Research Article

Preliminary phytochemical and pharmacological screening of *Pogostemon benghalensis* for antioxidant and antibacterial activity

Mukesh R. Pimpliskar[1], Rahul Jadhav[2], Yogesh Ughade[2], R. N. Jadhav[3*]

[1]*Biotechnology Department, KME's, G.M. Momin Women's College, Bhiwandi-421 305 (Thane), Maharashtra. India*

[2]*Ramnarain Ruia College, Matunga, Mumbai, 400 019 3 Maharashtra, India*

[3]*Vidyavardhini's, E. S. A. college of Science, Vasai road (Palghar), 401 202, Maharashtra, India*

Received: 19 March 2020 Revised: 21 August 2020 Accepted: 19 September 2020

Abstract

Background: *Pogostemon benghalensis* is an important aromatic herb. Since the ancient time plant is being used as medicines, agrochemicals and pharmaceutical by large number of tribal and rural people. Almost all parts of the plant are helpful to treat different kinds of aliments; very few of these claims have been reported and scientifically studied. **Objectives:** The present study focused on the phytochemical analysis, antioxidant activity, RBC haemolysis and antimicrobial activity. **Materials and methods:** Aqueous and methanol extracts of leaves of *P. benghalesis*, used for Phytochemical analysis revealed major components such as flavonoids, saponins, tannins, glycosides, alkaloids and phenolic compounds present in both PBE. **Results and conclusion:** Results of the study showed that *P. benghalensis* extract showed significant antioxidant and RBC haemolysis activity. Antibacterial analysis of *P. benghalensis* showed moderate to high activity against *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Streptococcus pyogenes* and *Klebsiella pneumonia,* respectively. Therefore, the phytoconstituents in this plant are found to be with high medicinal value and can be explored for further studies. There seems enormous potential and scope for future research and further pharmacological investigation on *Pogostemon benghalensis*.

***Keywords*:** *Pogostemon benghalensis,* phytochemical analysis, antimicrobial activity, antioxidant, RBC haemolysis

Introduction

Medicinal plants have been used by mankind for its therapeutic value. Impressive number of modern drugs has been isolated from natural sources which were based on the uses of the agents in traditional medicine. This plant-based, traditional medicine system continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care. According to World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs (Doughari and El-mahmood, 2008).

Pogostemon benghalensis belongs to family Lamiaceae. It is worldwide in distribution. It is perennial, aromatic, pubescent, under shrub; most common in Melghat at higher elevations forming large patches in shaded valleys and along the river banks (Naise and Bhadange 2014). The oil is used as a stimulant and styptic. Since the ancient time plant is being used as medicines, agrochemicals and pharmaceutical by large number of tribal and rural people. The study of primary phytochemical analysis of aqueous, methanolic extract of this plant showed the presence of various secondary metabolites like alkaloids, tannins, carbohydrates, sterol, terpenoids, quinon and flavonoids etc. Essential oil from leaf contains few monoterpene hydrocarbons, a moderate content of sesquiterpenes and high content of aliphatic hydrocarbons.

There are about 47 species mainly used for ethanomedicine and traditional medicinal system. It's mainly used for medicinal purpose such as diuretic, sedative, digestive, antiparasitic, carminative, appetizer, anticonvulsant, anti-inflammatory, and stimulant. Lamiaceae members are well known for their medicinal properties (Chopra, 1956).

Pogostemon benghalensis was selected for the study strictly on the basis of its ethanobotanical uses confirmed from the traditional healers of study area. The present paper deals with its in vitro antibacterial, antioxidant, phytochemical and haemolysis activity.

Address for Corresponding Author:
Rahul Nasikrao Jadhav
Department of Zoology, Vidyavardhini's E. S. A. College of Science, Vasai Road, 401202 Maharashtra, India
Email: jadhav2010@rediffmail.com

DOI: https://doi.org/10.31024/ajpp.2021.7.1.7

2455-2674/Copyright © 2021, N.S. Memorial Scientific Research and Education Society. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Methods and materials

Plant material collection

Plant sample was collected from a field near Vajreshwari , Thane district, Maharashtra. It is 31 km away from the nearest railway station of Virar. The damage less leaves were dried in hot air oven at 60°C-65°C for 12hrs and blended into powder, this powder of *Pogostemon bengalensis* were used for aqueous and methanol extraction.

Phytochemicals analysis

Standard methods were used for the preliminary phytochemical screening of phyto-components like, Flavanoids, Saponins, Tanins, Glycosides, Phenols, and Alkaloids (Harborne, 1973).

Antioxidants assay (DPPH)

Cheap plastic "disposable" cuvettes/eppendorf tubes, which are not attacked by methanol or ethanol, are commonly used for the assay (Bondet, 1997)**.** The prepared DPPH should be maintained at pH in the range 5.0 to 6.5.The initial DPPH concentration should give absorbance values less than 1.0 for 0.05-0.1mM concentration Method was used for antioxidant determination (Mandal et.al, 2009; Blois, 1958**).**

Methanolic extract of plant material was used with varying concentration ranging from (50-300ug/ml). Stock solution was added according to varying concentration in test and colour blank tube. After adding stock and diluents, DPPH reagent was added in dark in test and incubated for half an hour. Absorbance was measured at 517 nm spectrophotometrically. Ascorbic acid was used as a standard.

The percentage DPPH radical scavenging activity was calculated from the absorption value by the following equation:

$$\text{Percentage radical scavenging} = \frac{\text{Absorbance of control} - (\text{Absorbance of test} - \text{Absorbance of Blank})}{\text{Absorbance of Control}} \times 100$$

The Graph of scavenging activity versus the concentration of plant extract was plotted and the IC50 value was determined. The antioxidant activity was compared with similarly calculated antioxidant activity of the standard.

Antimicrobial assay

Antibacterial assay performed by agar disc diffusion method (Samy and Ignacimuthu, 2000). All the microbial media used in this experiment were obtained from (Himedia Laboratories, Mumbai). Overnight culture was prepared by inoculating approximately in 2ml nutrient broth with 2-3 colonies of each organism taken from nutrient agar. Broths were incubated overnight 35 0C with shaking. Inoculate were prepared by diluting overnight bacterial cultures approximately 10 cells per ml in sterile saline. The suspension of tested bacterial strains (0.1ml of cells per ml) was spread on Muller-Hinton agar plates (Bauer et al., 1966). Filter paper discs (6mm diameter) were impregnated in 20 microliter of the plant extract and dried aseptically. The disc are placed on the bacterial lawn of agar plates and incubated at 37°C for 24 hrs. The diameter of the inhibition zones were measured using a scale in millimeters. For comparative evaluation, Streptomycin (HiMedia Laboratories Pvt. Ltd., 10mcg/disc) were used as a positive reference standard. Then the cultured plates were incubated for 48 hrs at Room Temperature (RT). After incubation, inhibition of the bacterial growth was measured in Millimeters.

Red blood cells haemolysis assay

This test was performed according to ECVAM DB-ALM INVITTOX Protocol No. 37. Materials used for haemolysis assay was human blood obtained from LATA LABS, Vasai-(W.). The blood sample was diluted by adding into approximately 20ml of phosphate buffer saline. RBC was centrifuged at room temperature. Supernatant (plasma) was aspirated carefully from the surface of the RBC and washed three to four times with Phosphate Buffer Saline (pH7.4). This washing procedure was carried out in order to remove the bulk of the white blood cells, any traces of plasma and Buffy coat.

After adding test sample, total volume was made up to 1ml by adding *P. benghalensis* extract. From this 25µl of mixture was discarded. Rapidly 25µl of RBC suspension was added in each tube containing $8x10^9$ cells/ml incubate for 10 minutes. After incubation, the reaction was terminated by a rapid high speed centrifugation at 3500 rpm for 1 minute (for removing intact cells and debris from the medium). After centrifugation, supernatant was transferred to another eppendorf tube and absorbance was measured at 540 nm, 560 nm and 575 nm. Blank was set which contain test sample and *P. benghalensis* extract (540 nm and 575 nm for protein denaturation and 560nm for haemolysis). Three controls were set which include positive, negative and SDS control. Positive control gives the 100% haemolysis value which contains 975µl of D/W+25µl of RBC. Negative control which contains 975µl of PBS+25µl of RBC gives zero haemolysis value. SDS control was set for protein denaturation. After calculating % Haemolysis and denaturation index of each concentration, L/D ratio was determined (Naim et al., 1976).

$$\%\ \text{Haemolysis} = \frac{\text{Absorbance of test (560nm)}}{\text{Absorbance of D/W (560nm)}} \times 100$$

Results and discussion

Phytochemicals analysis

The preliminary analysis of phytochemicals in methanol and aqueous extract of *P. benghalensis* leaves showed the presence of phytoconstituents such as flavonoids, phenols, alkaloids, saponins, tannins and glycosides (Table1).

DPPH radical scavenging activity (Antioxidant assay)

On the basis of the results of the study, *P. benghalensis* has significant antioxidant activity and reducing power. Even though antioxidant activity was low than the standard, it's considerably significant. The antioxidant activity may be attributed to flavonoids and other compounds. However further work be performed on the isolation and identification of these antioxidant components. The antioxidants have potential for application in food, Pharmaceuticals and cosmetics (Table 2).

The DPPH radical scavenging activity obtained in a study on *P. cablin* showed that the ethanolic extract had approximately a threefold increase in DPPH radical scavenging activity with IC50= 18±0.90g/mL (Table 3).

The results were found that flavonoid content showed positive correlations with the antioxidant activity. Flavonoids, including flavones, flavanols, and Condensed tannins are plant secondary metabolites and their antioxidant activities depend on the presence of free OH groups, especially 3- OH. Evaluated the scavenging effect of the essential oils in DPPH reduction was investigated against a positive control (Standard Ascorbic Acid) (Jadhav et.al.2018). The more antioxidants occurred in the oils, the more DPPH reduction will occur. High reduction of DPPH is related to the high scavenging activity performed by particular sample. At a higher concentration, these essential oils may exhibit more significant free radical scavenging activity.

Table 1. Phytochemical screening of extract of *P. benghalensis*

S. No.	Test	PBME
1	Flavonoids	++
2	Saponins	++
3	Tannins	++
4	Glycosides	+
5	Phenols	+
6	Alkaloids	+

Abbreviations: (+ = less), (++ = abundant); (- = absent); PBME: methanol extract of *P. benghalensis* leaves

Table 2. Calculation of antioxidant activity for Standard ascorbic acid

Concentration of Ascorbic acid (µg/ml)	OD of Test	% Radical scavenging
1	0.109	47.019
10	0.098	58.278
15	0.093	63.576
25	0.087	70.198
50	0.082	74.834
75	0.078	79.470
100	0.072	86.754

O.D. of control – 1.586, wavelength – 517 nm

Table 3. Calculation of antioxidant (DPPH radical scavenging) activity

Concentration of *P. benghalensis* extract (µg/ml)	Test	Radical Scavenging (%)
100	1.103	30.453
200	0.905	42.938
300	0.579	63.493
400	0.239	84.930
500	0.120	92.433
600	0.073	95.397

Antimicrobial activity

The antibacterial activity of *Pogostemon bengalensis* against test organisms is shown in (Table 4).

Results obtained in the present study revealed that the four extracts of plant *Pogostemon bengalensis* has better potential antibacterial activity against *S. aureus, E. coli, S. typhi, S. pyogens* and *K. pneumoniae*. When tested by the Agar cup method, the hot methanolic extract showed highest antibacterial activity against all organisms, more than 12mm. Hot aqueous and cold methanol showed a significant activity was recorded against *S. aureus, S. pyogens and K. pneumonia* around 8-12mm. There was the lowest activity of aqueous cold extract. Cold aqueous extract did not showed activity against *E. coli, S. typhi* and *K. pneumoniae*. Antibacterial activity was compared with standard antibiotic streptomycin (30U) (Table 5).

Studies on Antimicrobial Activities and Phytochemical Analysis of the Plant, the methanol extract of the stem and root bark of *P. benghalensis* demonstrated activity against pathogenic bacterial strains like *Bacillus subtilis, Staphylococcus aureus, E. coli, Klebsiella pneumonia* and *S. typhi* in the agar diffusion test system for the first time which validated its potential in broad spectrum antibacterial activity and these extract either individually or in combination can be used as formulation to treat the infectious diseases caused by the test organism . Whereas Antimicrobial activity of the plant under study showed that all the extracts were showing zone of inhibition against the test bacteria and maximum zone of inhibition was observed with methanolic extract. The comparative analysis was done using standard antibiotic streptomycin and thus the investigation showed that *P. benghalensis* plant have significant antimicrobial activity.

There are several reports indicating the antimicrobial potential of various medicinal plants. However the antibacterial activity against pathogenic bacterial strains was reported by very few workers (Koche et al., 2012).

Table 4. Antimicrobial effect of *P. bengalensis* extracts (Zone of inhibition in mm)

Extracts	*S. aureus*	*E. coli*	*S. typhi*	*S. pyogenes*	*K. pneumoniae*
Cold Water	+	-	-	+	-
Hot water	++	+	+	++	++
Cold Methanol	+	++	+	++	+
Hot Methanol	+++	+++	+++	+++	+++

Key: (-) No zone of inhibition; (+) Zone of inhibition upto 8mm; (++) Zone of inhibition 8mm-12mm; (+++) Zone of inhibition more than 12 mm

Table 5. Antimicrobial effect of standard antibiotic

Test microorganism	Diameter of Zone of Inhibition (mm)
	Streptomycin (10mcg/disc) (Standard Positive control)
Gram positive bacteria	
S. pyogenes	23
S. aureus	15
Gram negative bacteria	
E. coli	20
S. typhi	11
K. pneumoniae	14

RBC'S haemolysis

RBC haemolysis activity of *P. benghalensis* was less than 50% at 15mg/ml concentration (Figure 1 and Table 6).

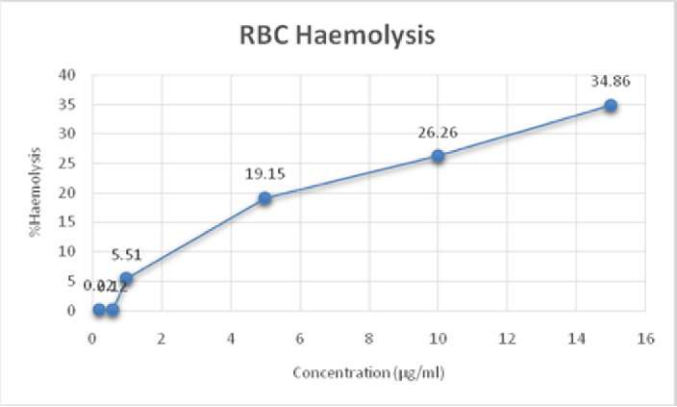

Figure 1. RBC haemolysis activity of *P. benghalensis*

So it is less irritant and can be used in production of skin oilments and cosmetics. The efficacy and stability of the extracts in these formulations, however, needs to be studied further.

In Vivo hemolysis can be caused by a large number of medical conditions, including many Gram-positive bacteria (e.g., *Streptococcus, Enterococcus,* and *Staphylococcus*), some parasites (e.g., Plasmodium), some autoimmune disorders (e.g., drug-induced hemolytic anemia), some genetic disorders (e.g., Sickle-cell disease or G6PD deficiency), or blood with

too low a solute concentration (hypotonic to cells). In Vitro hemolysis can be caused by improper technique during collection of blood specimens, by the effects of mechanical processing of blood, or by bacterial action in cultured blood specimens (Orf and Cunnington, 2015).

Table 6. Calculation of hamolysis % for various PBE concentrations

Concentrations (mg/ml)	Test	% Haemolysis
0.2	0.0903	0.22
0.6	0.1368	0.12
1	0.04047	5.51
5	1.4234	19.15
10	2.2131	26.26
15	3.2675	34.86

O.D taken at – 560 nm; Positive Control O.D - 2.9365; Negative Control O.D- 0.0437

Conclusion

The phytochemical screening study revealed the presence of major phytochemical constituents such as saponins, tannins, flavonoids, glycosides and phenolic compounds in *P. benghalensis*.

Exploitation of these pharmacological properties requires further investigation of these active ingredients by implementation techniques of extraction, purification, separation, crystallization and identification.

A good antimicrobial activity was observed of *P. benghalensis* against common disease causing agents, both gram positive i.e. *S. aureus, S. pyogens*, and gram negative i.e. *E. coli, K. pneumoniae,* and *S. typhi.* Thus, it can be used as a good source for the development of new medicines and natural therapies against these potent microorganisms. On the basis of the results of the study, *P. benghalensis* has significant antioxidant activity and reducing power. Even though antioxidant activity was low than the standard, it's considerably significant. The antioxidants have potential for application in food, Pharmaceuticals and cosmetics. RBC haemolysis activity of *P. benghalensis* was less than 50% at 15mg/ml concentration. So it is, less irritant and can be used in production of skin oilments and cosmetics. The efficacy and stability of the extracts in these formulations, however, needs further investigations.

Conflict of interest

The authors alone are responsible for the content and writing of this article.

Acknowledgement

The authors are thankful the Principal and Management to support and provide all facilities to do this work.

References

Bauer AW, Kieby W, Sherris JC, Turck M. 1966. Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology. 45:493-496.

Blois MS. 1958. Antioxidant determinations by the use of a stable free radical. Nature, 181:1199–1200.

Bondet V, Brand-Williams W, Berset C. 1997. Kinetics and mechanisms of antioxidant activity using the DPPH free radical method. Lebensmitt Wissenschaft Technologie Food Science Technology, 30:609–615.

Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants .Publication and Information Directorate, CSIR, New Delhi, 1956.

Doughari JH. El-mahmood. 2008. African Journal of Pharmacy and Pharmacology, 2(1): 7-13.

Harborne JB. Phytochemical Methods, A guide to modern technique of plant analysis. Chapman and Hall. 1973.

Koche DK, Kokate PS, Suradkar SS, Bhadange DG. 2012. Preliminary phytochemistry and antibacterial activity of *Ocimum gratissimum*. Bioscience Discovery, 3(1):20-24.

Mandal S, Yadav S, Yadav S, Kumar R. 2009. Antioxidants: a review. Journal of Chemical and Pharmaceutical Research, 1(1):102-104.

Naim M, Gestener B, Bondi A. Birk Y. 1976. Antioxidant and anti-hemolytic activities of soyabean isoflavones, Journal of Agriculture and Food Chemistry 24:1174-1177.

Naise MG, Bhadange DG. 2014. Preliminary phytochemical screening of Pogostemon benghalensis (N.Burman) Kuntz. International Journal of Phytotherapy, (4)1: 14-15.

Orf K, Cunnington AJ. 2015. Infection-related hemolysis and susceptibility to Gram-negative bacterial co-infection. Frontier Microbiology, 6:666.

Royden L, Pimpliskar MR Jadhav RN. 2018. Essential oils of azadirachta indica and vitex negundo leaves evaluation for phytochemical analysis, antioxidant activity and antimicrobial activity. International Journal of Recent Scientific Research, 9(8A):28284-28289.

Samy RP, Ignacimuthu S. 2000. Antibacterial activity of some folklore medicinal plants used by tribal in Western Ghats in India. Journal of Ethnopharmacology, 69:63-71.