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BIOACTIVITY STUDIES OF THE INDIVIDUAL INGREDIENTS OF THE DASHAMULARISHTA

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ABSTRACT:

The bioactivity studies of the individual ingredients of Dashamularishta – a classical Ayurvedic preparation were done with the aqueous extracts of the individual ingredients. The *Aegle marmelos* Correa. exhibited severe toxicity to the brine shrimp (BST) nauplii, wheat rootlet growth (WRG) inhibition bioassay and lettuce seed germination (LSG) bioassay. It exhibited no inhibition to the growth of PPR and Reo virus in vero cell line. The *Oroxylum indicum* exhibited moderate toxicity to the BST and WRG, but it is not toxic to the LSG. It exhibited no inhibition to the growth of PPR and Reo virus. The *Stereospermum suaveolens* exhibited severe toxicity to the BST and LSG, but it is not toxic to the WRG. It exhibited total inhibition to the growth of Reo virus, but it has not effect on the PPR virus. The *Premna integrifolia* showed severe toxicity to the BST, but it was not toxic to the WRG and LSG. It exhibited no inhibition to the growth of PPR and Reo virus. The *Gmelina arborea* exhibited severe toxicity to the BST and WRG, but it is not toxic to the LSG. It exhibited no inhibition to the growth of PPR and Reo virus. The *Solanum xanthocarpum* showed mild toxicity to the BST, WRG and LSG. It exhibited 75% inhibition to the growth of Reo virus. The *Solanum indicum* showed no toxicity to the BST, WRG and LSG. It exhibited 75% inhibition to the growth of PPR virus. The *Desmodium gangeticum* showed no toxicity to the BST, but moderate toxicity to the WRG and LSG. It exhibited total inhibition to the growth of PPR virus. The *Uraria lagopoides* showed no toxicity to the BST, WRG and LSG. It exhibited total inhibition to the growth of Reo virus. The *Tribulus terrestris* showed no toxicity to the BST, but showed moderate toxicity to the WRG and LSG. It exhibited 75% inhibition to the growth of both PPR and Reo virus.

INTRODUCTION

Dashamularishta (DMK) is a classical Ayurvedic preparation. It contains ten plants - *Aegle marmelos* Correa., *Oroxylum indicum* (Linn.) Vent., *Stereospermum suaveolens* Linn., *Premna integrifolia* Linn., *Gmelina arborea* Linn., *Solanum xanthocarpum* Burm. f., *Solanum indicum* Linn., *Desmodium gangeticum* DC., *Uraria lagopoides* DC., *Tribulus terrestris* Linn. In the Ayurvedic system of medicine it is used as analgesic, antiarthritic, against cough, rheumatism, etc. (Anonymous, 1992).

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The brine shrimp larvae (nauplii) is now suggested as a convenient probe for the pharmacological activities in plant extracts, which may be manifested as toxicity towards the newly hatched nauplii. It has the advantages of being rapid (24 hours following introduction of shrimp), inexpensive, and simple (e.g., no aseptic techniques are required). It easily utilizes a large number of continuously available organisms for statistical considerations and requires no special equipment or training and a relatively small amount of test sample (50 mg for crude extracts, even 2-20 mg or less). Active compounds thus obtained could then be subjected to more elaborate bioassays for specific pharmacological activities. Furthermore, it does not require animal serum as is needed for cytotoxicities. Animal rights advocates have not yet objected to the use of these invertebrates in experimental work (Meyer, *et al.,* 1982 and McLaughlin *et al.,* 1998).

In this communication, we have done some *in vitro* bioassays and antiviral activities of the individual ingredients of Dashamularishta, from which we could get ideas about some specific biological properties of the ingredients and may proceed for further studies with different solvent extracts with other different *in vitro* and *in vivo* pharmacological studies.

MATERIAL AND METHODS

Method of the Preparation of the Extract:

The plants - *Aegle marmelos* Correa. (BMT), *Oroxylum indicum* (Linn.) Vent. (SMT), *Stereospermum suaveolens* Linn. (PMT), *Premna integrifolia* Linn. (PMT), *Gmelina arborea* Linn. (GMR), *Solanum xanthocarpum* Burm. f. (KNT), *Solanum indicum* Linn. (BHT), *Desmodium gangeticum* DC. (SLP), *Uraria lagopoides* DC. (CKL), *Tribulus terrestris* Linn. (GKR) were collected from identified sources and then they were further identified with the help of the taxonomists of the herbariums of Bangladesh Forest Research Institute and Bangladesh National Herbarium. Except the *Tribulus terrestris* Linn., all others are stem-bark, only the *Tribulus terrestris* Linn. was seed. Then these were powdered by grinding machine. Then the aqueous extracts (kwaths) were prepared by the procedure described in the National Ayurvedic Formulary (Anonymous, 1992). Briefly, 100 gm of the powdered plant materials were boiled in 1600 ml water and were evaporated to 400 ml and thus the aqueous extracts were ready. These extracts were stored in freezer (below 0°C) to be used for the experiments.

Measurement of the Concentration of the Extract:

1 ml of the aqueous extracts were taken in watch glass and dried in low temperature (20°C) by a hot air dryer. Then the dried material was weighed with digital balance (Mettler, Switzerland). It was the mg of dry material present per ml of each of the extract.

Bioactivity Studies:

Brine Shrimp Lethality Tests (BST):

Seawater was made by 38 gm (3.8%) sea salt was dissolved in one liter of distilled water and then filtered off. This seawater was taken in a small tank and brine shrimp (*Artermia salina* Leach) eggs were added to one side of the divided tank and this side was covered. The eggs were allowed for two days (48 hrs) to hatch and mature as nauplii (larvae). The hatched shrimps were attracted to the lamp on the other side of the divided tank through the perforations in the dam. These nauplii were taken for bioassay. Then 100, 200, 300, 400, 500 and 600 µg/ml conc. of the test materials were taken in each vial with 5 ml of sea-water was added to each vial containing 10 brine shrimp nauplii. Three vials were used for each concentration and a control was used containing 100 µl of solvent and 10 nauplii in 5 ml of seawater. A magnifying glass was used for convenience of counting of the nauplii. After 24 hours, the vials were observed and the number of survivors in

each vial were counted and noted. From this data, the percentage of mortality of nauplii was calculated at each concentration (McLaughlin *et al*., 1998).

Wheat Rootlet Growth Inhibition Bioassay (WRG):

Wheat grains (*Triticum aestivum* L., cv 'Buck Ñandú') were germinated in tap water during 48 hours in the dark at room temperature. Ten germinated seeds, selected a random, were placed on filter paper in petri dish (10-cm diameter) containing certain amounts (100, 200, 300, 400, 500 and 600 µg/ml) of aqueous extracts. These Petri dishes were incubated for 5 days under the same conditions as above. Triplicates were performed for all concentrations and controls. The longest rootlet of each seed was measured and inhibition calculated as a percentage relative to the length of the rootlets in the controls with tap water. Triplicates were performed for all concentrations and controls. Data treatment assumed a normal distribution and the average rootlet length for each concentration was used in the percentage of growth inhibition calculated (Mongelli *et al.*, 1995). The IC_{50} values were calculated using the probit analysis method with the help of MS Excel 2000®™.

Lettuce Seed Germination Bioassay (LSG):

Assays were conducted in 10-cm Petri dishes against lettuce seedling (Green Wave®™, Takji, Japan) root growth with a sample concentration range of 100, 200, 300, 400 and 500 µg/ml in 0.5% agar (Pastaur Lab., France). All the seeds were first germinated on 0.5% agar in a growth chamber set at 18°C, 8-h nights, 22°C, 16-h days. Germinated seedlings were transferred to the prepared petri dishes and incubated for 72 hours at which time control showed good growth (Oguntimein and Elakovich, 1991). Triplicates were performed for all concentrations and controls. The lengths of roots were measured to the nearest millimeter and then the IC_{50} values were calculated using the probit analysis method with the help of MS Excel 2000®™.

Study of Antiviral Activity:

Cell Culture and Cytotoxicity Assays

The cell line used was VERO (green monkey kidney cells) for growing PPR and Reo virus. Cells were grown in monolayer in a 5% carbon dioxide and 95% atmosphere at 37ºC, in Eagle's minimum essential medium (EMEM) (Gibco®, UK) with 10% foetal bovine serum (FBS) (Gibco®, UK), 1% non-essential amino acid (Gibco®, UK), streptomycin, neomycin and gentamycin sulfate were added.

To test for cytotoxicity, monolayers of the cells were grown in 96-well microtitre plates, and exposed to twofold serial dilution (dilution factor) of the plant extracts, starting from neat (mg/ml) to 10^8. The treated cells were then incubated at 37ºC for 3 days after which they were checked for cytopathologic effect (CPE) and stained with crystal violet to show effect on cells if any. CPE was indicated by clearance of cells in the wells (Kudi and Myint, 1999).

Antiviral assay

For the antiviral assay two animal viruses were used. They were PPR virus and Reo virus. The ability of dilute plant extract to inhibit virus specific CPE was used as a measure of antiviral activity.

To screen for antiviral activity, monolayer of the cells described earlier was grown in the 96-well microtitre plates. Twofold serial dilution (dilution factor) of the plant extracts was made just as in the cytotoxicity assay. Then 100 µl of 10^5 viral particles was added to each test well. The cultures were then incubated at 37ºC in 5% carbon dioxide for an hour to allow for viral adsorption after which 100 µl/well of the plant extracts were added and then the plates were

reincubated at the same conditions usually for 3 days to allow development of CPE if any. Controls were set consisting of only cells and cells with virus only.

An extract was said to have antiviral activity if there was absence of viral CPE. The partial inhibition of the virus (marked reduction in infectivity of the virus exposed to an extract when compared to the virus only control) was also recorded (Kudi and Myint, 1999). Triplicates were performed for all concentrations and controls.

RESULTS AND DISCUSSIONS

Brine Shrimp Lethality Bioassay (BST):

Brine shrimp (*Artemia salina* Leach) larvae (nauplii) have been used for over thirty years in toxicological studies, and international symposia have been held discussing their biology and potential usefulness (Persoone, 1980). The brine shrimp bioassay is also useful in the science of food toxicology (McLaughlin, 1991). The eggs of brine shrimp, *Artemia salina* Leach, are readily available at low cost in pet shops as a food for tropical fish, and they remain viable for years in the dry state. Upon being placed in a brine solution, the eggs hatch within 48 hours, providing large numbers of larvae (nauplii). Brine shrimp have been previously utilized in various bioassay systems (Meyer *et al.,* 1982). This bioassay determines the lethalities of materials toward brine shrimp larvae and in doing so predicts the ability to kill cancer cells in cell cultures, kill insects, and exert a wide range of pharmacological effects (McLaughlin, 1991).

A positive correlation exists between brine shrimp lethality and 9KB (human nasopharyngeal carcinoma) cytotoxicity ($p=0.036$ and kappa=0.56); thus many researchers use brine shrimp as a prescreen of plant extracts for antitumor compounds. Many researchers use the brine shrimp test as a prescreen for a panel of six human solid tumor cell lines (McLaughlin *et al.,* 1998).

Median effective dose (ED_{50}) values for cytotoxicities are generally about one-tenth the median lethal concentration (LC_{50}) values found in the brine shrimp test. It is possible to detect and then monitor the cytotoxicity of the fractionation (bioactivity guided fractionation), as well as 3PS (P388) (*in vivo* murine leukemia) active extracts using the brine shrimp lethality bioassay rather than more tedious and expensive *in vitro* and *in vivo* antitumor assays (McLaughlin *et al.,* 1998).

The bioactivity study using the brine shrimp lethality bioassay of the aqueous extracts of the individual ingredients of the DMK was done. Most of the individual ingredients of the DMK are toxic to the 24 hour hatched nauplii of the brine shrimp. Because the lethal concentrations of the extracts were less than 1000 μg/ml to be accounted as toxic or bioactive.

The median lethal concentrations (LC_{50}) of the individual ingredients of the DMK are – GMR (290.65 μg/ml), SMT (66.95 μg/ml), GMT (74.44 μg/ml), BMT (20.09 μg/ml), PMT (2.38×10^{-3} μg/ml), SLP (44.77×10^{3}), CKL (10.93×10^{3}), GKR (6.3×10^{3}), BHT (3.16×10^{3}), and KNT (>400). Of which the median lethal concentration (LC_{50}) is less than 1000 μg/ml, is counted as toxic to the brine shrimp nauplii. Of these PMT exhibited the highest toxic effects to the 24 hour hatched brine shrimp nauplii. Other extracts, such as, GMR, SMT, GMT and BMT are comparatively lesser toxic than the PMT. Some others, such as, SLP, CKL, GKR, BHT and KNT are non-toxic. The LC_{50} values of these extracts are above 1000 μg/ml.

The Table-1 represents the results from the brine shrimp lethality bioassay including the LC_{50} and ED_{50}.

Table 1
Tabular Presentation of the Effect of the Individual Components of DMK on BST
(LC_{50} and ED_{50} values in μg/ml)

Samples	Log conc.					LC_{50}	ED_{50}
	2.000	**2.301**	**2.477**	**2.602**	**2.700**		
GMR	25.0	28.57	72.73	76.92	100.0	290.65	29.07
SMT	0.0	69.23	100.0	100.0	100.0	66.95	6.7
GMT	26.67	100.0	100.0	100.0	100.0	74.44	7.44
BMT	0.0	85.71	92.86	93.33	100.0	20.09	2.01
PMT	69.23	83.33	73.33	91.66	93.75	2.38×10^{-3}	0.238×10^{-3}
SLP	0.0	44.0	40.0	17.65	58.33	44.77×10^{3}	4.48×10^{3}
KNT	0.0	0.0	0.0	0.0	0.0	>500	>50
BHT	0.0	0.0	0.0	0.0	20	3.16×10^{3}	0.316×10^{3}
GKR	0.0	2.86	47.06	59.10	78.26	6.3×10^{3}	0.63×10^{3}
CKL	0.0	19.23	25.81	45.83	60.0	10.93×10^{3}	1.09×10^{3}

Wheat root-let growth inhibition bioassay (WRG):

The aqueous extracts of GMR (26.23×10^{-3} μg/ml), SMT (36.47 μg/ml) and BMT (2.49×10^{-7} μg/ml) are toxic to the wheat rootlet growth. Because the median inhibitory concentrations (IC_{50}) of these extracts are less than 1000 μg/ml, which are counted as toxic to the cells of the wheat rootlet.

But the median inhibitory concentrations (IC_{50}) of other two extracts GMT (201.33×10^{3} μg/ml) and PMT (98.04×10^{3} μg/ml) are not toxic to the wheat rootlet growth. Because the median inhibitory concentrations (IC_{50}) of these extracts are more than 1000 μg/ml.

Three aqueous extracts [SLP, KNT and GKR] of the individual ingredients of the DMK are toxic to the wheat rootlet growth. Because the inhibitory concentrations of SLP (0.026 μg/ml), KNT (23.61 μg/ml) and GKR (2.49×10^{-7} μg/ml) are less than 1000 μg/ml, which are also counted as toxic or bioactive. But the inhibitory concentrations of the other two aqueous extracts [BHT and CKL] are more than 1000 μg/ml.

The inhibitory concentrations of the individual ingredients on the wheat rootlet growth were GKR = 2.49×10^{-7}, SLP = 0.026, KNT = 23.61, BHT = 10.16×10^{3}, and CKL = 98.04×10^{3}. These are not toxic to the rootlet growth.

Of these the most toxic extracts are BMT and GKR (2.49×10^{-7} μg/ml) to the wheat root cells. Probably these extracts may have inhibitory effects on the mitotic phase (M phase) of the cell growth cycle.

The Table-2 represents the results from the WRG bioassay including the IC_{50}.

Table 2
Tabular Presentation of the Effect of the Individual Components of DMK on WRG
(IC_{50} values in μg/ml)

Samples	Log concentrations					IC_{50}
	2.000	**2.301**	**2.477**	**2.602**	**2.700**	
GMR	52.4	63.52	21.08	11.26	58.41	26.23×10^{-3}
SMT	66.67	32.0	26.33	17.33	15.0	36.47
GMT	11.56	-1.97	22.58	55.10	43.57	201.33×10^{3}
BMT	75.71	72.87	81.95	73.35	76.09	2.49×10^{-7}
PMT	26.28	46.65	54.41	34.95	50.71	98.04×10^{3}
SLP	52.4	63.52	21.08	11.26	58.41	0.026
KNT	36.99	69.75	75.11	83.62	95.0	23.61
BHT	11.56	-1.97	22.58	55.10	43.57	10.16×10^{3}
GKR	75.71	72.87	81.95	100.0	100.0	2.49×10^{-7}
CKL	26.28	46.65	54.41	34.95	50.71	98.04×10^{3}

Lettuce Seed Germination Bioassay (LSG):

The aqueous extracts of the BMT (1.25×10^{-3} μg/ml) and PMT (103.85 μg/ml) are toxic to the lettuce seed germination. Because the median inhibitory concentrations (IC_{50}) of BMT and PMT are less than 1000 μg/ml, which is counted as toxic or bioactive. But the median inhibitory concentrations (IC_{50}) of the aqueous extracts of the GMR (7.102×10^{3} μg/ml), SMT (730.79×10^{3} μg/ml) and GMT (434.03×10^{3} μg/ml) are more than 1000 μg/ml, which are counted as non-toxic.

Three aqueous extracts [SLP, KNT and GKR] are toxic to the lettuce seed germination. Because the inhibitory concentrations of SLP, KNT and GKR are less than 1000 μg/ml, which are counted as toxic or bioactive. But the median inhibitory concentrations of the other two aqueous extracts [BHT and CKL] are more than 1000 μg/ml. The inhibitory concentrations of these extracts were GKR = 0.0, SLP = 0.03, KNT = 23.61, BHT = 20.13×10^{3} and CKL = 98.04×10^{3}.

In this experiment, the aqueous extracts of the BMT exhibited highest toxic effect to the lettuce seed germination.

The Table-3 represents the results from the LSG bioassay including the IC_{50}.

Table 3
Tabular Presentation of the Effect of the Individual Components of DMK on LSG
(IC_{50} values in μg/ml)

Samples	Log concentrations					IC_{50}
	2.000	**2.301**	**2.477**	**2.602**	**2.700**	
GMR	16.91	13.64	60.0	44.55	62.73	7.102×10^{3}
SMT	7.82	16.64	27.27	9.1	57.55	730.79×10^{3}
GMT	1.82	50.0	40.27	40.0	37.36	434.03×10^{3}
BMT	21.18	33.82	-12.73	11.73	-1.18	1.25×10^{-3}
PMT	73.64	34.55	33.64	3.36	16.36	103.85
SLP	52.4	63.52	21.08	11.26	58.41	0.03
KNT	36.99	69.75	75.11	83.62	93.16	23.61
BHT	11.56	-1.97	22.58	55.10	43.57	20.13×10^{3}
GKR	75.71	72.87	81.95	73.35	76.09	256.96
CKL	26.28	46.65	54.41	34.95	50.71	98.04×10^{3}

Antiviral Activities of the Aqueous Plant Extracts on Animal Viruses

From the combination of the Dashamularishta (DMK) – a group of aqueous extracts of the plant parts [barks from the BMT (*Aegle marmelos*), SMT (*Oroxylum indicum*), PMT (*Stereospermum suaveolens*), GMR (*Gmelina arborea*), and GMT (*Premna integrifolia*)] did not exhibit any antiviral activity against the Peste des Petits Ruminants (PPR) virus.

Only the aqueous extracts of the bark of PMT (*Stereospermum suaveolens*) exhibited potent antiviral activity against the Reo virus in the VERO cell lines cultures.

The aqueous extracts of other plant parts from the combination DMK [barks from the BMT (*Aegle marmelos*), SMT (*Oroxylum indicum*), GMR (*Gmelina arborea*), and GMT (*Premna integrifolia*)] did not show any antiviral activity against the Reo virus in the VERO cell lines.

The aqueous extracts of the whole plants of SLP (*Desmodium gangeticum*), BHT (*Solanum indicum*) and the seeds of the GKR (*Tribulus terrestris*) has moderately potent antiviral activity against the Peste des Petits Ruminants (PPR) virus and the aqueous extracts of the whole plants of CKL (*Uraria lagopoides*), GKR (*Tribulus terrestris*) and KNT (*Solanum xanthocarpum*) has moderately potent antiviral activity against the Reo virus.

The whole plants of the CKL (*Uraria lagopoides*) and KNT (*Solanum xanthocarpum*)] and the whole plants of the BHT (*Solanum indicum*) and SLP (*Desmodium gangeticum*)], did not show any antiviral activity against the PPR virus and Reo virus, respectively, in the VERO cell lines.

The Table-4 represents the effect of the individual components of DMK on virus propagation in tissue culture of Green Monkey Kidney (VERO) cells. Photos showing different stages of VERO cell culture and effects of plant extracts on the cell line infected with virus.

Table 4
Tabular Presentation of the Effect of the Individual Components of DMK on Virus Propagation in Tissue Culture of Green Monkey Kidney (VERO) Cells

Groups	Primary			Animal Viruses			
	Cyto-toxicity	Conc. (mg/ml)	Dil. Factor	PPR virus	Lowest Effective Conc.	Reo virus	Lowest Effective Conc.
BMT	NT	10	2	-	0.0	-	0.0
SMT	NT	10	2	-	0.0	-	0.0
GMT	NT	10	2	-	0.0	-	0.0
GMR	NT	10	2	-	0.0	-	0.0
PMT	NT	10	2	-	0.0	++++	100.0 ng/ml
SLP	NT	10	2	++++	10.0 ng/ml	-	0.0
KNT	NT	10	2	-	0.0	+++	1.0 µg/ml
BHT	NT	10	2	+++	10.0 µg/ml	-	0.0
GKR	NT	10	2	+++	1.0 µg/ml	+++	100.0 ng/ml
CKL	NT	10	2	-	0.0	++++	1.0 µg/ml

Note: ++++, Total Inhibition, +++, 75% Inhibition, ++, 50% Inhibition, +, <50% Inhibition, -, No Inhibition, NT, Non-cytotoxic to the Vero cell line at primary concentration.

CONCLUSION

In all the individual ingredients of Dashamularishta – *Stereospermum suaveolens* exhibited the highest toxic effects to the 24 hours hatched brine shrimp nauplii. It may have highly significant cytotoxic activity with other biological activities. It also showed highly significant antiviral activity against Reo virus.

The most toxic effects on the wheat root cells exhibited by the aqueous extracts of *Aegle marmelos* and *Tribulus terrestris*. Probably the extracts *A. marmelos* and *T. terrestris* may have inhibitory effects on the mitotic phase (M phase) of the cell growth cycle. Other biological activities (e.g., allelopathy, phytotoxicity, etc.) may also present with this activity.

In the lettuce seed germination inhibition bioassay, the aqueous extracts of the *Aegle marmelos* exhibited highest toxic effect to the lettuce seed germination, which support the previous result from the wheat rootlet growth inhibition bioassay.

The aqueous extracts from the whole plants of *Desmodium gangeticum*, *Solanum indicum* and the seeds of the *Tribulus terrestris* has moderately potent antiviral activity against the Peste des Petits Ruminants (PPR) virus in the vero cell lines.

From these studies it is suggested that, most of the individual ingredients of Dashamularishta have profound biological activities, which must be ensured by vast research works. Especially *Stereospermum suaveolens*, *Desmodium gangeticum*, *Uraria lagopoides*, *Tribulus terrestris*, etc., need much more research works. Pure compound isolation from these plants and plant parts could be done for proper identification of bioactivity.

These results led us to do the *in vivo* pharmacological studies on the central nervous system, gastrointestinal and urinary systems.

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REFERENCES

Anonymous (1992). Preparation of kwath and Dashmulakwath, in *Bangladesh National Formulary of Ayurvedic Medicine*, (Approved by the Govt. of Bangladesh vide Ministry of Health and Family Welfare, Memo No. Health-1/Unani-2/89/(Part-I) 116, dated 3-6-i1, 20-32.

Kudi, A.C. and Myint, S.H. (1999). Antiviral activities of some Nigerian medicinal plant extracts. *J. Ethnopharmacol*, **68:** 289-294.

McLaughlin, J.L., Rogers, L.L. and Anderson, J.E. (1998). The Use of Biological Assays to Evaluate Botanicals. *Drug Inform J.* **32**(2): 513-524.

McLaughlin, J.L. (1991). Bench-top Bioassays for the Discovery of Bioactive Compounds in Higher Plants. *Brenesia*, **34**: 1-14.

Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobssen, L.B., Nichols, D.E. and McLaughlin, J.L. (1982). Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents. *Planta Med*, **45:** 31-34.

Mongelli, E., Desmarchelier, C., Giulietti, A., Coussio, J. and Ciccia, G. (1995). Bioactivity of certain medicinal latexes used by the Ese'ejas. *J. Ethnopharmacol*, **47:** 159-163.

Oguntimein, B.O. and Elakovich, S.D. (1991). Allelopathic activity of the essential oils of Nigerian medicinal plants. *Intern J. Pharmacogn*, **29**(1): 39-44.

Persoone. (1980). Proceedings of the International Symposium on Brine Shrimp, *Artemia salina*, Universal Press, Witteren, Belgium, pp.1-3.