European Journal of Medicinal Plants
6(3): 181-190, 2015, Article no.EJMP.2015.054
ISSN: 2231-0894

SCIENCEDOMAIN *international*
www.sciencedomain.org

In vitro Evaluation of Membrane Stabilizing Potential of Selected Bryophyte Species

Akinpelu Bolajoko Ayinke[1*], Makinde Amos Morakinyo[2], Isa Musibau Olalekan[2], Taiwo Olakunle Philip[1], Ojelabi Olajumoke Mariam[1] and Oyedapo Oluboade Oluokun[1]

[1]Department of Biochemistry, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria.
[2]Department of Botany, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria.

Authors' contributions

The entire work was done jointly in a collaborative manner with active participation of all the authors. Authors ABA and OOO designed the experiments, performed the statistical analysis and wrote the protocol. Field work and identification of the samples were done by the authors MAM and IMO. Authors TOP, OOM and ABA performed all the experiments. The corresponding author ABA prepared the draft manuscript and did the literary review. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2015/7629
Editor(s):
(1) Sabyasachi Chatterjee, Department of Biotechnology, Burdwan University, India.
(2) Marcello Iriti, Professor of Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Anonymous, University of Nigeria, Nsukka, Nigeria.
(2) Anonymous, University of Belgrade, Serbia.
(3) Anonymous, University of Belgrade, Serbia.
(4) Anonymous, Payame Noor University, Iran.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=913&id=13&aid=7765

Original Research Article

Received 30th October 2013
Accepted 19th November 2014
Published 13th January 2015

ABSTRACT

Aims: The purpose of the current study was to investigate the possible anti-inflammatory properties of the three bryophytes species: *Archidium ohioense, Bryum coronatum and Racophilum africanum* with a view to utilizing the extracts of the plant s in the treatment of inflammatory related ailments.
Place and Duration of Study: The research was carried out in Ile-Ife, Osun-State, Nigeria between May 2012 and March 2013.
Methodology: The study adopted membrane stabilizing technique of red blood cells exposed to both heat and hypotonic induced lyses with varying concentrations of the extracts (chloroform, acetone, ethyl acetate and ethanolic) of the plants.

**Corresponding author: E-mail: badeoye@oauife.edu.ng;*

Results: The results of the membrane stabilizing activity assay showed that acetone extracts contained principles that protected red blood cells effectively against heat and hypotonic induced lyses. *A. ohioense* extracts protected red blood cells at all concentrations used while *B. coronatum* protected better at lower concentrations. However, *R. africanum* extracts (with the exception of the acetone extract) promoted lysis of red blood cells at lower concentrations.
Conclusion: The study showed that the anti-inflammatory activities of the extracts of A. *ohioense, B. coronatum and R. africanum* were concentration dependent and comparable to those of non-steroidal anti-inflammatory drugs.
This study indicates the possibility of generating an alternative source of novel anti- inflammatory compounds from the studied bryophytes, which might overcome the ever expensive synthetic drugs with long term side effects.

Keywords: Membrane stabilization; anti- inflammatory; bryophyte; red blood cells; Archidium ohioense; Bryum coronatum and Racophilum africanum.

1. INTRODUCTION

Bryophytes are considered to be among the oldest land plants that do not have true vascular tissue and are therefore called non-vascular plants that produce spores rather than seeds [1]. Examples are mosses, liverworts and hornworts. Due to lack of economic importance, insignificant number, size and inconspicuous distribution has made bryophyte apparently of no therapeutic use when compared to it's tracheophyte counterparts [2,3]. These small, slow-growing groups of plants are often associated with disturbed habitat, barren rock surface and extreme climatic condition. Bryophytes are abundant in many different types of plant communities and have a substantial and distinctive influence on the functioning of ecosystem where they occur most especially in moist areas possessing adaptive mechanisms to survive periods of water stress [4]. Bryophytes are known to contain numerous potentially useful compounds, including oligosaccharides, polysaccharides, sugar alcohols, amino acids, fatty acids, aliphatic compounds, phenylquinones and aromatic and phenolic substances [5].

A number of bryophytes have been identified, classified and reported to express interesting bioactivities [6,7,8,9,10]. These plants had been investigated extensively for their active constituents and pharmacological activities such as cytotoxic, anticancer and antitumor activity [11,12,13], antifungal [14,15], antioxidant [16], anti-inflammatory [17] and antibacterial [16,17,18].

Traditional uses of bryophytes include treatment of liver ailments, ringworm, heart problems, inflammation, fever, urinary and digestive problems, infections, lung disease, skin problems, tonsillitis, bronchitis, ulcer, tympanitis (inflammation of inner ear) and as filters and cleansing agents against pollutants [19,20]. Himalayan Indians use *Marchantia polymorpha* or *M. palmata* to treat boils and *Riccia* species to treat ringworm [5]. Sphagnum is used to treat hemorrhages [21]. *Rhodobryum giganteum* and *R. roseum* are used to treat cardiovascular diseases and nervous prostration while *Haplocladium microphyllum* is used in treatment of cystitis, bronchitis, tonsillitis and tympanitis [2]. Also, *Fissidens* is used as an antibacterial agent for swollen throats and other symptoms of bacterial infection [2]. Bryophytes used in treatment of inflammation include *Marchantia polymorpha (*liverwort) used in treatment of jaundice and as an external cure to reduce inflammation [22,23], *Barbula unguiculata, Bryum capillare*, as well as larger mosses like *Octoblepharum albidum*, are used as external applications for fever and body aches [24]. Also, *Polytrichum commune* has been used in China to reduce inflammation and fever [25].

This study reported, for the first time, the red blood cells membrane stabilizing potentials of *A. ohioense, B. coronatum and R. africanum* extracts against heat and hypotonic induce lyses, with a view to investigating the possible therapeutic potentials.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Materials

Fresh plants of *R. africanum, A. ohioense* and *B. coronatum* were collected in the Biological Garden and the base of hill II near Road 8, O. A. U Senior staff quarters, Obafemi Awolowo

University Campus, Ile-Ife Osun State. The plants were identified and authenticated at IFE Herbarium by Dr A. M. Makinde, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria.

2.2 Reagents and Chemicals

All reagents and chemicals used were of analytical grades, and were obtained from various sources such as British Drug House (BDH) England and Sigma Chemical Company, Germany. All solutions, buffers and reagents were prepared using glass distilled water. Panadol (Standard anti-inflammatory drugs) were used as reference drugs, each was weighed and dissolved separately in distilled water to obtain concentrations of 0.25 to 2.0 mg/ml respectively.

2.3 Preparation of Plants Extracts

The fresh plants of *R. africanum, A. ohioense* and *B. coronatum* were harvested, rinsed and air dried. The plants were powdered using manual grinding machine and 20 g of each powdered material was soaked separately in 400 ml ethyl acetate, ethanol, acetone and chloroform for 72 hr. Suspension of each powdered plant materials was filtered and evaporated to dryness under reduced pressure at 35℃ on rotatory evaporator to yield ethylacetate extract (EAE), ethanolic extract (EE), acetone extract (AE) and chloroform extract (CE). Solutions of each plant extract was prepared by weighing appropriate quantity and dissolved in 0.5 ml Tween 20. The volume was adjusted to 2 ml with isosaline and diluted further to give varying concentrations (0.25, 0.5, 1.0, 1.5 and 2.0 mg/ml) of each extract.

2.4 Phytochemical Screening

Extracts were screened for the presence of secondary metabolites using standard procedures as described by Oyedapo et al [26]. Alkaloids (Mayer's, Wagner's and Dragendorf reagents), saponins (froth test), flavonoids (ethanolic KOH/ethylacetate), cardiac glycosides (chloroform/H_2SO_4), tannins (ferric chloride reagent), xanthoprotein (dilute H_2SO_4/benzene /ammonia solution), anthraquinones (ethanolic NaOH), phlabotannins (HNO_3/ammonia solution), triterpenes (chloroform/conc. H_2SO_4) and steroids (conc. H_2SO_4).

2.5 Preparation of Bovine Erythrocytes

Bovine erythrocytes were prepared according to the procedure reported by Oyedapo and Famurewa [27]. Fresh blood sample was collected into anticoagulant bottle containing trisodium citrate (3.8% w/v) and mixed thoroughly to prevent lysing. The anticoagulated blood was poured into clean centrifuged tubes and centrifuged at 3000 rpm for 15 min. The supernatant was carefully removed with sterilized Pasteur pipettes. The packed cells of erythrocytes was re-suspended in fresh isosaline, mixed carefully and centrifuged at 3000 rpm for 10 min. This process was repeated until a clear supernatant was obtained. Then a 2% (v/v) erythrocytes suspension was prepared by adding 98 ml isosaline to 2.0 ml of packed red blood cells.

2.6 Assay of Membrane Stabilizing Activity

The procedure for the membrane stabilizing assay was carried out according to the method of Sadique et al. [28] as reported by Oyedapo *et al* [29]. The assay mixture consisted of 0.5 ml hyposaline (0.42% w/v NaCl), 1.0 ml 0.15M sodium phosphate buffer, pH 7.4, varying volumes of isosaline (0.85% w/v NaCl), and 0.5 ml erythrocyte suspension.

The drug control was pipette as above without 2% (v/v) erythrocyte suspension while the blood control contained all the reagents except the drug or the extract. The reaction mixtures were mixed properly and incubated at 56℃ for 30 min. The tubes were cooled under running water and then centrifuged at 3000 rpm for 5 min. The supernatant was collected and absorbance was read at 560nm against the test blank. The buffered sodium chloride solution served as blank.

The percentage membrane stability activities were estimated from the expression.

$$100 - \frac{\{(\text{drug test value} - \text{drug control value}) \times 100\}}{\text{Control value}}$$

The control represents 100% lysis.

2.7 Statistical Analysis

Values are expressed as mean ± SEM of 3 consistent readings. The statistical significance

differences were analyzed using Student" t" test, with $p<0.05$ taken as statistically significant.

3. RESULTS AND DISCUSSION

The vitality of cells is known to depend on the integrity of their membrane [30]. Exposure of red blood cell to injurious substances such as hypotonic medium caused excessive accumulation of fluids within the cell and this result in lyses of its membrane accompanied by heamolysis and oxidation of heamoglobin. Such injury to red blood cell membrane will further render the cell more susceptible to secondary damage through free radical induced lipid peroxidation [30,31].

Lysosomes play a key role in the inflammatory processes. During inflammation, lysosomal hydrolytic enzymes are released into the cytosol, causing damages to the surrounding organelles and tissues with attendance variety of disorders [25]. Anti-inflammatory agents or drugs normally act by stabilizing lysosomal membrane by inactivating already released enzymes into the cytosol. Since erythrocytes membrane structurally resembles that of lysosomal membrane, as such it has been used as a model system by many workers in the study of interaction of drugs with membranes. The effect of drugs on the stabilization of erythrocytes could be extrapolated to the stabilization of lysosomal membranes [32,33,34]. The membrane stabilizing activity of red blood cell membrane exhibited by some drugs serves as a useful *in vitro* method for assessing the anti-inflammatory activity of various compounds [35].

Phytochemical analyses of chloroform, acetone, ethylacetate and ethanolic extracts of the investigated mosses (Table 1) revealed that their chemical composition include alkaloids, flavonoids, saponins, cardiac glycosides, triterpenes and steroids. Investigations have revealed that flavonoids, triterpenoids, alkaloids and host of other secondary metabolites exhibited analgesic and anti-inflammatory effects as a result of their membrane stabilizing action in various experimental animal models [36,37]. Flavonoids had been reported to posses' *in vitro* anti-inflammatory property [38]. Also, the stabilization of erythrocyte membranes and other biological macromolecules as a result of binding of saponins to cations had also been documented [39,40,41,42,43].

In Fig. 1 (a-d) are the membrane stabilizing profiles of acetone, chloroform, ethanolic and ethyl acetate extracts of *A. ohioense* on bovine erythrocytes exposed to heat and hypotonic induced lyses. *A. ohioense* acetone extract, with biphasic at all concentrations used, exhibited a minimum activity stability of 24.39±1.53% and maximum activity of 94.66±28.73%. Chloroform extract of *A. ohioense* (Fig.1b) exerted the minimum stability of 5.08±2.42% and maximum activity of 84.88±2.78%. It's mode of response was biphasic except at 2.5 mg/ml which exhibited monophasic mode of protection. *A. ohioense* ethyl acetate extract (Fig.1c) exerted minimum membrane stability of 11.56% and maximum activity of 90.95±1.30% while the ethanolic extract (Fig.1d) exerted minimum membrane stability of 11.53±0.40 and maximum activity of 64.65±2.52.

Table1. Phytochemical constituents in *Archidium ohioense*, *Bryum coronatum* and *Racopilum africanum* extracts

Metabolites	Extracts		
	A. ohioense	***B. coronatum***	***R. africanum***
Alkaloids	AE, EE, EAE	AE, EAE, EE	EAE,EE
Flavonoids	CE, AE, EAE, EE	CE, AE, EE	AE,EAE,EE
Saponins	CE, AE, EAE, EE	CE, AE, EAE, EE	CE,AE,EE
Tannins	—	—	—
Anthraquinones	—	—	—
Xanthoproteins	—	—	—
Phlobatannins	AE	—	—
Cardiac glycosides	CE, AE, EAE, EE	CE, AE, EAE, EE	CE, AE, EAE, EE
Triterpenes	CE, AE, EAE, EE	EAE, EE	—
steroids	CE, AE, EAE	EAE	EAE

— - Absent CE – Chloroform Extract AE – Acetone Extract
EAE – Ethylacetate Extract EE – ethanolic Extract

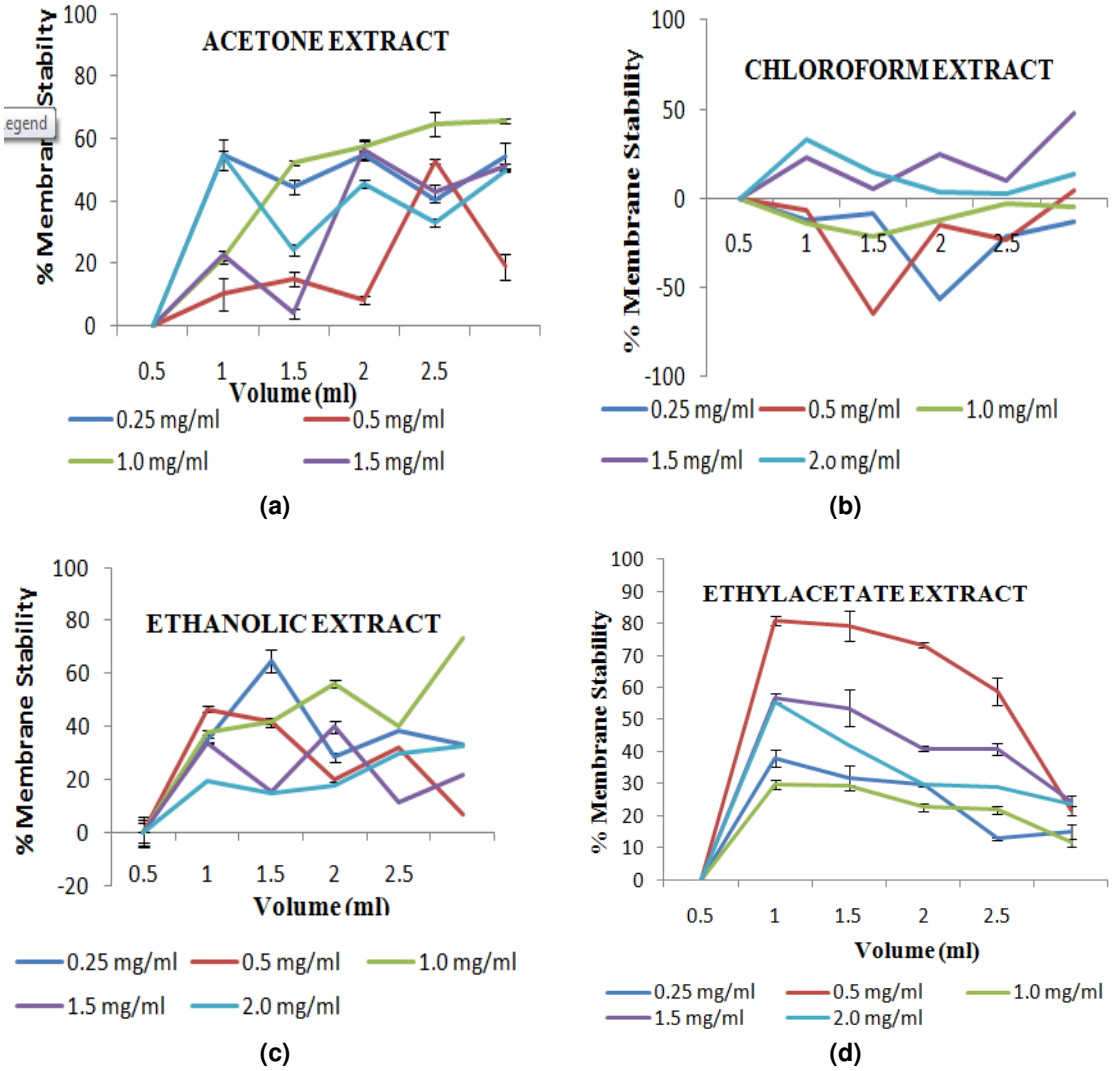

Fig. 1a – d. Membrane stabilizing profiles of *A. ohioense* on bovine erythrocytes exposed to both heat and hypotonic induced lyses. Each value represented the mean ± SEM of 3 readings

Membrane stability profiles of acetone, chloroform, ethanolic and ethylacetate extracts of *B. coronatum* was shown in Figs. 2(a-d). *B. coronatum* acetone extract gave membrane stability 28.56±1.04% and 84.34±2.30% as minimum and maximum percentage activities respectively (Fig. 2a). The response was biphasic at all the tested concentrations (Fig. 2a)

Its chloroform counterpart exerted minimum membrane stability of 11.21±0.38% and maximum of 72.95±1.15% (Fig. 2b).The response of these two extracts was biphasic at all tested concentrations (Figs. 2a and b). Ethanolic and ethylacetate extracts of *B. coronatum* only stabilized bovine red blood cells at lower concentrations (i.e. 0.25 and 0.5 mg/ml) as shown in Figs. 2c and d. The minimum membrane stability exerted by ethanolic extract was 39.86±16.18% and maximum activity of 58.20±2.01 while ethylacetate extract exerted minimum membrane stability of 3.63±0.96% and maximum activity of 63.05±1.94%.

Fig. 3(a-d) showed the membrane stability profiles of *R. africanum*. The profile showed that acetone extract of *R. africanum* protected the bovine erythrocytes at all concentration tested. It exerted minimum and maximum percentage stability activities of 4.01±0.31% and 64.77±2.45% respectively and the mode of protection was biphasic except at 1.0 mg/ml

which was monophasic. *R. africanum* chloroform and ethylacetate extracts showed similar pattern of protection [biphasic (Figs. 3 c and d)]. These two extracts only stabilized bovine red blood cells at high concentrations. Chloroform extract exerted minimum membrane stability of 2.57±1.43% and maximum activity of 47.47±0.96% (Fig. 3b), while ethylacetate extract exerted minimum activity of 23.02±3.05% and maximum activity of 55.38±0.00% (Fig. 3d). R. africanum ethanolic extract only stabilized the stressed erythrocytes at 0.5 and 2.0 mg/ml (Fig. 3c). It exerted maximum and minimum membrane stabilizing activities of 1.73±1.45% and 31.84±0.82% respectively.

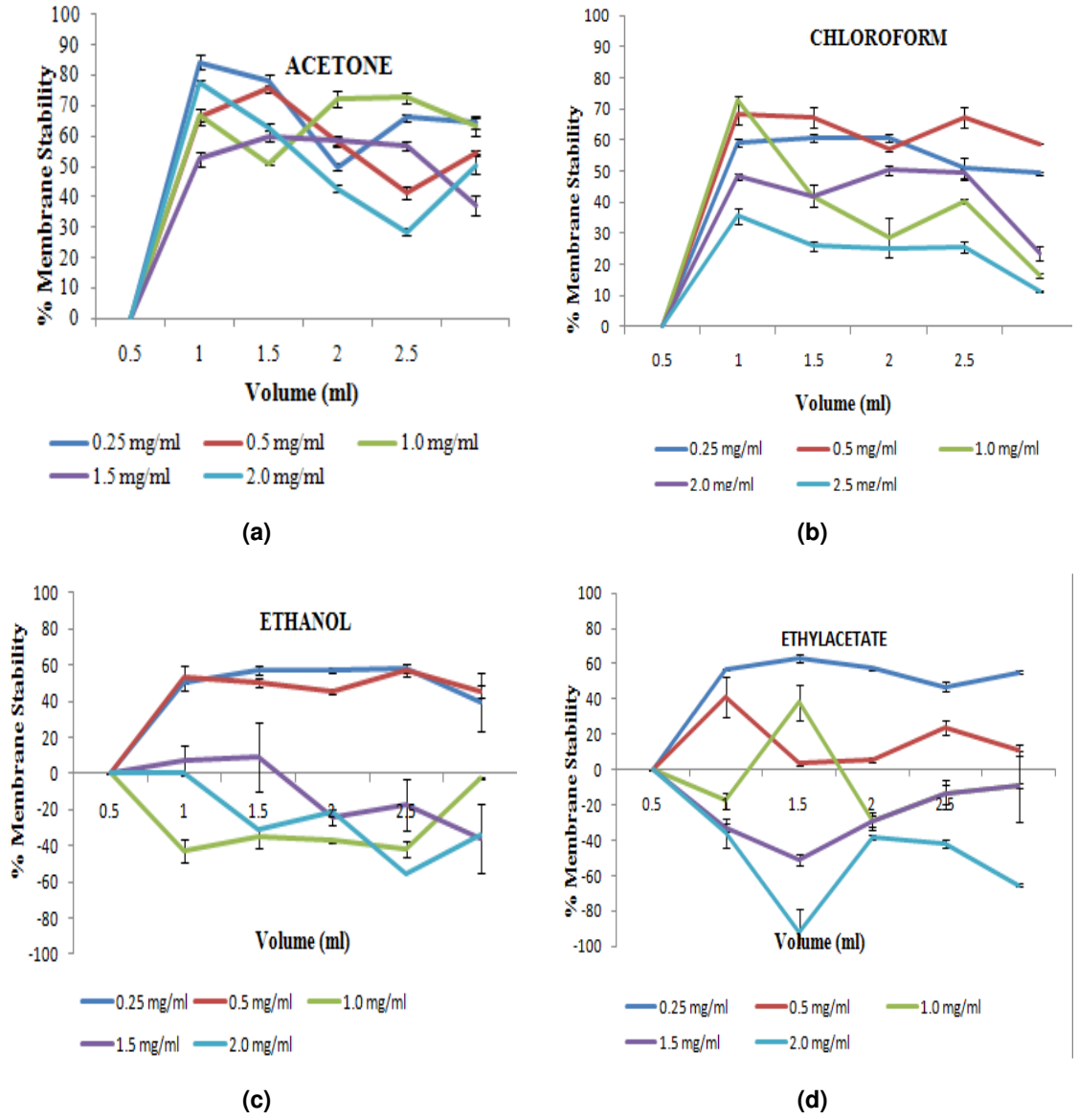

Fig. 2a–d. Membrane stabilizing profiles of *B. coronatum,* on bovine erythrocytes exposed to both heat and hypotonic induced lyses. Each value represented the mean ± SEM of 3 readings

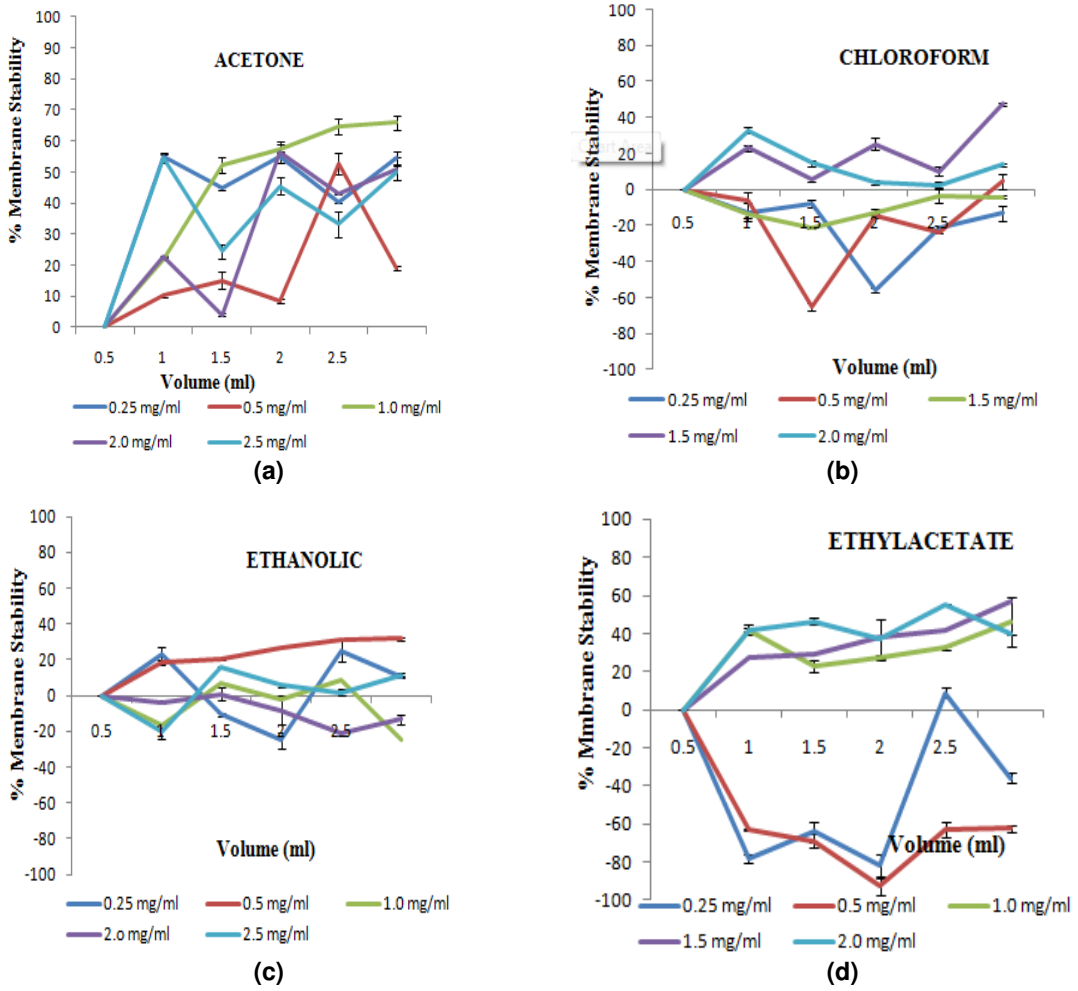

Fig. 3a – d. Membrane stabilizing profiles of *R. africanum* on bovine erythrocytes exposed to both heat and hypotonic induced lyses. Each value represented the mean ± SEM of 3 readings

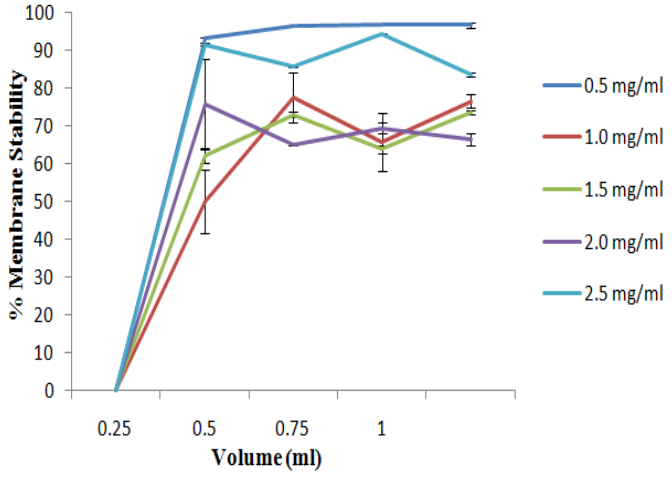

Fig. 4. Membrane stabilizing profiles of Panadol on bovine erythrocytes exposed to both heat and hypotonic induced lyses. Each value represented the mean ± SEM of 3 readings

Finally, the standard anti-inflammatory drug (Panadol, Fig. 4) at 1.0 mg/ml and 0.5 mg/ml exerted minimum and maximum membrane stability activities of 50.00±0.00 and 96.57±0.23%, respectively. The response was biphasic on the bovine red blood cells.

The study showed that all the *A. ohioense* extracts*, B. coronatum* acetone and chloroform extracts and *R. africanum* acetone extract protected the stressed bovine erythrocyte membrane at all concentration used but did not compare favourably with Panadol (standard drug). It was also noted that acetone and chloroform extracts contains principles that protected the erythrocyte membrane effectively compared to ethanolic and ethylacetate extracts. On the basis of these results, it could be inferred that the extracts of the studied bryophytes contains principles that were capable of stabilizing bovine red blood cell membrane exposed to heat and hypotonic-induced lyses and could therefore serve as a useful therapy in management and treatment of inflammatory related diseases. However, further investigation on the toxicity of the plants should be investigated.

4. CONCLUSION

The results of the study suggest that extracts of *A. ohioense, B. coronatum* and *R. africanum* may offer some beneficial effects in the management of inflammatory related ailments.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Uno G, Storey R, Moore R. Bryophyte and Ferns: The seedless plants in*:* Principles of botany 1st Edn. McGraw-Hill; 2001.
2. Glime JM. Economic and ethnic uses of bryophytes. In: Flora of North America: North of Mexico, Flora of North America editorial committee (Eds.). Part 1, Oxford University Press, New York. 2007;27:14-41.
3. Harris ESJ. Ethnobryology: Traditional uses and folk classification of bryophytes. Bryologist. 2008;111:169-217.
4. Dilks TJK, MCF Proctor. The pattern of recovery of bryophytes after desiccation. J. Bryology. 1974;8:97-115.
5. Pants G, Tewari SD. Bryophytes and mankind. Ethnobotany. 1990;2:97-103.
6. Dulger B, Hacyo lu N, Ugar G. Evaluation of antimicrobial activity of some mosses from Turkey. Asian J. Chem. 2009;21:(5) 4093–4096.
7. Chobot V, Kubicova L, Nabbout S, Jahodar L, Hadacek F. Evaluation of antioxidant activity of some common Mosses. Z. Naturforsch. C. 2008;63:476-482.
8. Sabovljevic AM, Sokovic J, Glamoclija A, Ciric M, Vujicic B, Pejin, M Sabovljevic. Bio-activities of extracts from some axenically farmed and naturally grown bryophytes. J. Med. Plants Res. 2011;5:656-671.
9. Singh M, Rawat AKS, Govndarajan R. Antimicrobial of some Indian Mosses. Fitoterapia. 2007;78:156-158.
10. Tonguc-Yaintas O, Mercili-Yapia B. *In vitro* antimicrobial activity of *Brachythuciusam campestre* and *Eurhynchium pulchellum* extracts. Asian J. Chem. 2009;21:2193-2197
11. Shen J, Li G, Liu Q, He Q, Gu J, Shi Y, Lou H. Marchantin C: A potential anti-invasion agent in glioma cells. Cancer Biol. Ther. 2010;9:33-39.
12. Shi YQ, Zhu CJ, Yuan HQ, Li BQ. Marchantin C, a novel microtubule inhibitor from liverwort with anti-tumor activity both *in vivo* and *in vitro*. Cancer Lett. 2007;276:160-170.
13. Shi YQ, Liao YX, Qu XJ, Yuan HQ, Li S, Qu JB, Lou HX. Marchantin C, a macrocyclic bis bibenzyl, induces apoptosis of human glioma A172 cells. Cancer Lett. 2008;262:173-182.
14. Niu C, Qu JB, Lou HX. Antifungal bis [bibenzyls] from the chinese liverwort *Marchantia polymorpha*. Chem. Biodivers. 2006;3:34-40.
15. Veljic M, Ciric A, Sokovic M, Janackovic P, Marin PD. Antibacterial and antifungal

activity of the liverwort (*Ptilidium pulcherrimum*) methanol extract. Arch. Biol. Sci. 2008;62:381-395.

16. Mukhopadhyay ST, Mitra S, Biswas A , Das N, Poddar-Sarkar M. Screening of antimicrobial and antioxidative potential of selected eastern himalayan mosses. EJMP. 2013;3(3):422-428.
17. Ivanova V, Kolarova M, Aleksieva K, Dornberger KJ, Haertl A. Sanionins: Anti-inflammatory and antibacterial agents with weak cytotoxicity from the Antarctic moss *Sanionia georgicouncinata*. Prep. Biochem. Biotechnol. 2007;37:343-352.
18. Mitre GB, Kamiya N, Bardon A, Asakawa Y. Africane-type sesquiterpenoids from the Argentine liverwort *Porella swartziana* and their antibacterial activity. PubMed. 2004;67(1):31-6.
19. Khanan R, Chaudary BL, Khanam S. Studies on antibacterial activity of *Asterella angusta* (Steph.) Kachroo. Against some pathogenic bacteria. Journal of Expermental science. 2011;2(2):43-46.
20. Banerjee RD, Sen SP. Antibiotic activity of bryophytes. Bryologist 1997;82(2):141-153.
21. Bland J. Forests of Lilliput. Prentice-Hall, Inc. Englewood Cliffs. 1971;210.
22. Hu R. Bryology. Higher Education Press, Beijing China. 1987;465.
23. Miller NG, Miller H. Make ye the bryophytes. Horticulture.1979;57(1):40-47.
24. Sturtevant W. The Mikasuki Seminole: Medical beliefs and practices. Ph. D. Dissertation. Yale University. 1954;203.
25. Ding H. Medicinal spore-bearing plants of China. Shanghai. 1982;409.
26. Oyedapo OO, Akinpelu BA, Akinwunmi KF, Adeyinka MO, Sipeolu FO. Red blood cell membrane stabilizing potentials of extract of *Lantana camara* and its fractions. Int. J. Plant Phy. and Biochem. 2010;2(4);46-51.
27. Oyedapo OO, Famurewa AJ. Antiprotease and membrane stabilizing activities of extracts of *Fagara Zanthoxyloides Olax subscorpioides and Tetrapleura tetraptera.* Int. J. Pharmacogn. 1995;33:65-69.
28. Sadique J, Al-Rqobah NA, Bughaith MF, El-Gindy AR. The bioactivity of certain medicinal plants on the stabilization of RBC membrane system. Fitoterapia LX, 1989;525-532.
29. Oyedapo OO, Akinpelu BA, Akinwunmi KF, Adeyinka MO, Sipeolu FO. Red blood cell membrane stabilizing potentials of extracts of *Lantana camara* and its fractions. International Journal of Plant Physiology and Biochemistry. 2010;2(4):46-51.
30. Ferrali M, Signorni C, Ciccoli L, Comporti M. Iron release and membrane damage in erythrocytes exposed to oxidizing agents, phenylhydrazine divicine and isouramil. Biochemical Journal. 1992;285:295-301.
31. Augusto O, Kunze KL, Montellano PR. N-phenylprotoporphyrin formation in the
32. haemogolobin- phenylhydrazine reaction. The Journal of Biological Chemistry. 1982;257:6231-624.
33. Kumar PE, Sadique J. The biochemical mode of action of Gynaudronis gynandra in inflammation. Fitoterapia LVII, 1987; 379-385.
34. Horie T, Sugiyama V, Awazu S, Hanano M. The correlation between drug binding to human erythrocyte and its hemolytic activity. J. Pharmacology. 1979;4:116-122.
35. Litman GW, Litman RT, Henry CJ. Analysis of lipophilic carcinogen membrane interaction using human erythrocyte membrane system model. Cancer Res. 1976;243:4364-4371.
36. Naibi RA, Sukumar, Sethuraman V, Suluchana N, Sadique J. Satellite Symposium on Traditional Medicine as Asian congress of Pharmacology. Tamil University Thanjavur. 1985;140.
37. Jorge RM, Leite JPV, Oliveira AB, Tagliati CA. Evalaution of antinociceptive anti-inflammatory and antiulcerogenic activities of *Maytenus illicifolia*. J. Ethnopharmacol. 2004;94:93-100.
38. Middleton EJ. Effect of plant flavonoids on immune and inflammatory cell function. Adv Exp Med Biol. 1998;439:175-82.
39. Kam PCA and Liew S; Traditional Chinese herbal medicine and anaesthesia. Anaesthesia. 2002;57:1083-1089.
40. El-Shabrany OA, El-Gindi OD, Melek FR, Abdel-Khalk SM, Haggig MM. Biological properties of saponin mixtures of *Fagonia cretica* and *Fagonia mollis.* Fitoterapia LXVIII. 1997;219-222.
41. Hess SM, Milloning RC. In “Inflammation, Mechanism and Control”, Lepow, LH,

Wards PA. Eds, Academic Press, New-York. 1972;1-72.

42. Middleton Jr E. Biological properties of plant flavonoids: An overview. Inter. J. Pharmacognosy. 1996;34:344-348.

43. Pathak D, Pathal K, Singla AK. Flavonoids as medicinal agents: Recent Advances. Fitoterapia LXII. 1991;371-376.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=913&id=13&aid=7765