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Full Length Research Paper

Synergistic effect of combined extract of *Bryophyllum pinnatum* and *Aloe barbadensis* enhances anti-microbial activity in-vitro

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This study was conducted between June and December 2012 in Achievers University, Owo-Nigeria to determine in-vitro antimicrobial activity of combined extract of *Bryophyllum pinnatum* and *Aloe barbadensis* on some clinical bacterial isolates and control organisms. *The* leaves from both herbs were collected, washed, grinded, filtered and the filtrates dried, weighed re-dried and re-weighed again until the weights were constant. Dried filtrate of each extract was grinded with pestle and mortar into powdery form. One gramme of each powdery extract was dissolved in ten milliliters of sterile distilled water to produce 100mg/ml. *Bryophyllum pinnatum* extract and *Aloe barbadensis* extract were mixed together at ratio of 6:4 to prepare various concentrations (10^{-1} to 10^{-9} mg/ml). of admixed extract. Each prepared concentration was tested on different bacteria isolates and control organisms using punch-hole antimicrobial sensitivity method .Findings from this study showed that a billionth concentration (mg/ml) of the mixed extract inhibited the growth of all common clinical isolates examined except *Pseudomonas aeruginosa* and *Klebsiella Pneumoniae* that were inhibited at high concentration (10^{-3}) of the extract. Assessing the efficacy of the fresh and stored extracts, the results showed no significant difference in the zones of inhibition of the bacteria isolates with respect to varying concentrations of fresh and stored extracts (P>0.05). By comparing the activity of the extract on the test isolates and control organisms there was insignificant variation in the zones of inhibition produced by the organisms (P>0.05). In conclusion, the synergism between the two herbs increased their antimicrobial potentials as a result of positive interaction emanating from their combination.

Keywords: Synergism, *Bryophyllum pinnatum*, *Aloe barbadensis*, anti-bacterial

INTRODUCTION

Bryophyllum pinnatum is a perennial herb growing widely and used in folkloric medicine in tropical Africa, tropical America, India, China, and Australia It is commonly refer to as life plant, miracle leaf, Air plant and the katakataka plant (Engler *et al.*,1926). The plant is widely used in traditional medicine for the treatment of variety of ailments and well known for its haemostatic and wound healing properties. The divine herb contains a wide range of active compounds, otherwise known as phytochemicals such as; alkaloids, triterpenes, glycosides, flavonoids, steroids, bufadienolides, lipids and organic acids.(Marriage *et al.*,1971) (Gaind *et al.*, 1972). The extracts from this plant possess medicinal

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properties such as; immune-modulator, CNS depressant, analgesic, anti-inflammatory, antiallergenic, antianapyhlactic, anti-ulcerous, gastroprotective, insecticidal and sedative (Akinpelu, 2000).(Misra *et al*.,1979) (Pal *et al*.,1991)

Similarly*, Aloe barbadensis* a is a stemless or very short-stemmed succulent plant growing to 60–100 cm tall, The leaves are thick and fleshy, green to grey-green, with some varieties showing white flecks on the upper and lower stem surfaces (Yates,2002) The margin of the leaf is serrated and has small white teeth. The flowers are produced in summer on a spike up to 90cm tall, each flower being pendulous, with a yellow tubular corolla 2–3 cm long. Like other *Aloe* species, *Aloe vera* forms arbuscular mycorrhiza, a symbiosis that allows the plant better access to mineral nutrients in soil.(Gong et al.,2002) The species has a number of synonyms: *A. barbadensis* mill., *Aloe indica and Aloe perfoliata* Lam. var. *vera* ,Common names include Chinese Aloe, Indian Aloe, True Aloe, Barbados Aloe, Burn Aloe, and First Aid Plant (Vogler and Ernst (1999).

Bacterial resistance to antibiotics is becoming a concern to public health. The increasing resistance of many microorganisms to many antibiotics has prompted the searches for new antimicrobial and antifungal agents either by synthesis of new agents or through the discovery of new natural antimicrobial agents (Cock, 2008).The concept of combinational therapy of herbal products is probably one of the ways to reduce drug resistance

Several studies (Ofokansi *et al*., 2005, Okwu *et al*., 2004, and Satish *et al.,* 1999) had documented antimicrobial activities of *Bryophyllum pinnatum* and *Aloe barbadensis* on some common isolates of microorganisms when separately administered. However, combinatorial effect of both plant extracts on microorganisms is yet to be documented. The aim of the study therefore is to determine the in-vitro antimicrobial activity of the combined extract of *Bryophyllum pinnatum* and *Aloe barbadensis* on some common bacterial isolates.

MATERIALS AND METHODS

Collection of herbs and preparation of crude extract

Fresh *Bryophyllum pinnatum* leaves were collected from Isale Aluko garden near Baboko market in Ilorin while the fresh *Aloe barbadensis* pant was collected from potted plant at Adewole Estate in Ilorin. All the plants and leaves were taken to Professor Oladele in the Plant Biology Unit of Kwara State University for identification. Manual cold extraction was employed on each of the plants.

The *Bryophyllum pinnatum* leaves were washed and grinded in sterilized electric blender, the paste obtained was squeezed out through a sterilized mesh of about 0.20mm in size and the residue (extract) was kept in an electronic oven, maintained at 60^0c for drying. The resultant fire granule(powdery) was weighed, re-dried and weighed again until constant weight was obtained the same procedure was repeated for the *Aloe barbadensis* leave to obtain a coarse resultant granules which was later grinded with sterilized ceramic mortar and pestle .

Preparation of the fortified extract

One gram of the granulated extract of *Bryophyllum pinnatum* was weighed and dissolved in 10millimiters of sterile distilled water to obtain 100mg per millimeter concentration of bryophyllum pinnatum. Similarly, 1 gram of powdery *Aloe barbadensis* was weighed and dissolved in 10millimeter of sterile distilled water to obtain 100mg per millimeters concentration of aloe barbadensis, 0.6ml of the *Bryophyllum pinnatum* concentration was mixed with 0.4ml of the *Aloe barbadensis* to obtain 100mg per ml of the combined extract.

Collection and preservation of clinical Bacteria isolates

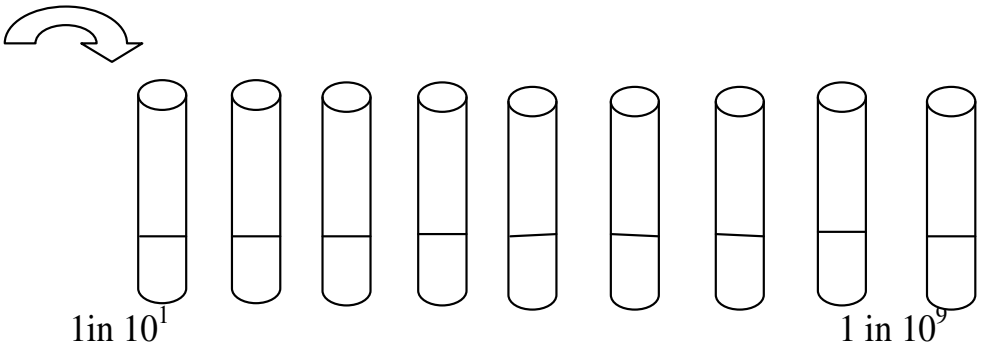
Bacterial isolates was collected from Microbiology Laboratory Unit in the pathology Department of Federal Medical Centre Owo. Confirmed laboratory bacterial isolates (*pseudomonas, E.coli, klebsiella spp, staphylococcus, proteus spp, and salmonella*.) were inoculated into already prepared agar slant, labeled properly and incubated at 37^0c for two days and later preserved and stored inside the refrigerator at 2-4^0C

Collection and preservation of control bacterial

The control organisms used were; *oxford Staphylococcus aureus* (NCIB 8588) and *Escherichia coli*(NCIB 950). The control organism were gotten from National Institute of Medical Research, Yaba - Lagos Nigeria.

Dilution of the extract and procedure for antimicrobial testing

Dilution of the extract was made by serial 10-fold dilution method starting with 1in 10^1 to
1 in 10^9 of 1mg/ml of the fortified extract with molten nutrient agar as diluents.



Sensitivity testing medium was prepared by dissolving 14g of the nutrient agar powder in 500ml of distilled water in one-liter conical flask. The mixture was gently warmed and stirred until the powder dissolved completely. The mixture was then autoclaved at 1.5 atmosphere and temperature of 121^0C. Sensitivity plates were prepared from the sterilized molten nutrient medium and were kept in the refrigerator at 4^0C when not used immediately the clinical isolates and control bacterial were stored in separate bijou bottles of peptone water. The organisms in the peptone water were allowed to stand in the incubator at 37^0C for 20 minutes before flooding each organism on the dried sensitivity testing plates

Punch- hole diffusion technique was employed in this study as described by Baker and Silverton (2000). Nine holes were made on each plate with sterilized base of Pasteur pipette and each hole was filled separately with drops 1 in 10^1 to 1 in 10^9 concentrations of 1mg/ml of the extract. The plates were allowed 15minuted on laboratory bench for proper absorption of the extract onto the agar while excess diluted extract were removed by inverting the plates and incubated at 37^0C overnight. Different zones of inhibition were measured after 24 hours of incubation with the aid of a pair of divider and ruler in milliliters to determine the MIC of the extract against particular isolate. Minimum inhibitory concentration (MIC) is the lowest concentration of the extract that inhibited the visible growth of a particular bacterial isolate after overnight incubation

Statistical analysis of data

Mean of the diameters of zones of inhibition, Standard deviation and Student's test were the statistical tools employed for the analysis of data collected The mean is the central tendency of the data obtained which is the summation of the diameters divided by the size of the collection. Standard deviation which was calculated based on the appropriate formula. Student- t test was applied to assess the difference in the extract activities on clinical isolates and control organisms

RESULTS

Antimicrobial activities of *Aloe barbadensis* extract on clinical isolates and control organisms is as shown in Table 1. Assessing the impact of the extract on the isolates and control organisms, the extract was only effective at higher concentration of 10^{-1} and 10^{-3}. When the mean diameter of zones of inhibition of the clinical isolates of *Staphylococcus aureus* was compared with the mean diameter of the oxford Staphylococcus aureus at the same concentration of the extract, statistical analysis by student-t test showed no significant difference ($P>0.05$)

Table 2 shows antimicrobial activities of fresh *Bryophyllum pinnatum* on clinical isolates and control organisms. After the extract application at varying concentrations, only concentration of 10^{-1} was effective on all the organisms tested. Statistical analysis showed that there was no significant difference in the mean zone of inhibition of the clinical Escherichia coli isolates and that of the control at the same concentration of the extract ($P>0.05$)

Table 3 depicts the activities of the fresh extract of combined extract of *Bryophyllum pinnatum and Aloe barbadensis* on gram positive and gram negative bacterial isolates. the extract produce a higher inhibition of *Staphylococcus aureus,* Hemolytic streptococci*, E.coli, Proteus spp* and *Salmonella typhi* with a reducing zone of inhibition as the dilution increased from 10^{-1} to10^{-9} . The extract however showed lower efficacy on *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* with the extract concentration between 10^{-3}and10^{-9}showing no effect on *Pseudomonas aeruginosa* while *Klebsiella pneumoniae* was resistant to the extract at concentration lower than 10^{-5} mg/ml

In-vitro antimicrobial sensitivity of some bacterial isolates to varying concentration of combined extract of *Bryophyllum pinnatum and Aloe barbadensis* stored at 4^0 C for one month is as shown in Table 4. The varying concentrations of the stored extract was efficacious on inhibiting the growth of *Staphylococcus aureus, Hemolytic streptococci,* E. coli, Proteus spp. and

Table 1. Antimicrobial susceptibility of fresh extract of *Aloe barbadensis* on some bacterial isolates

Bacterial Isolate	No Exam	EXTRACT CONCENTRATION BY ZONE OF INHIBITION±SD (mm)				
		10^{-1}	10^{-3}	10^{-5}	10^{-7}	10^{-9}
Staphylococcus aureus	45	1.9±0.03	1.6±0.2	0	0	0
Heamolytic strept	20	1.9±0.3	1.87±0.01	0	0	0
E. coli	40	1.92±.17	1.82±0.42	0	0	0
Pseudomonas aeruginosa	15	1.8±032	1.73±0.2	0	0	0
Klebsiella spp	26	2.65±0.1	1.84±0.35	0	0	0
Proteus spp	22	2.5±0.13	1.92±0.03	0	0	0
Salmonella typhi	10	*2*.03 ±.1 5	1.92±0.04	0	0	0
Control Organisms						
Oxford staph (NCIB 8588)	40	2.65±0.01	2.18±0.34	0	0	0
E.coli (NCIB 950)	34	2.50± 0.1	1.86± 0.14	0	0	0

No-number SD-Standard deviation

Table 2. Antimicrobial susceptibility of fresh extract of *Bryophillum pinnatum* on some bacterial isolates

Bacterial Isolate	No Exam	EXTRACT CONCENTRATION BY ZONE OF INHIBITION±SD (mm)				
		10^{-1}	10^{-3}	10^{-5}	10^{-7}	10^{-9}
Staphylococcus aureus	45	1.2±0.03	1.0±0.2	0	0	0
Heamolytic strept	20	1.8±0.3	1.47±0.01	0	0	0
E. coli	40	1.62±.17	1.12±0.42	0	0	0
Pseudomonas aeruginosa	15	1.1±032	0	0	0	0
Klebsiella spp	26	2.65±0.1	1.84±0.35	0	0	0
Proteus spp	22	2.5±0.13	1.92±0.03	0	0	0
Salmonella typhi	10	*2*.03 ±.1 5	0	0	0	0
Control Organisms						
Oxford staph (NCIB 8588)	40	1.55±0.01	1.28±0.34	0	0	0
E.coli (NCIB 950)	34	1.60± 0.1	1.36± 0.14	0	0	0

No-number SD-Standard deviation

Table 3. Antimicrobial susceptibility of fresh combined extract of *Bryphyllum pinnatum* and *Aloe barbadensis* on bacterial isolates

Bacterial Isolate	No Exam	EXTRACT CONCENTRATION BY ZONE OF INHIBITION±SD (mm)				
		10^{-1}	10^{-3}	10^{-5}	10^{-7}	10^{-9}
Staphylococcus aureus	45	1.5±0.03	1.3±0.2	0.86±1.3	0.46±0.1	0.5±0.02
Heamolytic strept	20	1.6±0.3	1.67±0.01	1.13±0.03	0.86±0.1	0.77±0.3
E. coli	40	1.22±.17	1.0±0.42	0.96±0.23	0.84±0.1	0.70±01
Pseudomonas aeruginosa	15	1.2±032	0.73±0.2	0	0	0
Klebsiella spp	26	10.65±0.1	1.4±0.35	0.8±0.11	0	0
Proteus spp	22	2.1±0.13	1.33±0.03	1.4±0.3	1.8±0.03	0.37±0.13
Salmonella typhi	10	1.03 ±.1 5	0.9±0.04	0.76±.21	0.64±0.03	0.60±01
Control Organisms						
Oxford staph (NCIB 8588)	40	1.65±0.01	1.4±0.34	0.8± 0.13	0.62±0.01.	0.44±0.03
E.coli (NCIB 950)	34	1.60± 0.1	1.56± 0.14	0.2±0.02	0182±0.03	0.15±0.01

No-number SD-Standard deviation

Table 4. Antimicrobial susceptibility of stored combined extract of *Bryphyllum pinnatum* and *Aloe barbadensis* on bacterial isolates

Bacterial Isolate	No Exam	EXTRACT CONCENTRATION BY ZONE OF INHIBITION±SD (mm)				
		10^{-1}	10^{-3}	10^{-5}	10^{-7}	10^{-9}
Staphylococcus aureus	45	1.5±0.03	1.3±0.2	0.86±1.3	0.46±0.1	0.5±0.02
Heamolytic strept	20	1.6±0.3	1.67±.01	1.13±0.03	0.86±0.1	0.77±0.3
E. coli	40	1.2±0.1	1.0±0.4	0.96±.02	0.84±0.1	0.70±01
Pseudomonas aeruginosa	15	1.2±0.32	0.73±0.2	0	0	0
Klebsiella spp	26	1.65±0.1	1.4±0.3	0.8±0.1	0	0
Proteus spp	22	2.1±0.13	1.33±0.03	1.04±0.3	1.8±0.03	0.37±0.13
Salmonella typhi	10	0.9±0.04	0.86±0.2	0.72±0.01	0.61±0.02	0.35±0.2
Control Organisms						
Oxford staph (NCIB 8588)	40	1.65±0.01	1.4±0.3	0.8± 0.1	0.53± 0.03	0.41± 0.06
E.coli (NCIB 950)	34	1.60± 0.1	1.56± 0.14	0.2±0.24	0.16±0.01	0.15±0.01

No-number SD-Standard deviation

Table 5. Comparison of antimicrobial activities of the fresh combined extract of *Bryphyllum pinnatum* and *Aloe barbadensis* against clinical and control isolates of *Staphylococcus aureus*

Conc. of extract	zone of inhibition (mm)		P-value	Significance level
	Control Organisms	Clinical Isolates		
10^{-1}	1.65±0.01	1.5±0.03	0.91	P>0.05
10^{-3}	1.4±0.3	1.3±0.2	1.62	P>0.05
10^{-5}	0.8± 0.1	0.86±1.3	1.86	P>0.05
10^{-7}	0.62 ±0.01	0.46±.1	1.82	P>0.05
10^{-9}	0.44±0.03	0.50±0.02	1.91	P>0.05

Degree of freedom = 8
Critical value (T_{tab}) = 2.306

Table 6. Comparison of antimicrobial activities of the fresh extract of combined extract of *Bryphyllum pinnatum* and *Aloe barbadensis* against clinical and control isolates of *Escherichia coli*

Conc. of extract	zone of inhibition (mm)		P-value	Significance level
	Control Organisms	Clinical Isolates		
10^{-1}	1.60±0.1	1.6±0.3	0.91	P>0.05
10^{-3}	1.56±0.14	1.67±0.01	1.62	P>0.05
10^{-5}	1.2±0 0.4	1.13±0.03	1.86	P>0.05
10^{-7}	0.18±0.04	0.26±0.6	1.882	P>0.05
10^{-9}	0.15± 0.03	0.1±0.02	1.91	P>0.05

Degree of freedom = 8
Critical value (T_{tab}) = 2.306

Samonella typhi. Also the stored extract was only effective on *Pseudomonas aeruginosa* at the concentrations not below 10^{-3} while *Klebsiella pneumoniae* was sensitive to the extract at concentrations not lower than 10^{-5}

Antimicrobial sensitivity of clinical isolates and control *Staphylococcus aureus* to fresh combined extract of *Bryophyllum pinnatum and Aloe barbadensis* is as shown in Table 5 Comparing the mean diameter of zones of inhibition of the clinical isolates of *Staphylococcus aureus* with the mean diameter of the control *Staphylococcus aureus* at the same concentration of the extract, statistical analysis by student-t test showed no significant difference (P>0.05)

Table 6 shows antimicrobial activities of fresh combined extract of *Bryophyllum pinnatum and Aloe barbadensis* on clinical isolates and control organisms. Statistical analysis showed that there was no significant difference

in the mean zone of inhibition of the clinical *Escherichia coli isolates* and that of the control (*E.coli* NCIB 950) at the same concentration of the extract (P>0.05)

DISCUSSION

Bacterial resistance to several antibiotics is becoming a source of challenge and concern to public health. In view of the increasing rate of anti-microbial drug resistances ravaging not only the African continent but the world at large; alternative, effective and affordable substitutes are essential if bacterial infections are to be properly controlled. Several authors like Volger and Ernst (1999), Yemitan and Salaahdeen (2006) Sapratman *et al*., (2001), Salahdeen and Yemitan (2005) and Oyewole (2005) had earlier documented the medicinal potentials of the two divine herbs employed in this study. This study was therefore focused on combinatorial effect of both natural herbs to discover new and effective replacements of ineffective ones.

The findings from this present study revealed a synergistic effect of both herbs when combined together on some bacterial isolates. The in-vitro activities of the fortified *Bryophyllum-Aloe* extract was observed on both gram positive and gram negative bacteria. At a billionth (nano)concentration of the fortified extract*, Staphylococcus, aureus* , Hemolytic streptococcus, *Escherichia coli, Proteus species* and *Salmoella species* were effectively inhibited while two other organisms namely *Pseudomonas aureginosa* and *Klebsiella preumoniae* resisted the extract at nano-concentration but were inhibited in higher concentration of 10^{-3}.

Several authors like Obaseiki –Ebor (1985); Satish *et al*., (1999); Cock (2008) had previously documented the antibiotic potentials of each of these divine herbs particularly when employed as mono-therapy at high concentrations . Applying the concept of nanomedcine however, combination of both herbs has produced synergistic interaction that drastically reduced the concentration capable of complete inhibition of the same type of organisms.

Generally, as the concentration of the extract reduced, its activities on the bacteria isolate also reduce d insignificantly ($P > 0.05$). However, the lowest concentration of the extract

(10^{-9}) still produced significant zone of inhibition of most microorganism examined except *Pseudomonas aerugenosa* and *Klebsiella pneumoniae.*

Assessing the efficiency of the fresh fortified extract and the one month stored extract on bacteria isolates, the study showed no significant difference in the zones of inhibition produced by isolates with respect to varying concentrations ($P > 0.05$) .

Comparing the activities of the fresh extract on clinical isolates *of Staphylococcus aureus* and *Escherichia coli* to that on their control organisms, there was no significant variation in the zone of inhibition produced ($P > 0.05$). This implies that the action of the extract against the test and control organisms is uniform without any bias.

In view of the limitations of the present study, we want to make the following suggestions for future studies.

(a) Further studies to determine the mode of action of the combined extract and its biochemical targets in the microorganisms are desirable.

(b) Biochemical and histological investigations on the extract using animal model to ascertain the safety for human consumption.

(c) Since this study was conducted in-vitro, in-vivo susceptibility test should also be conducted using Laboratory animal to further confirm the reliability of the extract.

(d) Study on the best route of administration of the extract that will produce maximum efficiency is suggested.

(e) Impact of combined extract on malaria parasites and fungi should also be investigated.

(f) Comparative investigation on the efficacy of the extract by cold, hot and alcoholic extraction on bacteria isolates is advocated since the present study employed only cold extraction method.

(g) Research on effect of the combined extract on non-infectious diseases such as diabetics, asthma, hypertension, and peptic ulcer is advocated.

CONCLUSION

The result from this study revealed a synergism in the activities of the two divine herbs. Proper exploration and application of this positive interaction could probably improve treatment of many disease conditions as against mono-therapy with individual herbs. The findings also showed that simple storage of the extract at 4^0C could maintain the potency of its active components for up to thirty days.

REFERENCES

Akinpelu DA (2000). Antimicrobial activity of Bryophyllum Pinnatum leaves Fitoterapia. 71(2): 193-194

Baker, Silverton (2000): Introduction to medical laboratory technology (3rd Edition) Butterworth Publications London

Cock T (2008). the in vitro antimicrobial activities of the various fractions against a range of microorganisms J. Aloe vera study 2:12-34

Engler D (1926). aturrlichen Pflanzenfamilien nebst ihren Gattugen and wichtigen Arte insbesondre der Nutpflanzen. Leipzig: W. Engelmann.4: 402-412

Gong M, Wang F, Chen Y (2002). Study on application of arbuscular-mycorrhizas in growing seedings of Aloe vera in Chinese. Zhong yao cai Zhongyaocai Journal of Chinese medicinal materials 25 (1): 1–3.

Gaind K, Gupta R (1972). Alkanes ,Alkanols, Triterpenes, and Sterols of Kalanchoe Pinnata. Phytochemistry. 11: 1500-1502

Marriage PB, Wilson DG (1971). Analysis of Organic acids of Bryophyllum pinnatum. Can. J. Biochem. 49: 282-295

Misra S, Dixit SN (1979). Antifungal activity of leaf extract of some higher plants. Acta Botanica Indica. 7: 147-150

Ofokansi KC, Esimone CO, Anele CK (2005). Evaluation of the in vitro combined antibacterial effect of the leaf extracts of Bryophyllum pinnatum (Fam: crassulaceae) and Ocimum gratissimum (Fam: labiatae). Plant Prod. Res. J. 9: 23-27

Okwu DE (2004). Evaluation of the chemical composition of indigenous spices of southeastern Nigeria. Global J. Pure Appl. Sci. 7: 455-459

Obaseiki-Ebor (1985). Preliminary report on the invitro antibacterial activity of Bryophyllum pinnatum leaf juice. Afr. J. Med. Med. Sci. 14(3¬4):199-202 (1985).

Oyewole JAO (2005). Antinociceptive, anti-inflammatory and antidiabetic effects of Bryophyllum pinnatum leaf aqueous extract. J. Ethno pharmacol. 99: 13-19

Pal S, Nag Chaudhari AK (1991). Studies on the Anti-Ulcer activity of a Bryophyllum Pinnatum leaf extract in experimental animals. J. Ethnopharmacol. 33: 97- 102

Salahdeen HM, Yemitan OK (2005). Neurosedative and muscle relaxant activities of aqueous extract of Bryophyllum pinnatum. Fitoterapia. 76: 187-193

Supratman U, Fujita T, Akiyama K (2001). Anti-tumor promoting activity of bufadienolides from Kalanchoe pinnata and K. daigremontiana x tubiflora .Biosci. Biotechnol. Biochem. 65 (4): 947–949

Satish S, Raveesha KA, Janardhana GR (1999). Antibacterial activity of plant extracts on phytopathogenic Xanthomonas campestris pathovars. Letters in Appl. Microbiol. 28 (2):34-36

Vogler BK, Ernst E (1999). Aloe vera: a systematic review of its clinical effectiveness. Br. J. Gen. Pract.49 (447):823-828.

Yates A (2002). Yates Garden Guide. Harper Collins Press, Australia

Yemitan OK, Salahdeen HM (2006). Neuropharmacological Effects of Aqueous Leaf Extract of Bryophyllum Pinnatum in Mice. African Journal of Biomedical Research. 9: 101-107