Pharmaceutical Constituents of Stem of Bryophyllum Pinnatum

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Abstract
Bryophyllum pinnatum laeves extract is used by the traditional medicinal practitioners in the treatment of many diseases. In this research, effect of the stem extract of B.pinnatum is investigated on many disease-causing micro-organisms to find out its anti-microbial efficacy. Phytochemical screening of Bryophylum pinnatum stem showed the presence of the following; flavonoids (0.53); alkaloids (0.29), tannin(0.64); saponins(1.05); phenol(0.32); phytate (0.64) and HCN(14.07). The methanolic, ethanolic and aqueous extracts of the stem were found to inhibit the four Gram +ve bacteria, four Gram –ve bacteria and four fungi. The four Gram+ve bacteria are Staphylococcus aureus, Bacillus subtilis, Streptococcus pyogenes and Listeria monocytogen. The four Gram –ve bacteria are Echerichia coli, Pseudomonas auregenosa, Klebsiella and Salmonella typhi. The four test fungi are Pennicillum, A. niger, Fusarium and Candida. The minimum inhibitory concentration (MIC) of methanolic, ethanolic and aqueous extracts of the stem on four Gram +ve bacteria are:S. aureus (200,200,200mg/ml); B. subtilis (150,100,200mg/ml); S. pyogenes (150,200,250 mg/ml);L. monocytogen (200,200,250mg/ml). The MIC of methanolic, ethanolic and aqueous extracts of the stem on four Gram –ve bacteria are E. coli (Nil,200,250mg/ml);P.auregenosa(200,200,250mg/ml); Klebsiella (200,150,250mg/ml); S. typhi (250,250,250mg/ml). The MIC of methanolic, ethanolic and aqueous extracts of the stem on four test fungi are Pennicillum (250,250 200mg/ml); A. niger (250,200,250mg/ml); Fusarium (200,250,Nil mg/ml) and Candida (200,150,200mg/ml) . The maximum bacteria concentration (MBC) for Gram +ve bacteria in methanolic, ethanolic and aqueous extracts are: S. aureus (650,700,700mg/ml); B. subtilis (600,550,750mg/ml); S. pyogenes (600,750,800mg/ml) and L. monocytogen (600,750,750mg/ml). MBC for Gram -ve bacteria in methanolic, ethanolic and aqueous extracts are: E. coli (700,500,650mg/ml); P. auregenosa(250,200,350mg/ml); Klebsiella (150,200,300mg/ml); S. typhi (750,600,800mg/ml) .MFC for the four test fungi are Pennicillum (800,600,850mg/ml); A. niger (600,500,700mg/ml); Fusarium (700,600,750mg/ml) and Candida(600,450,500mg/ml). Elemental analysis shows that stem of B. pinnatum contains an appreciable quantity of calcium, magnesium, phosphorus, sodium and potassium.
Keywords: Stem of B. pinnatum, crude extracts, phytochemical analysis, elemental analysis, anti-microbial activity, MIC and MBC.

1. Introduction

Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agent for the prevention of diseases and ailments (Sharma e tal, 2008). Medicinal plants have been referred to plant species that contain medicinally active ingredients in all or any of their parts (Sofowora,1993). Numerous researchers on medicinal plants and herbal drug production reported that medicinally active ingredients of medicinal plant occur in the leaves, flowers, roots, stem, bark or wood (Anon, 2005; Sofowora, 1993). Despite the immense technological advancement in modern medicine, many people in the world (approximately 75% of the population) still rely on traditional healing practices and medicinal plants for their daily health needs (WHO, 1996). In most of the developing countries of the world, rural and urban dwellers, literate or illiterate rely heavily on herbal preparation for the treatment of various diseases despite the availability of orthodox medicine (Nwabuisi, 2002). B. pinnatum has been noted for its versatile medicinal value in traditonal medicine in Nigeria. It has been employed for the treatment of earache, burns, abscesses, ulcer, insects bites, whitlow, diarrhea and lithiasus (Okwu, 2007; Okwu et al, 2006). In southern Nigeria, the herb is used to facilitate the dropping of the placenta of newly born baby (Okwu, 2007). The lightly roasted leaves are used externally for skin fungus and inflammations and the lead infusion is an internal remedy for fever (Egereonu et al, 2005). The herb is considered a sedative, wound-healer, diuretic and cough suppressant (Egereonu et al, 2005). B. pinnatum is used for the treatment of all sorts of respiratory conditions; asthma, bronchitis and cough (Medicineatourfeet.com. 2008; Daxiel, 1936). In Eastern Nigeria, the decoction is used presently by herbalists for the treatment of gonorrhea, genital, vaginal and muscosal conidiasis as well as asthma and cough (Okwu, 2007; Egereonu et al, 2006). Scientific basis for the efficacy of plants in phytomedicine has been studied (Okili et al, 2001). Bryophyllum pinnatum commonly known as (life plant, air plant (Mexican) love plant, coanterbury bells, catherdral bells, etc) is a perennial herb growing widely and used in India, China, Australia and tropical America (Engler, 1926; Balzer, 1949). B. pinnatum is a succulent plant, 50 – 200cm tall and about 3.2cm wide,

rarely branched producing vegetatively by adventitions shoots from base, they are medium green above blotched with purple underneath. It has flashy, dark green leaves. Its flower is in paniculate cymes 20 – 80cm long. It has fruit whose follicles are 10 -14mm long enclosed in the persistent papery calyx. The seeds are numerous in each fruit.

2. Experimental

The stems of B. pinnatum were obtained form Agulu in Anocha L.G.A. of Anambra State. The stems were dried under air and mild sunshine for three weeks and ground unto powder with manual grinder, it was then stored in polyethylene bottle until needed for analysis. The organoleptic, phytochemical and extraction of the active components were determined by the methods outlined by (Harbon, 1975). Microbial screening was done using the methods outlined by (Akinyele et al, 1997; Bryant, 1972).

3. Result and Discussion

The results of the analysis carried out on stems of B. pinnatum for its active constituents present are given in tables 1-10.

Table 1: Result of the Organoleptic Characteristic of the Stem

Parameter	***Inference***
Colour	Green
Texture (dried and grounded)	Powdering and soft
Taste	Astringent
Odour	Spicy
Moisture	10.07 ± 0.040%
Ash content	4.35 ± 0.030%

Table 2: Phytochemical Composition of the Stem Crude Drug

Class of phytocompound	***Inference***
Alkaloid	+
Flavonoid	+
Saponin	+
Tannin	+
HCN	+
Phytate	+
Phenol	+

Key

+ = present
- = absent

Table 3: Quantitative Estimates of Phytochemical Constituents of Stem of *B. Pinnatum*

Phytocompound	**Quantity %**
Alkaloids	0.029±0.010
Flavonoids	0.53±0.010
Saponins	1.05±0.010
Tannin	0.64±0.001
HCN	14.07±0.030
Phytate	0.46±0.002
Phenol	0.32±0.001

Table 4: Elemental Constituents of Stem of B. Pinnatum

Elemental Constituent	**Quantity %**
Calcium	46.76±2.30
Magnesium	11.20±1.38
Phosphorus	294.40±0.50
Sodium	18.33±0.12
Potassium	118.70±0.40

Table 5: Result of Antibacterial Activities of Crude Extracts of Stem of B. Pinnatum on four gram +ve Bacteria

Test organisms	Average Diameter (mm) Of Inhibition Zone			Control		
	Methanol extract	Ethanol extract	Aqueous extract	50% MeOH	50% EtOH	Distil H_2O
S. aureus	11.30mm	9.70mm	7.00mm	NA	NA	NA
B. subtilis	13.70mm	18.00mm	11.30mm	NA	NA	NA
S. pyogenes	13.70mm	11.70mm	7.70mm	NA	NA	NA
L. monocytogen	9.70mm	11.30mm	6.30mm	NA	NA	NA

Key: MeOH means Methanol
EtOH means Ethanol
NA mean No action

Table 6: Result of Anti-Bacterial Activities of Crude Extract Leaves of B. *Pinnatum* on four gram –ve Bacteria

Test organisms	Average Diameter (Mm) Of Inhibition Zone			Control		
	Methanol extract	Ethanol extract	Aqueous extract	50% MeOH	50% EtOH	Distil H_2O
E. coli	0.00mm	12.30mm	9.70mm	NA	NA	NA
P. auregenosa	13.30mm	13.70mm	11.00mm	NA	NA	NA
Klebsialla	24.70mm	14.70mm	9.70mm	NA	NA	NA
S. typhi	8.70mm	10.30mm	7.70mm	NA	NA	NA

Table 7: Result of Anti-Fungal Activities of Crude Extract of Leaves of B. Pinnatum on Four Test Fungi

Test organisms	Average Diameter (mm) Of Inhibition Zone			Control		
	Methanol extract	Ethanol extract	Aqueous extract	50% MeOH	50% EtOH	Distil H_2O
Pennicillum	9.30mm	10.00mm	7.00mm	NA	NA	NA
A. Niger	10.00mm	12.30mm	7.70mm	NA	NA	NA
Fusarium	10.30mm	9.30mm	0.00mm	NA	NA	NA
Candida(Yeast)	12.70mm	14.30mm	10.70mm	NA	NA	NA

Table 8: Minimum Inhibitory Concentration (MIC) and Maximum Bacterial Concentration (MBC) of Four Gram +ve Bacteria

Test organisms	Methanol extract (MIC)	Ethanol extract (MIC)	Aqueous extract (MIC)	Methanol extract (MBC)	Ethanol extract (MBC)	Aqueous extract (MBC)
S. aureus	200mg/ml	200mg/ml	200mg/ml	650mg/ml	700mg/ml	700mg/ml
B. subtilis	150mg/ml	100mg/ml	200mg/ml	600mg/ml	550mg/ml	750mg/ml
S. pyogenes	150mg/ml	200mg/ml	250mg/ml	600mg/ml	750mg/ml	800mg/ml
L. monocytogen	200mg/ml	200mg/ml	250mg/ml	600mg/ml	750mg/ml	750mg/ml

Table 9: Minimum Inhibitory Concentration (MIC) and Maximum Bacterial Concentration (MBC) Of Four Gram -ve Bacteria

Test organisms	Methanol extract (MIC)	Ethanol extract (MIC)	Aqueous extract (MIC)	Methanol extract (MBC)	Ethanol extract (MBC)	Aqueous extract (MBC)
E. coli	0.00mg/ml	200mg/ml	250mg/ml	0.00mg/ml	600mg/ml	700mg/ml
P. auregenosa	200mg/ml	200mg/ml	250mg/ml	650mg/ml	700mg/ml	800mg/ml
Klebsiella	200mg/ml	150mg/ml	250mg/ml	600mg/ml	550mg/ml	750mg/ml
S. typhi	250mg/ml	150mg/ml	250mg/ml	800mg/ml	250mg/ml	750mg/ml

Table 10: Minimum Inhibitory Concentration (MIC) and Maximum Bacterial Concentration (MBC) of Four Gram -ve Bacteria

Test organisms	Methanol extract (MIC)	Ethanol extract (MIC)	Aqueous extract (MIC)	Methanol extract (MBC)	Ethanol extract (MBC)	Aqueous extract (MBC)
Pennicillum	150mg/ml	100mg/ml	200mg/ml	800mg/ml	500mg/ml	850mg/ml
A. niger	100mg/ml	50mg/ml	200mg/ml	600mg/ml	500mg/ml	700mg/ml
Fusarium	200mg/ml	150mg/ml	200mg/ml	700mg/ml	600mg/ml	750mg/ml
Candida	100mg/ml	50mg/ml	150mg/ml	600mg/ml	450mg/ml	500mg/ml

4. Discussion

Table 1 shows the organoleptic test result of the stems of ***B. pinnatum***. The percentage ash content of 4.35 ±0.030 dipicts that the stems contain reasonable quantity of elements. Table 2 and 3 brought before the sight, the presence of these phytocompounds in the stem: alkaloids (0.29±0.010); flavonoids (0.53±0.010); saponins (1.05±0.010); tannin (0.64±0.001); HCN (14.07±0.030); phylate (0.64±0.002) and phenol (0.32±0.001). The presence of these phytochemicals justifies the use of this herbs in the treatment of various ailments as pointed out in the introductory section of this paper. Table 4 indicates that stems of ***B. pinnatum*** contain necessary micro-constituent elements needed by the human body for the maintainance of osmotic pressure of the blood. Table 5 – 7 exposed the result of anti-microbial activities of stems of ***B. pinnutum*** on four:

i. Gram +ve bacteria
ii. Gram –ve bacteria
iii. Fungi

The four gram +ve bacteria are: *S. aureus, B subtilis, S. pyogenes and L. monocytogens*. The four gram –ve bacteria are: *E. coli, P. auregenosa, Klebsiella and S. typhi* and the four test fungi are: *Pennicillum, A. niger, Fusarium and Candida*. The tables showed that the crude drug is a wonderful antibiotic. It can inhibit the twelve micro-organisms comfortably. The twelve microorganisms and their zone diameter (ZD) in (mm) for methanolic, ethanolic and aqueous extracts are as follows: *S. aureus* (11.30, 9.70, 7.00); *B. subtilis* (13.70, 18.00, 11.30). *S. pyogenes* (13.70, 11.70, 7.70); *L. monocytogen* (9.70, 11.30, 6.30); *E. coli* (0.00, 12.30, 9.70); *P. auregenosa* (13.30, 13.70, 11.00); *Klebsiella(* 14.70, 14.70, 9.70); *S. typhi* (8.70, 10.30, 7.70); *Pennicillum* (9.30, 10.00, 7.00); *A. niger* (10.00, 12.30, 7.70); *Fusarium* (10.30, 9.30, 0.00) and *Candida* (12.70, 14.30, 10.70) respectively.**** It is interesting to note that methanolic extract showed the highest activity on a gram-ve bacterium – *Klebsiella* with ZD of 14.70mm and the least activity was shown on one gram-ve bacterium- ***E. coli*** with ZD of 0.00mm. In order words methanolic extract has no effect on the bacterium. Ethanolic extract showed the highest activity on a gram+ve bacterium – **B. subtilis** with zone size of 18.00mm and the least activity was shown on gram+ve bacterium- S.aureus with ZD of 9.70mm. Aqueous extract showed the highest activity on a gram+ve bacterium ***B. subtilis*** with zone size of 11.30mm but the least activity was shown on the gram+ve bacterium- ***L. monocytogen*** with ZD of 6.30mm.

Again methonolic axtract showed the highest activity on one of the test four fungi – ***Candida*** with ZD of 12.70mm but the least activity was exhibited on the fungus ***A. niger*** with ZD of 10.00mm. Ethanolic extract exhibited the highest activity on the fungus – ***Candida*** with ZD of 14.30mm but the least activity was shown on **Fusarium** with ZD of 9.30mm. Aqueous extract showed the highest activity on the fungus ***Candida(Yeast)*** but the least activity was exhibited on ***Fusarium*** with ZD of 0.00mm. 50% methanol, 50% ethanol and distill water used as the control had no effect on the twelve test micro-organisms.

5. Conclusion

This analysis exposed the fact that the stem extracts of B. pinnatum are wonderful antibiotics. The stems of B. pinnatum have both anti-bacteria and ant-fungal effect. Result of this investigation portrayed the fact that the traditional medicinal use of the stem of B. pinnatum should continue and the bioactive ingredients responsible for the antimicrobial properties of the stem extracts should be elucidated.

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