATP which is involved in a wide range of metabolic functions including the tricarboxylic acid cycle, fatty acid oxidation, the urea cycle, amino acid metabolism, and haem synthesis among others that are central to the normal operation of most cells [3]. Thus, it is no longer an over statement that ROS and RNS may be important initiators and mediators in many types of cancer[4], heart diseases, endothelial dysfunction[5], atherosclerosis and other cardiovascular disorders, inflammation and chronic inflammation[6], burns[7], intestinal tract diseases[8], brain degenerative impairments[9], diabetes[10]. Eye diseases [11], and ischaemic and post ischaemic pathologies [12]. Antioxidants play an important role in inhibition of, and/or scavenging for radicals, thus providing protection against diseases. Antioxidants inhibit many oxidation reactions caused by ROS and RNS such as singlet oxygen, superoxide radicals, peroxy radicals, hydroxyl radicals and peroxy nitrate [13]. Plant derived antioxidants have increasingly generate a lot of interest

due to the several scientific evidence supporting their role in the management of cancer, neurodegenerative diseases and other ailments whose pathophysiology is associated with oxidative stress. *Anchomanes difformis* (Blume) Engl..(Family :Araceae) is a herbaceous plant with stout prickly stem 2 m in height, having a much divided leaf, and spathe of 20 – 25 cm long. Commonly called wild yam or forest anchomanes, it is found occurring in the West African forest belt from Sierra leone to Western Cameroons[14] with the local names; Oje (Igbo), Ogirisako, Igo (Yoruba)[15], nkokot(Ibibio, South-South, Nigeria), Ebaenang(Efik, South-South, Nigeria), and Cakara (Hausa) are documented. *Anchomanes difformis* is an important medicinal plant in West Africa. The rhizome is eaten but only after special preparation that entails prolonged washing and cooking of early shooting stage [14]. Aqueous extract of the tubers are used to cure dysentery by traditional healers [16]. In French Guinea, rubefacients and vesicants are made from the rhizome for external application against measles and smallpox [17]. The root, leaves and stems are purgatives. It has also been reported in the treatment of kidney-pains, oedemas and as a diuretic for treating urethral discharge, jaundice and as poison antidote [17-18]. Abscesses can be treated with application of the root pulp with potter's clay while lactogenic ability is found in the rhizome and eye-medicine can be obtained from the stem-sap [18, 19]. Rhizome aqueous extract is used in the treatment of pain, inflammation and fever [18, 20]. The use of the powdered root mixed with palm oil has been reported as a remedy for respiratory diseases in children in Zaire (DRC), whereas in Benin republic, the root is used as a diuretic, and to treat diabetics, oral and anal legions, tuberculosis and malaria [21]. Phytochemical screening of rhizome of A*nchomanes difformis* revealed the presence of the following phytochemicals: alkaloids, tannins and saponins[16]. Phytochemical screening of its leaves revealed presence of tannins, steroids, alkaloids, saponins, flavonoids and cardiac glycosides [22]. Scientific studies have been carried out on the phytochemical and antimicrobial properties of the leaf, tuber and stem of *Anchomanes difformis* [16, 22]. Literature on antioxidant properties of root extracts of *Anchomanes difformis* have been reported [23, 24]. Literature on scientific investigation of the medicinal properties of the stem of *A. difformis* are scarce. In view of this, and considering the ethnomedicinal use of this plant in the treatment of diseases associated with ageing and degeneration, this present study is a bioactivity guided free radical scavenging fractionation evaluation of the stem ethanol extract as a preliminary step towards isolating and characterising the antioxidant compounds which could serve as possible leads in the development of drugs for the treatment of diseases associated with oxidative stress.

MATERIALS AND METHODS
Sample collection and identification
The fresh stem of *Anchomanes difformis* was collected from Rumuokparali forest Choba, Rivers State. This was authenticated by D.E. Esimonekhai of the Department of Botany, University of Ibadan, were a voucher specimen with herbarium number UIH-22361 was deposited. A voucher specimen was also deposited at the Herbarium of the Department of Pharmacognosy and Phytotherapy, University of Port Harcourt for future reference.

Reagents, solvents, Apparatus and instruments used
Analytical grade reagents used in this study include: ethanol, n-hexane, dichloromethane, methanol, acetone, ethyl acetate, sulphuric acid, distilled water, ferric chloride, ammonia, sodium hydroxide, Fehling's solution A and B, Meyer's reagent, Dragendorff's reagent, picric acid, Molisch reagent, glacial acetic acid, acetic anhydride, normal saline, blood solution, Diphenyl picrylhydrazine (DPPH). Apparatus and instruments used include : chromatography tanks, chromatography columns, UV-lamps, spectrophotometers(Spectrum Lab 752s), spray gun, water bath, weighing balances, marcerating tanks, non-heparinized micro capillary tubes, pipettes, beakers, crucibles, test tubes, glass funnels, filter papers, volumetric flasks, bijou bottles, measuring cylinders.

Sample preparation
The freshly cut stem of *Anchomanes difformis* was collected and sliced into smaller pieces with a sharp knife and dried under ambient condition. The dried stems were then pulverized using an electric blender and stored in airtight polythene bags before use.

Extraction of plant material
The dried pulverized sample (50 g) was weighed out and cold macerated in 350 mL of absolute ethanol for about 14 days and then filtered with a clean white muslin cloth. This was further filtered using a Whatmann No 1 filter paper. This final filtrate was dried using crucibles of known weights over a water bath with the temperature set at 40 ^{o}C to obtain the crude ethanol extract (ADES).

Determination of median lethal dose (LD$_{50}$)
Median lethal dose (LD$_{50}$) is the dose of a test substance that is lethal for 50% of the animals in a dose group. The median lethal dose (LD$_{50}$) of the plant crude ethanol extract (ADES) was determined by the method reported by Lorkes[25]. This was done in two stages. The first stage which consists of three groups A-C of three mice each were administered 10, 100, and 1000 mg extract /kg body weight orally respectively . They were observed for 24 hours for signs of toxicity. The second stage also had three groups of one mouse each. Based on the no death recorded from stage one, they were administered: 1600, 2900, and 5000 mg extract /kg body weight orally as specified by the

Lorke's method. The animals were then observed for mortality and signs of toxicity within each group over a 24hour period. The LD_{50} was calculated as the geometric mean of the highest non-lethal dose and the lowest lethal dose.

Fractionation of crude ethanol extract

The solvent-solvent partitioning technique was used in fractionating the crude ethanol extract (ADES) with n-hexane, and dichloromethane as organic solvents used successively in increasing order of polarity. The ADES was suspended in aqueous methanol (10 % v/v) and transferred into a separating flask. This was partitioned with 3 x 35 mL portion of n-hexane. The n-hexane layers were pooled together as the n-hexane (non-polar) fraction coded ADHS. The leftover aqueous layer after partitioning with n-hexane was further partitioned with 3 x 35 mL portions of dichloromethane. The dichloromethane layers were pooled as dichloromethane (intermediate polar) fraction ADDS while the remaining aqueous (polar fraction) layer was coded ADAS.

Quantitative antioxidant assay methods

The quantitative antioxidant assay was done using the DPPH spectrophotometric assay method [26-28]. Briefly, stock solutions of 10 mg/ mL of the crude ethanol extract (ADES) and the various fractions (ADHS, ADDS and ADAS) obtained from the crude ethanol extract were prepared in methanol. DPPH (0.02 g) was dissolved in 100 mL of methanol resulting in DPPH solution with concentration of 0.2 mg/mL following the double dilution approach the concentrations: 10.0, 5.0, 2.50, 1.250, 0.625 and 0.313 mg/mL of the crude ethanol extract and its fractions were separately obtained from their respective stock solutions. Using a pipette, 5 mL aliquots of the 0.2 mg/mL DPPH stock solution was added respectively to each concentration of the various samples solutions (5 mL), mixed and allowed to stand for 30 minutes in a dark environment. The absorbance of the various concentrations of the test samples were measured using a UV/Visible spectrophotometer (Spectrum LAB 752s) in duplicates at 517 nm. Methanol was used as reagent blank while methanol: DPPH stock solution (1:1 v/v) was used as negative control. Ascorbic acid was used as reference antioxidant standard agent for comparison. Antioxidant activity was recorded as the percentage reduction in the absorbance of DPPH by either the test samples or standard. The IC_{50} which is the concentration of the sample which produces 50 % of reduction in absorbance value was then extrapolated from a plot of percentage inhibition of DPPH activity against concentration.

% inhibition = [(Ac-As) / Ac] × 100
Where Ac= absorbance of negative control and
As = absorbance of sample

Phytochemical screening

Preliminary phytochemical tests were carried out on the crude plant ethanol extract (ADES) and fractions(ADHS, ADDS and ADAS) using standard phytochemical screening reagents [29-30] to determine the presence or absence of secondary plant metabolites such as: alkaloids (using Drangendorff's, Meyer's and Hager's reagents), carbohydrate derivatives (using Molisch and Fehling's reagents), phenolic compounds(using ferric chloride test), anthraquinone derivatives (using Bontrager's test), triterpeniods (using Liebermann Burchard's and Salkowski test), cardiac glycosides (Keller Killiani tests), saponins (using Frothing and blood haemolysis tests)

STATISTICAL ANALYSIS

The mean antioxidant activity at the various test concentrations were compared with that obtained for the standard using the student t-test of significance at 95 % confidence level and differences considered significant at p-value <0.05.

Chromatography separation of the antioxidant component ADD1 from the bioactive Dichloromethane fraction ADDS

The bioactive dichloromethane fraction ADDS was further fractionated using open column chromatography. Normal phase silica gel (Mesh size 70 – 230) was used as stationary phase. The wet packing technique was used to pack the column. The column was packed with 30 g of the silica gel followed by loading with 1g of the dichloromethane fraction ADDS. Elution was done using a 10 % stepwise gradient of n-hexane: ethyl acetate as mobile phase in increasing order of polarity. 50 mL of each gradient was used for the elution with eluted fractions collected in 25 mL portions. The eluted fraction were spotted on TLC plates and developed in two different solvent systems; methanol: chloroform (1:1) and n-hexane: dichloromethane (1:1) and viewed under 254 and 365nm UV lamp. The developed TLC plates were also sprayed with DPPH to identify constituents with antioxidant activity. Based on similarity from TLC examination, column fractions eluted with n-hexane: dichloromethane 45:5 to 10: 40 v/v were pooled together coded DCMP and purified using preparative thin layer chromatography (TLC) to isolate the antioxidant component coded ADD1 based on its characteristic green fluorescence under UV irradiation at 254nm and formation of yellow coloured bands on a white background after spraying with the 0.1% of 2,2-diphenylpicycrylhydrazyl (DPPH) in methanol. For the preparative TLC, a slurry of silica gel 60G for prep TLC was prepared and spread to a thickness of 1mm on a thin layer glass plates (10 cm x 20 cm) using a TLC spreader and allowed to set after which the coated plate was activated in an oven at 100°C for about 3 hrs before use. The coated plate was marked 4 cm from the base and 5 cm from the top resulting in a solvent front distance of 11 cm. The pooled column fractions coded

DCMP due to the column chromatography fractionation of the dichloromethane fraction (ADDS) was spotted along the marked base line and allowed to air dry. A mobile phase containing n-hexane: methanol: acetic acid (10:10:1 v/v/v) was prepared and poured into a TLC tank. The tank was allowed to equilibrate by lining the walls with filter paper and the lid replaced and made air-tight by greasing. After equilibration for about an hour the lid was quickly removed and the spotted plate was then introduced into the equilibrated tank with the lid replaced firmly. The plate was then allowed to develop by ascent/capillary action over a solvent front distance of 11cm. The developed TLC plate (n-hexane:methanol acetic acid (10:10:1 v/v/v as mobile phase)was then visualized under UV lamp to identify the antioxidant component coded ADD1 (R_f= 0.86)) based on its characteristic green fluorescence under 254 nm. This band was then marked, scrapped out and eluted by maceration in dichloromethane overnight. After which it was filtered and the filtrate air dried in a fume cupboard to obtain a yellowish brown viscous residue ADD1. The antioxidant activity of the ADD1 was then further confirmed qualitatively (by developing on pre-coated silica gel HF$_{254}$ plates which serve as adsorbent with n-hexane:methanol acetic acid (10:10:1 v/v/v as mobile phase) from its characteristic yellow coloured reaction on a white background after spraying with the 0.1% of 2,2-diphenylpicycrylhydrazyl (DPPH) in methanol. The ADD1 was partially characterized using Fourier transform infra-red (FTIR) spectroscopy. For the FTIR spectroscopy of the ADD1, this was briefly done as follows: A portion of the sample was mixed evenly with KBr and placed on the KBr disc. The KBr disc containing the sample was then placed on the FTIR spectrophotometer to acquire the FTIR spectrum.

Chromatography separation of the antioxidant component ADD1 from the bioactive aqueous fraction ADAS

The less bioactive aqueous fraction ADAS was further fractionated using open column chromatography. Normal phase silica gel (Mesh size 70 – 230) was used as stationary phase. The wet packing technique with the column wet packed with 20 g of silica gel. The aqueous fraction ADAS (0.7 g) was pre-adsorbed on silica gel by mixing with silica gel in ratio 1:1 and allowed to air dry. This was then loaded on the top of the packed adsorbent and then further fractionated by eluting with the mobile phase gradient of ethyl acetate and methanol (100:0, 50:50, and 0:100 v/v) in increasing order of polarity. 100 mL of each gradient was used for the elution with eluted fractions collected in 50 mL portions. The eluted fractions where collected, spotted on TLC plates and developed in two different solvent systems; n-hexane: dichloromethane (1:1) and chloroform: methanol (1:1). The developed plates were viewed under UV light at 254 nm and thereafter sprayed with DPPH reagent to detect antioxidant constituents. The eluents obtained from the aqueous fraction ADAS by eluting with ethyl acetate and methanol 50:50 to 0:100 having similar R_f values after TLC examination were pooled together and allowed to air-dry during which the formation of a colourless crystal was observed. This was purified by washing with 99 % methanol to form a colourless crystalline residue coded ADA2. The methanol soluble portion was filtered and allowed to air dry to form yellowish-brown residue coded ADA1. The ADA2 and ADA1 were partially characterized using FTIR spectroscopy. For the FTIR spectroscopy of the ADA2 and ADA1, this was briefly done as follows: A portion of the sample was mixed evenly with KBr and placed on the KBr disc. The KBr disc containing the sample was then placed on the FTIR spectrophotometer to acquire the FTIR spectrum.

RESULTS AND DISCUSSION

Anchomanes difformis and other aroids are medicinal plants whose stem and leaves are used traditionally in re-conditioning of the health of elderly people and to treat ailments associated with ageing and oxidative stress [16, 21]. Absolute ethanol was used in the extraction of the dried stem of *A. difformis* by maceration yielding 0.92 % w/w of the crude ethanol extract (ADES). For the acute toxicity test, the plant crude ethanol extract (ADES) was administered through the oral route to a set of mice in two stages. At the end of the two stages within the dose range: stage 1(10, 100 and 1000 mg/kg bw) and stage 2(1600, 2900 and 5000 mg bw), no death was recorded. This shows that the plant crude ethanol extract (ADES) is relatively safe with a median lethal dose (LD_{50}) of > 5000 mg/kg bw. This study is relevant in the establishment of the toxic dose of the plant extract to serve as a guide in the early and late stages of drug discovery and development, for the identification of possible adverse effects and the limit of exposure level at which such effects could occur. After solvent-solvent partitioning of the crude ethanol extract (ADES) using n-hexane, and dichloromethane successively in increasing order of polarity, three fractions: n-hexane (ADHS), dichloromethane (ADDS) and aqueous (ADAS) fractions were obtained. These fractions alongside the parent crude ethanol extract (ADES), were subjected to quantitative bio-guided antioxidant evaluation using the spectrophotometric DPPH assay method. The dichloromethane (ADDS) and the aqueous (ADAS) fractions exhibited significant (p<0.05) promising concentration dependent DPPH inhibition activity as shown in Table 1. The observed trend in free-radical scavenging activity was: ADHS (DPPH IC_{50} > 10.0 mg/mL)< ADES (DPPH IC_{50} > 10.0 mg/mL) < ADAS (DPPH IC_{50}= 4.0 mg/mL) < ADDS (DPPH IC_{50}= 1.8 mg/mL) < ascorbic acid (DPPH IC_{50} <0.3125 mg/mL). This result is suggestive that the dichloromethane (ADDS) and aqueous (ADAS) fractions are containing constituents that could act as good free radical scavengers and probably have the ability to inhibit the progression of, and tissue damage due to oxidative

stress. Thus they could be beneficial in the treatment of various diseases in which oxidative stress is an important mechanism for pathogenesis. The DPPH assay is based on the measurement of the radical scavenging capacity of antioxidants towards it. The odd electron of nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidants. Thus when DPPH reacts with a donor, its reduced form is generated, accompanied by the disappearance of the violet colour to form a yellow colour[27, 28]. Phytochemical examination of the the crude ethanol extract (ADES) and its fractions (ADHS, ADDS and ADAS) as shown in Table 2 revealed depending on the extract or solvent fraction, the presence of saponins, triterpenoids and carbohydrate derivatives in the stem of *A. difformis* with the absence of phenolics, anthraquinones, alkaloids, and cardiac glycosides. Whereas the polar aqueous fraction ADAS like the parent powdered stem contained saponins, triterpenoids and carbohydrate derivatives, the intermediate polar dichloromethane (ADDS) and the non-polar n-hexane (ADHS) fractions are devoid of saponins which are highly polar glycosides with surfactant and haemolytic action with triterpnoids as aglycone. The saponins and triterpenoids detected in the stem of *A. difformis* could implicate these classes of phytochemicals as important bioactive agents of the stem part of this plant and might be involved in the therapeutic action of the plant part as they have been reported to have antioxidant activity[31, 32].The antioxidant activity of the component ADD1 which gave a characteristic green fluorescence under UV irradiation at 254 nm (Rf 0.86, silica gel HF_{254} adsorbent with n-hexane: methanol: acetic acid (10:10:1 v/v/v as mobile phase) was isolated from the bioactive dichloromethane ADDS fraction based on its characteristic yellow coloured reaction on a white background after spraying with the 0.1% of 2,2-diphenylpicycrylhydrazyl (DPPH) in methanol. The use of DPPH as a chromogenic spray reagent on developed TLC plate is a qualitative assay protocol for the preliminary identification of antioxidant components on TLC. The vibrational frequencies (V cm^{-1}, neat) observed in the FTIR spectrum of ADD1 (see as rationalized in Table 3) showed the presence of hydrogen bonded O-H stretching at 3424 cm^{-1}, typical of hydroxylated organic compounds like carbohydrates and alcohol and further confirmed from the characteristic C-O stretching in the fingerprint region at 1114 cm^{-1} characteristic of carbohydrates and alcohols. Also evident in the FTIR spectrum of ADD1 are the aliphatic C-H stretching in the region 2927 cm^{-1} with the corresponding C-H deformation band at 1404 cm^{-1} while the vibrational band at 1652 $cm^{-1}$is most likely due to an aromatic C=C stretching because of the confirmatory out-of-plane C-H deformation band at 781 cm^{-1} without a corresponding 2v overtone band where it to be an olefinic C=C stretching. This band at 1652 cm^{-1} could also not be attributed to a ketone(cyclic) because of this evident presence of this out-of-plane C-H deformation band at 781 cm^{-1} which should be absent

for ketones. Correlating these spectra data with the result of the phytochemical screening in Table 2 for the crude ethanol extract and the fractions, it further lend credence to the absence of phenolics or enolic derivatives. This supported the suggestion that ADD1 is more likely to be an aromatic (but not phenolic) compound having a hydroxylated aliphatic side chain or glycosylated side chain and not a phenolic derivative because of the observed negative test for phytophenolics derivatives (see Table 2). The antioxidant activity of the component ADA1 from the aqueous fraction ADAS of the crude ethanol extract ADEs of *A. difformis* was also identified based on its characteristic yellow coloured reaction on a white background after spraying with the 0.1% of 2,2-diphenylpicycrylhydrazyl (DPPH) in methanol from TLC(observed R_f =0.43) in a similar manner as explained for ADD1 above. Unlike ADA1, ADA2 however did not show antioxidant activity. The vibrational frequencies (V cm^{-1}, neat) observed in the FTIR spectrum of ADA1 (see as rationalized in Table 4) showed the presence of hydrogen bonded O-H stretching at 3354 cm^{-1}, typical of hydroxylated organic compounds like sugar derivatives, and alcohol and further confirmed from the characteristic C-O stretching in the fingerprint region at 1083.6 cm^{-1} characteristic of sugar derivatives and alcohols. Also evident in the FTIR spectrum of ADA1 are the aliphatic C-H stretching in the region 2970 cm^{-1} with the corresponding C-H deformation band at 1372 cm^{-1} while the vibrational band at 1635 cm^{-1} could not be attributed to neither an aromatic C=C stretching nor an olefinic C=C stretching because of the absence of the confirmatory out-of-plane C-H deformation band usually in the region 700-980 cm^{-1} and their associated 2v overtone band where it to be an olefinic C=C stretching. This band at 1635 cm^{-1} could be due to ketone (cyclic) because of this evident absence the out-of-plane C-H deformation band and associated overtone bands. Correlating these spectra data with the result of the phytochemical screening in Table 2 for the aqueous fraction ADAS, it further lend credence to ADA1 likley be a glycoside derivative (probably a saponin) with a saturated ketone aglycone (non-sugar) moiety. The FTIR spectrum of ADA2(see as rationalized in Table 5) showed the presence of O-H stretching(3436 cm^{-1}) and aliphatic C-H stretching (2836 cm^{-1}) with the evident absence of confirmatory vibrational bands in the fingerprint region unlike as seen for ADA1 and ADD1. This precludes ADA2 from being an organic alcohol nor a sugar derivative but a compound with the hydroxyl group not bonded to a carbon atom (hence the absence of a C-O stretching vibration in the fingerprint region 1000-1200 cm^{-1}). Thus the non-antioxidant ADA2 could be an organometallic compound with a water of crystallization.

CONCLUSION

The free radical scavenging activity of the stem of *Anchomanes difformis* observed and evaluated in this study could be attributed to phytochemical constituent's saponins and intermediate polar triterpenoids derivatives. Thus findings from this study warrants that *A. difformis* be further evaluated by elucidating the chemical structure of the isolated compounds using nuclear magnetic resonance spectroscopy and Mass spectrometry.

Table-1: Concentration dependent free radical scavenging activity profile of the crude ethanol extract and solvent fractions from the stem of *Anchomanes difformis*

Test concentration mg/ml	Mean % DPPH radical inhibition + standard deviation of the test samples				
	Ascorbic acid	ADAS	ADDS	ADHS	ADES
.3125	92.98±0.0038	29.00 ± 0.153	36.33 ± 0.162	8.54 ± 0.241	7.40 ± 0.306
0.625	95.71±0.0099	33.16 ± 0.110	35.65 ± 0.167	38.97 ± 0.493	15.11 ± 0.160
1.25	94.42±0.0038	37.08 ± 0.071	45.77 ± 0.088	-14.95±0.808	38.14 ± 0.279
2.5	94.35±0.0034	41.69 ± 0.024	62.31 ± 0.042	25.38 ± 0.165	17.75 ± 0.109
5.0	93.05±0.0028	53.70 ± 0.097	77.12 ± 0.158	25.76 ± 0.174	32.25 ± 0.167
10	91.26±0.0011	69.94 ± 0.26	84.74 ± 0.218	27.49 ± 0.216	30.36 ± 0.131
IC_{50} (mg/ml)	< 0.3125	4.0000	1.8000	> 10.0000	> 10.0000

Table-2: Phytochemical screening results of the fractions (n-hexane, dichloromethane and aqueous) and powdered stem of *Anchomanes difformis*

Phytochemical method	n-hexane fraction (ADHS)	dichloromethane fraction (ADDS	aqueous alcohol fraction (ADAS)	Powdered stem
1)ALKALOIDS				
a) Dragendorf test	-	-	-	-
b) Mayer test	-	-	-	-
c) Hager test	-	-	-	-
2)CARBOHYDRATES				
a)Molisch test	-	+	+	+
b)Fehlings test		+	+	+
3)ANTHRAQUINONE				
Bontrager test	-	-	-	-
4) SAPONINS				
a) Frothings test	-	-	+	+
b) Haemolysis test	-	-	+	+
5)CARDIAC GLYCOSIDES				
Keller Killani test	-	-	-	-
6)Triterpenoids				
a)Liebermann Burchard test	+	+	+	+
b)Salkwoski test	+	+	+	+
7) Phenolics				
$FeCl_3$ test	-	-	-	-

Key: + present, - absent

Table-3: Fourier Transform Infra-Red spectroscopy of component ADD1 isolated from the dichloromethane fraction ADDS of the crude ethanol extract ADES of *A. difformis* stem

Observed vibrational frequencies (V cm^{-1}, neat	Inferred functional group
3424.3	H-bonded O-H stretching of carbohydrate or alcohol
2927.5	Aliphatic C-H stretching
1652.5	Aromatic C=C stretching
1404	Aliphatic C-H deformation
1114.8	C-O (s) of carbohydrate or alcohol
781.02	Out-of plane C-H deformation of m-substituted aromatic ring

Table-4: Fourier Transform Infra-Red spectroscopy of component ADA1 isolated from the aqueous fraction ADAS of the crude ethanol extract ADES of *A. difformis* stem

Observed vibrational frequencies ($\acute{V}$ cm^{-1}, neat	Inferred functional group
3354.3	H-bonded O-H stretching of polyol or sugar alcohol
2970	Aliphatic C-H stretching
1635.6	C=O of a carbonyl
1371.7	Aliphatic C-H deformation
1083.6	C-O stretching. of polyol or sugar alcohol

Table-5: Fourier Transform Infra-Red spectroscopy of component ADA2 isolated from the aqueous fraction ADAS of the crude ethanol extract ADES of *A. difformis* stem

Observed vibrational frequencies ($\acute{V}$ cm^{-1}, neat	Inferred functional group
3436.6	Very broad H-bonded-O-H stretching due to water of crystalisation
2835.9	C-H stretching

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