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Research Article

Comparative Study On Phytochemical Screening And Antimicrobial Activity Of *In Vitro* Grown And Wild *Pogostemon benghalensis* (Burm. F.) O. Kuntze

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ABSTRACT

The study was aimed for the *in vitro* propagation of *Pogostemon benghalensis* plant and comparison of its phytochemical content and antimicrobial activity with wild plants. In order to get *in vitro* developed plants, explants were cultured on MS medium supplemented with different concentrations and combinations of 6-benzyl amino purine (BAP), Indole-3-acetic acid (IAA) and α- Naphthalene acetic acid (NAA). Profuse callus induction was observed in the medium containing 2.0 mg/l 2,4-D + 1.0 mg/l BAP from nodal explants. Numerous microshoots were developed from callus tissue in MS medium supplemented with 1.5mg/l BAP + 1.0mg/l IAA. Further proliferation and elongation of microshoots was achieved in the same media composition. The dried leaf powder of natural and *in vitro* grown plants was subjected to sequential extraction using chloroform, methanol, carbon tetrachloride and water as solvent. Preliminary phytochemical analysis and antimicrobial activity of the leaf extracts of naturally grown and *in vitro* cultivated plants were carried out. Antimicrobial activity was performed against gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and gram negative bacteria (*Salmonella abony, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi*) by disc diffusion method. The results also indicated that both *in vitro* regenerated and naturally grown *Pogostemon benghalensis* plants possess various phytochemical compounds and both the plant showed potential antimicrobial activity against test organisms. This result has provided the basis for the wide use of this plant as the source of therapeutic medicine after purification of the extract.

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INTRODUCTION:

Plants have been used as source of curative agent from the beginning of human civilization. Now we know that, plants have infinite ability to synthesize numerous secondary metabolites having therapeutic properties for treating several ailments.[1] These substances also serve as plants defense against microorganisms, insects and herbivores .[1] Naturally produced secondary metabolites of higher plants may give a new source of antimicrobial agents with possibly novel mechanism of action . [2-3]

Pogostemon benghalensis is a perennial aromatic herb belongs to the family Lamiaceae. This species is common in many parts of Bangladesh particularly in greater Sylhet district and in the Chittagong Hill Tracts region. Different parts of this plant are used in Indian traditional medicine for the treatment of respiratory tract infection, tuberculosis, rheumatism and fever. [4] Antimicrobial resistance (AMR) is currently one of the major threats facing mankind. The emergence and rapid spread of multi and pan drug resistance organisms such as vancomycin resistant organism put the world in a dilemma. The health and economic burden associated with AMR on a global scale are dreadful. Available antimicrobials have been misused and are almost ineffective with some of these drugs associated with dangerous side effects in some individuals. Development of new, effective and safe antimicrobials is one of the way by which AMR burden can be reduced. Medicinal plants are potential sources of new antimicrobial molecules. There is renewed interest in antimicrobial activities of phytochemicals. [5] Correlation between the phytoconstituents and the bioactivity of plant is desirable to know for the synthesis of compounds with specific activities to treat various health ailments and chronic diseases as well. [6] The present study was carried out in order to mass scale *in vitro* propagation of *Pogostemon benghalensis* and to compare the phytochemical content and antimicrobial activity of the naturally grown and *in vitro* raised plant.

MATERIALS AND METHODS

Plant materials for *in vitro* studies:

Germplasm of wild *Pogostemon benghalensis* was collected from Moulvibazar and Chittagong Hill Tracts and is being maintained in the medicinal plant conservatorium of Botany Department, University of Chittagong. Juvenile parts of six months old plants were used as explants. Shoot apices, nodal and leaf segments of field grown plants were collected and thoroughly washed under running tap water. The materials were then separated into short pieces and surface sterilized with 5% savlon and liquid soap for 5 - 10 minutes with constant shacking. The materials were then washed 3 - 4 times with distilled water for complete removal of detergent and taken under running laminar airflow cabinet and transferred to 500 ml sterilized conical flask. After rinsing with 70% ethanol for less than 60 seconds, they were immersed in 0.1 %(w/v) $HgCl_2$ for 5 minutes. To remove every trace of the sterilant, the materials were then washed 4 – 5 times and were prepared for inoculation.

In Vitro Propagation

The explants were inoculated on Murashige and Skoog (MS) medium containing different concentrations and combinations of BAP (6-benzyl amino purine), IAA (Indole-3-acetic acid) and NAA (α- Naphthalene acetic acid) with 8% (w/v) agar and 3% sucrose. pH of the culture media was adjusted to 5.8 before adding agar. The culture room was maintained at 25 ± 2^0C and 16 h photoperiod. Shoots, after initial proliferation, were subcultured on the same fresh media or different media for further response at an interval of 15 – 20 days. For multiple shoot induction and proliferation, the basal media was supplemented with BAP (1.0 – 2.5 mg/L) either alone or in combination with IAA (0.5 – 1.0 mg/L) and/or NAA (0.5 – 1.0 mg/L). Shoots were elongated on the same medium along with profuse multiplication. Considerable elongation was reached after two subcultures in the same medium at an interval of 15 days. Cultures were maintained in the proliferation medium for continuous supply of *in vitro* plants.

Qualitative Phytochemical Screening

Fresh leaves collected from wild and *in vitro* propagated plants were used for phytochemical screening. Methanol and aqueous extracts were assessed for the presence of the bioactive compounds by following the standard methods. [7-12] This method is known as spot test method.

Test for alkaloids

Five gram of fresh finely chopped and pasted plant material of both wild and *in vitro* grown plants was mixed up to moistened with 10 ml 2% HCL and heated in water bath at 60° C for one hour. After cooling the extract was filtered through Whatman No.1 filter paper. Two drops of extract were put on each microscopic groove slide with one drop of the alkaloid detecting reagent, Dragendroff 's reagent; Hager's reagent; Mayer's reagent; Wagner's reagent; Tannic acid reagent respectively.

Tests for flavonoids

Shinoda test. Pieces of magnesium ribbon and concentrated HCl were mixed with aqueous crude plant extract. Pink scarlet color showed after few minutes which indicated the presence of flavonoid

Alkaline reagent test. 2 ml of 2% NaOH solution was mixed with aqueous plant crude extract. An intense yellow color was produced which turned colorless on addition of few drops of diluted acid to the mixture. This result showed the presence of flavonoids.

Test for glycosides

Keller-kiliani test: A solution of glacial acetic acid (4.0 ml) with 1 drop of 2% $FeCl_3$ mixture and 1 ml concentrated H_2SO_4 were mixed with 10 ml aqueous plant extract. A brown ring formed between the layers which showed the presence of glycosides.

Test for steroids

One ml of the methanolic extract was dissolved in 10 ml of Chloroform and equal volume of concentrated Sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of Steroids. Test for terpinoids

Salkowski test: 5 ml of the methanolic extract was treated with 2 ml of chloroform in a test tube. 7 ml of concentrated H_2SO_4 carefully added to the mixture to form a layer. An interface with a reddish brown coloration formed if terpinoid is present.

Test for quinine

For the quinine test 1ml of plant extract, 1ml of concentrated sulphuric acid was added and allowed to for some time to develop colour. Development of red colour shows the presence of quinine.

Test for phlobatannins

The deposition of a red precipitate denoted the presence of phlobatannins when 10 ml aqueous extract of plant was dissolved in few drops of 1% HCl were added in the boiling tube.

Test for coumarin

One ml of plant extract, 1ml of 10% NaOH was added and was allowed to stand for some time development of yellow color shows the presence of coumarin.

Extract preparation for antimicrobial assay

Fresh leaves collected from naturally grown plants and *in vitro* propagated plants were sun dried and ground into a fine powder. This powder of leaves was sequentially soaked in threefold quantity (w/v) of methanol, chloroform, carbon tetra chloride and distilled water for 72 hours and filtered using Whatman No.1 filter paper. The filtrate was concentrated under reduced pressure using rotary vacuum evaporator. Pest of sample was dissolved in DMSO solution. Thus sample was prepared for antimicrobial activity.

Microorganism used

To screen the antibacterial activity of the plant extracts, four human pathogenic bacteria organisms were used as test organisms. Among the six human pathogenic bacteria, two were gram positive (*viz. Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538) and four were gram negative (*Salmonella abony* NCTC 6017, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739, *Salmonella typhi* ATCC 6539).

Preparation of inoculum

The organism to be tested was taken from well the isolated colony and mixed with 2 ml sarile saline. The mixture was shaken well to obtain homogenous suspension. The the turdidity of the suspension was

adjusted by comparing with the 0.5 McFarland standard solution.

Antimicrobial assay

The *in vitro* sensitivity of the bacteria to the test materials was done by disc diffusion method. [13] Mueller Hinton agar (MHA) media was distributed in sterilized Petri dishes. Bacterial suspension (0.1 ml) was taken in the sterile Petri dish and about 15-20 ml agar media was poured. Then it was rotated clock and anti-clockwise and waited for solidification. The paper discs (5 mm in diameter) have been soaked (20 µl/disc) with leaf extracts for antibacterial analysis. In performing the sensitivity spectrum analysis agar medium plate have been selected uniformly with the test organisms. Then the discs prepared with definite solvent extract have been placed on the medium surface. On the other hand, disc containing each solvent was used as negative control (C) and standard antibiotic disc of azithromycin was used as positive control. These plates are then kept at low temperature (4^{o}C) for two to four hours to allow maximum diffusion of compound. During this time dried discs absorb water from the surrounding media and then the test materials are dissolved and diffused out of the media. The diffusion occurs according to the physical law that controls the diffusion of molecules through agar gel. The plates were then incubated at 37^{o} C for 24 hours at inverted position to allow maximum growth of the microorganisms. After incubation the plates have been observed and results were noted as the "Zone of Inhibition" (clear distinct zone around the discs) in mm in diameter with transparent scale including the diameter of the discs.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal concentration (MBC) of the plant extracts

MIC and MBC of the drugs that were found to be effective for the organisms were determined by applying different concentrations of antimicrobial substaces and the same bacterial loads in nutrient broth.

Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of antimicrobial substances that will inhibit the visible growth of a microorganism after overnight incubation and Minimum bactericidal concentrations (MBCs) as the lowest concentration of an antimicrobial compounds that will prevent the growth of an organism after subculture onto antibiotic-free media. The MICs are used by diagnostic laboratories mainly to confirm resistance, but most often as a research tool to determine the in vitro activity of new antimicrobials. The present study was performed to determine the MIC and MBC values of plant extracts against the test organisms.

Inoculums preparation

Inoculums preparation was done as described in the previous test. The inoculum turbidity was adjusted as to 0.5 McFarland standard.

Procedure

The MIC was determined by broth micro-dilution method. [14] The broth micro-dilution testing was performed according to CLSI" s directions. 50µL of 2-fold Luria Burtenii (LB) broth medium was poured into 2nd well of the 1st, 3rd, 5th and 7th rows of 2of a sterile 96 well U-shaped bottomed microtiter plate and 50µL 1-fold original LB broth medium (pH 7.2) was poured into each other wells of the plate. 50µL of antibiotic suspension was poured into the 2nd well of a row and mixed by steady circulation motions of micropipette tip. It resulted in 2-fold dilution. From this 50µL suspension was poured into the 3rd well of the row and mixed which again produce another 2-fold dilution. In this way, the gradual 2-fold dilution was done up to the 3rd well of the 2nd, 4th, 6th and 8th rows for 4 selected isolates. From these wells 50µL suspension was discarded without further addition to next well. The process described above was followed for the effective antibiotics of some renowned Pharmaceuticals. Inoculation was done by pipetting 50µL of inoculum suspension in each well of a microtiter plate. This resulted in another 2-fold dilution. The 1st and 4th wells of 2nd, 4th, 6th, 8th row were considered as positive control for growth of the bacteria. Then the micro titer plates were incubated at 37ºC for 20-22 hours. After incubation, the tested micro titer plates were assayed by tetrazolium sal.t [15] To each well, 10 µL of sterile 2, 3, 5-triphenyltetrazolium chloride (0.5% w/v) solution was added. Cultures were then incubated at 37ºC for 24 hours. A change in color from yellow to red indicated

growth of bacteria and the MIC was interpreted visually. The MIC was estimated by visual observation as the first dilution, which completely inhibits bacterial growth in LB broth medium. The main advantage of the Micro dilution method for the MIC determination lies in the fact that it can readily be converted to determine the MBC as well. It can be measured as following process:

One loop full of suspension from each of the three wells containing the three lower concentrations (including MIC) of an antibiotic that showed no visual growth was streaked on MHA plates and the plates were then incubated at 37ºC for 24 to 48 hours. The plates were observed for bacterial growth. The highest dilution at which at least 99% of bacteria were inhibited was considered as MBC.

RESULTS AND DISCUSSION

Multiple shoot buds (MSBs) induction from nodal explants in MS medium

The best response (86%) in respect to multiple shoot bud's induction, was noted when nodal segments were cultured on MS medium supplemented with 2.0 mg/l BAP + 1.0 mg/l NAA and 2.0 mg/l BAP + 1.0 mg/l IAA + 0.5 mg/l NAA. But average number of MSBs (3.5 ± 0.26) per explant was the highest in the medium containing 2.0 mg/l BAP + 1.0 mg/l IAA + 0.5 mg/l NAA (**Figure 1**). However, some workers [16] reported that KIN (1.0 mg/L) and NAA (0.5 mg/L) were the good media combination for direct shoot regeneration from nodes. Therefore, our study suggests a new combination of growth media that promote the induction of MSBs from nodes. Proliferated shoots were properly elongated in the same medium.

b

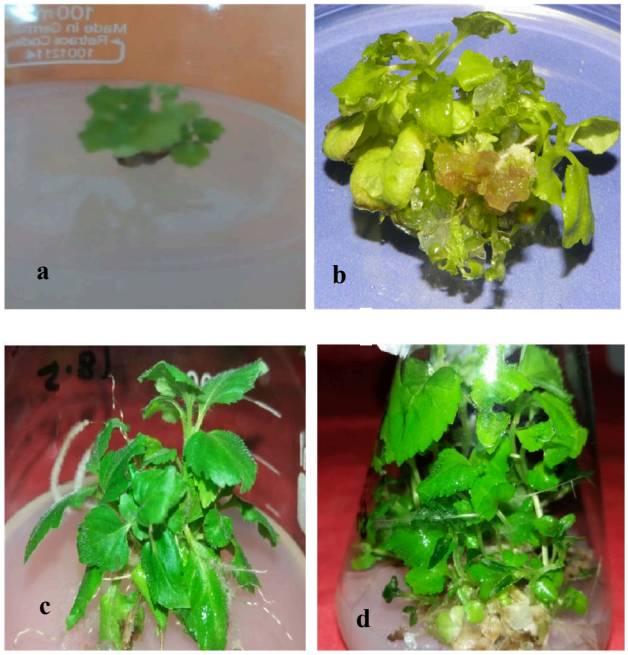

Figure 1: Multiple shoot induction from nodal explants of Pogostemon benghalensis, (a)shoot initiation (b) shoot multiplication (c) Elongation and (d) proliferation of shoot in 2.0 mg/l BAP + 1.0 mg/l IAA + 0.5 mg/l NAA.

Phytochemical analysis

The methanolic extracts of the naturally grown and *in vitro* cultivated plantlets were subjected to the preliminary phytochemical analysis (**Table-1**). The results showed that the methanolic extract of the *in vitro* regenerated plantlets contain alkaloid, terpenoid and quinine. Again the naturally grown plants contain alkaloid, flavonoid, terpenoid and quinine.

Table- 1. Qualitative test results for eight secondary metabolites of Pogostemon benghalensis.

Plant sample Used	Secondary metabolites (% of coloration)							
	Alk	Phl.	Flv.	Ter.	Str.	Gly.	Qui.	Cou.
***In vitro* developed plant**	+	-	-	+	-	-	+	-
Naturally grown plant	+	-	+	+	-	-	+	-

Notes: (+) indicates the presence and (-) indicates absence; Alk = Alkaloid, Phl. = Phlobatannins, Flv. = Flavonoids, Ter. = Terpenoids, Str. = Steroids, Gly. = Glycosides, Qui. = Quinine, Cou. = Coumarin

Antimicrobial activity

Antimicrobial or antibiotic resistant pathogen bacteria and fungi are causing major health problem worldwide. For these reasons the search for new antimicrobial agents with novel modes of action represents a major target in chemotherapy. So, in this study, antimicrobial activities of the different extracts of the tested plants (both *in vitro* and naturally grown) were carried out to determine their antimicrobial properties, which have been presented in the Table-2 and Figure 2, 3 and 4. From the result of the antimicrobial screening of the naturally grown and *in vitro* developed plants, it is observed that most of the plant extracts showed more or less antimicrobial activities against the tested organisms. In case of *in vitro* plant, CH_3OH extract showed comparatively better activity than that of others. It showed the highest activity (20 mm in diameter) against the *E. coli* which was more than that of positive control (Azithromycin) and the least activity (7 mm in diameter) was observed against the *B. subtilis*. Zone of inhibition was also observed by the $CHCl_3$ extract and water extracts of the *in vitro* plant against some of the test organisms. For the $CHCl_3$ extract, the best activity (10 mm in diameter) was found against *Salmonella abony* and for water extract, the highest activity (11 mm in diameter) was observed against *Staphylococcus aureus*. However, CCl_4 extract of the *in vitro* plant did not show any activity against test organisms except *Salmonella typhi*. Almost same pattern of antimicrobial activity was also exhibited by the different extracts obtained from naturally grown plant. CH_3OH extract of this plant showed comparatively better activity against all test organisms than that of others. This extract gave the highest zone of inhibition (19 mm in diameter) against *E. coli*, which is as same as the zone of inhibition showed by azithromycin (positive control used in the study) and the lowest activity (11 mm in diameter) showed by this extract against *Salmonella abony*. Both water and $CHCl_3$ extract showed moderate activity against most of the organisms. The best activity (13 mm in diameter) by the water extract was against *Staphylococcus aureus* and for $CHCl_3$ extract the highest zone of inhibition (10 mm in diameter) was observed against *Salmonella typhi.*

Table-2: Zone of inhibition observed against test organisms (bacteria) by the different solvent extracts.

Scientific Name of plants	Plant Sample Used	Solvents used	Zone of inhibition in diameter (mm) 200 µg dw./disc					
			S. typhi	*P. aeruginosa*	*S. aureus*	*E. coli*	*S. abony*	*B. subtilis*
Pogostemon benghalensis	***In vitro* developed plant parts**	CCl_4	6				-	-
		$CHCl_3$	8	9	6		10	-
		Water	-	9	11	-	-	8
		CH_3OH	10	16	15	20	113	7
	Naturally grown plant parts	CCl_4	-	-	-	-	-	-
		$CHCl_3$	10	7	5		9	-
		Water	-	10	13			10
		CH_3OH	14	12	14	19	11	17
	Standard Control	****Azithromycin** (200 µg /disc)	**18	**20	**18	**19	**19	**18

Note: Number indicates zone of inhibition. Minus sign (-) indicates no zone of inhibition.
S. typhi = Salmonella typhi , P. aeruginosa = Pseudomonas aeruginosa , S. aureus = Staphylococcus aureus , E. coli = Escherichia coli , S .abony = Salmonella abony , B. subtilis = Bacillus subtilis

However, CCl_4 extract of the naturally grown plant showed no activity against any of the test organisms. This is may be due to the reason that this plant contains no bioactive compound that dissolves in CCl_4. From these results, it can be concluded that CH_3OH is the best solvent in which maximum number of antimicrobial compounds present in both types of plant can be dissolved. As CH_3OH extract of the both types of plant showed the better antimicrobial activity against most of the test organisms, this extract had been selected for further studies [Determination of Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)]

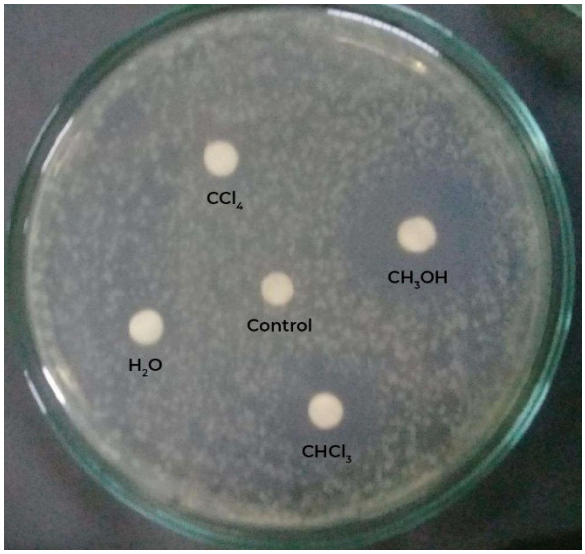

Figure 2. Zone of inhibition of Methanol (CH3OH) and Chloroform extract (CHCl3) of wild plant against Salmonella abony

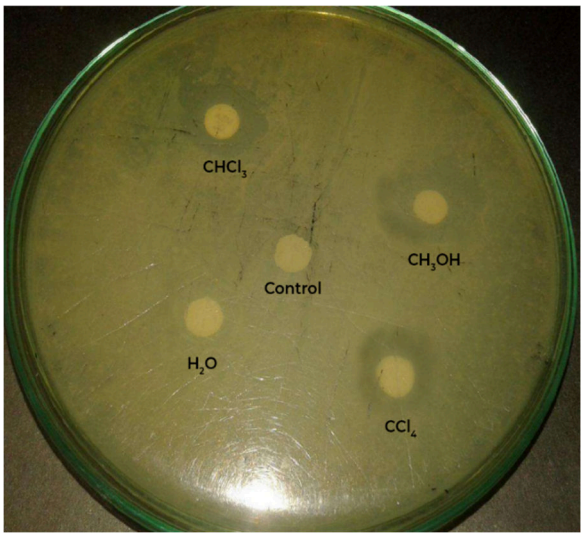

Figure 3. Zone of inhibition exihobited by Methanol (CH_3OH) and Chloroform extract ($CHCl_3$) of in-vitro developed plant against Salmonella typhi

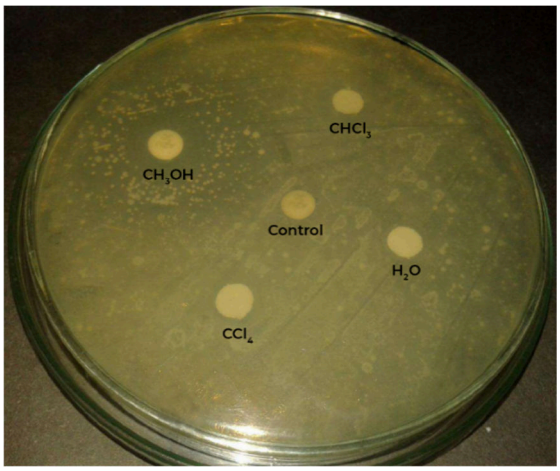

Figure 4. Zone of inhibition exhibited by the Methanol (CH_3OH) extract of in-vitro developed plant against against E.coli.

MIC and MBC of the CH_3OH extract of the plant: MIC of bacteria indicates the lowest concentration of substance that inhibits the growth of bacteria. On the other hand, MBC is the lowest concentration of substance that kills the bacteria. In this study MIC, MBC and ratio of these values (MBC/MIC) of CH_3OH extract of the both type of plant were determined that has been presented in the Table 3.

Table 3: Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of CH_3OH extract:

Bacteria	CH_3OH extract of Naturally grown *Pogostemon benghalensis* (µg/ml)			CH_3OH extract of *in vitro* developed *Pogostemon benghalensis* (µg/ml)		
	MIC	**MBC**	**MBC/MIC**	**MIC**	**MBC**	**MBC/MIC**
S. typhi	625	3000	4.8	1250	3500	2.8
P. aeruginosa	1250	5000	4	625	2500	4
S. aureus	625	2500	4	NF	NF	NF
E. coli	312.5	1250	4	312.5	625	2
S. abony	1250	5000	4	625	3000	4.8
B. subtilis	625	2500	4	2500	ND	ND

Note: NF = Not found; ND = Not Done. *S. typhi = Salmonella typhi , P. aeuginosa = Pseudomonas aeruginosa , S. aureus = Staphylococcus aureus , E. coli = Escherichia coli , S .abony = Salmonella bony , B. subtilis = Bacillus subtilis*

For naturally grown plant, minimum MIC (312.5 µg/ml) and MBC (1250 µg/ml) values was observed against *E. coli*, the maximum values (1250 µg/ml for MIC and 5000 µg/ml for MBC) were observed against *P. aeruginosa* and *S. abony*. In the same way, the lowest MIC (312.5 µg/ml) and MBC (625 µg/ml) values of the CH_3OH extract of the *in vitro* developed plant were also found against *E. coli* but this extract exhibited the highest MIC (2500 µg/ml) against *B. subtilis*. Antimicrobial substances are considered as bacteriostatic agents when the ratio MBC/ MIC > 4 and bactericidal agents when the ratio MBC/MIC ≤ 4. [17] Thus, CH_3OH extract of the naturally grown *Pogostemon benghalensis* plant can be considered as bacteriostatic for *S. typhi* but for rest of the test bacteria it can be bactericidal. On the contrary, CH_3OH extract of the *in vitro* developed *Pogostemon benghalensis* plant can be considered as bactericidal for *S.typhi*, *P.aeruginosa* and *E.coli* but for *S. abony* it exhibited bacteriostatic activity. However, this extract showed comparatively higher MIC value against *B. subtilis* and thus this extract had been excluded from determining MBC. Therefore, from this study it can be inferred that after purification of the CH_3OH extract of the studied plants, it can be used to treat different infectious diseases caused by these test organisms.

CONCLUSION

The present research work was undertaken with the view to develop protocols for rapid and mass scale propagation of *Pogostemon benghalensis* and to compare of *in vitro* regenerated and naturally grown plants with respect to phytochemical and antimicrobial properties. Although, there were little differences for phytochemical properties between *in vitro* and naturally grown plants, both types of plants showed almost same antimicrobial activities against most of the organisms. However, further research is needed for the purification and characterization of these plant extracts for developing potential new therapeutic drugs

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REFERENCES

1. Mallikharjuna PB, Rajanna LN, Seetharam YN and Sharanabasappa GK. Phytochemical Studies of Strychnos potatorum L.f.- A Medicinal Plant . Journal of Chemistry., 2007; 4(4): 510- 518.

2. Hamill FA, Apio S, Mubiru NK, Bukenya –Ziraba R, Mosango M, Maganyi OW and Soejarto DD. Traditional herbal drugs of Southern Uganda, II: literature analysis and antimicrobial assays Journal of Ethnopharmacology, 2003; 84(1): 57-78.3.
3. Barbour EK, Al Sharif M, Sagherian VK, Habre AN, Talhouk RS and Talhouk SN.Screening of selected indigenous plants of Lebanon for antimicrobial activityJournal of Ethnopharmacology, 2004; 93: 1-7.
4. Khade AB, Shigwan AV, Hatpakki BC, and Ghurghure SM. A Comprehensive Review on Pogostemon benghalensis (Burm. F.) O. Kuntze. Research and Reviews: Journal of Pharmacognosy and Phytochemistry, 2013; 1(1): 10-15.
5. Anyanwu MU and Okoye RC. Antimicrobial activity of Nigerian medicinal plants. Journal of Intercultural Ethnopharmacology, 2017; 6(2): 240-259.
6. Yadava M, Chatterji S, Watal G. Preliminary phytochemical screening of medicinal plants used in traditional medicine. International Journal of pharmacy and pharmaceutical science, 2014; 6(5): 534-542.
7. Trease GE and Evans WC."Phenols and phenolic glycosides," in Textbook of Pharmacognosy, Balliese, Tindall and Co Publishers, London, UK. 1989, vol. 12, pp. 343–383,
8. Trease GE , Evans WC. Pharmacognosy,15th ed. Springer, Berlin, 2002.
9. Harbone JB. Phytochemical methods. London: Chapman and Hall Ltd, 1973.
10. Sofowora A. Medicinal plants and traditional medicine in Africa. Spectrum Books, Ibadan, 1993; p. 150.
11. Webb LJ. An Australian phytochemical survey. I. alkaloids and cyanogenic compound in the queensland plants. C. S. I. R. O. Bill.260, Melborne. 1949.
12. Aplin TEH and Cannon JR (1971). Distribution of alkaloids in some Western Australian Plants. Economic. Bot.,1971; 25(4): 366-380.
13. Bauer AW, Kirby WM, Sherris JC and Turck M (1966). Antiboitic susceptibility testing by a standardized single disk method, Amer. J. clin. Pathol.,1966; 45 (4): 493-496.
14. Amsterdam D. Susceptibility testing of antimicrobials in liquid media. In: Loman V. Antibiotics in laboratory medicine,1996; 4: 52-111.
15. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med, 1998; 64: 711–713.
16. Bailung B, Gogoi HK. and Shrivastava RB (2010). *In vitro* propagation of *Pogostemon benghalensis* (Burm. F.) Kuntze through somatic embryogenesis. Asian Journal of Microbiology, Biotechnology & Environmental Sciences, 2010; 12(3): 659-662.
17. Gatsing D, Mbah JA, Garba IH, Tane P, Djemgou P and Nji-Nkah BF. "An antisalmonellal agent from the leaves of Glossocalyx brevipes Benth. (Monimiaceae)," Pakistan Journal of Biological Sciences, 2006 ; 9(1) : 84–87.

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