

Comparative study of Phytochemicals and anti-microbial activity of *K. pinnata* and *P. benghalensis*

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Abstract

Background: *Kalanchoe pinnata* and *Pogostemon benghalensis* are known to have many medicinal values. Both the plants are having many important phytochemicals. However, very few of these claims have been reported and scientifically studied.

Methods: The leaves of the studied plants were extracted for the further experiments and water extraction was carried out using classical method. Qualitative tests were conducted to find the presence of the important photochemical. The determination of the anti-microbial property was done by the disk diffusion method against two pure cultures of *Klebsiella pneumoniae* and *Staphylococcus aureus*.

Results: Qualitative analysis of the photochemical showed the presence of steroids, terpenoids, flavonoids and saponins in the leaves of *Kalanchoe pinnata* and the presence of steroids, flavonoids, glycosides, alkaloids and tannins in the leaves of *Pogostemon benghalensis*. The results found from the disk diffusion method clarifies that both the plants are having anti-microbial property. In Mueller Hinton Agar for *Klebsiella pneumoniae* the leaf extract of *Pogostemon benghalensis* (sample 1) showed a zone of inhibition was 9 mm (including diameter of disk is 6mm) and for *Kalanchoe pinnata* (sample 2) was 8 mm (including diameter of disk is 6mm). Similarly, the zone of inhibition for the culture of *Staphylococcus aureus* of *Pogostemon benghalensis* (sample 1) was 8 mm (including diameter of disk is 6mm) and that for *Kalanchoe pinnata* (sample 2) was 9 mm (including diameter of disk is 6mm).

Conclusion: The current study confirms many photochemical to be present in the studied plants. Also, their anti-bacterial properties were enumerated and thus support scientific basis for its use in traditional medicine.

Keywords: medicinal plants, phytochemical analysis, water extract, disk-diffusion method

1. Introduction

1.1 *Pogostemon benghalensis*

The *Pogostemon benghalensis* is a very common member of Lamiaceae family which shows Aromatic nature mainly found in open riverine forest. It is also cultivated in India, Bangladesh, Srilanka, Nepal, Myanmar, Thailand and China. Antibacterial, anti-fungal, anti-tubercular, anti-rheumatic, etc characters are shown by *Pogostemon benghalensis*. Most of the plant parts has medicinal usage.

Moreover, it is also used as manure for rice crop, and warding off moths during storage of clothes.

Preliminary phytochemical screening showed the presence of flavonoids, alkaloids, saponins, phenolic compounds, terpenoids and steroids in both extracts. The methanol extract of aerial parts of *P. benghalensis* Ktze syn. (Lamiaceae) was found to be active concentration of 2 g/ml of dried plant material in disc diffusion assay with chloramphenicol as a positive control against *Mycobacterium phlei* [1, 2, 3, 4, 5, 6, 7].

Taxonomy

Phylum: Tracheophyta.
Class: Magnoliopsida.
Family: Lamiaceae.
Genus: Pogostemon.
Order: Benghalensis.
Species: Pogostemon Benghalensis.



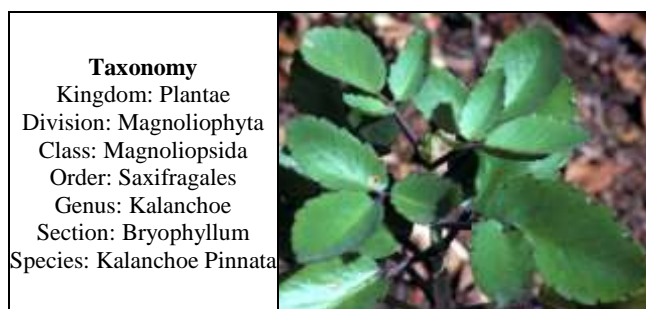
1.2 *Kalanchoe pinnata*

In this part of North-East of India, Assam, *Kalanchoe pinnata* is locally known as “Dupar Tenga” which has a tremendous medicinal value and grows 3-5 feet tall. *Kalanchoe pinnata* is also called as ‘air plant’, it consists tall hollow stems, fleshy dark green leaves that are distinctively scalloped and trimmed in red. And flower is bell-like pendulous. It is botanically classified as

Bryophyllum pinnatum and *Kalanchoe pinnatum* two main Latin names which refer to the same plant. High range of phytochemical and medicinal value is shown by *Kalanchoe pinnata* [1, 2, 3]. The *Kalanchoe pinnata* is high source of different classes such as alkaloid, diterpenoidal lactones, glycosides, steroids, phenolics, aliphatic compounds. It has reported antidiabetic, antioxidant, immunomodulatory anti-allergic, anti-cancerous activities. [8,9,10]. It is a medicinal

plant largely used in folk medicine for the treatment of kidney stones, gastric ulcer, pulmonary infection, rheumatoid arthritis etc. According to one article there is

evidence that shows the plant is anti-inflammatory, anti-parasitic, anti-bacterial, anti-cancer, anti-depressant [11, 12, 13, 14, 15, 16, 18, 19].



2. Material and Method

2.1 Plant material: 400 gram of leaves were collected of two species *Pogostemon benghalensis* and *Kalanchoe pinnata* separately from Guwahati, Assam and washed them properly by sterile water and dehydrate the leaves in hot air oven at 50 degree Celsius gradually and dehydrate leaves were grinded in motor pestle to make powder form of 50 gram.

2.2 Chemical: Ethanol, Agarose, Mueller Hinton Agar, chloroform, lead acetate, Glacial acetic acid and ferric chloride, concentrated sulphuric acid. All other general chemicals used with highest purity grade available commercially.

2.3 Extraction of Phytochemical: 5 g of the powder of leaves of two species were taken separately and add 50 ml of dist. Water and homogenised by the help of homogenizer and then kept it in shaking water bath for 4 hour at 50 degree Celsius and then mixture were filtered through whatman filter paper and filtered was subjected to freeze dry by the help of lyophilizer to get powder form. The different species extract (powder form) was collected, labelled and stored at -20 degree Celsius. Then 200 mg of each sample filtered powder were taken and add 20 ml of Dist water to each sample to make standard solution 10mg/ml of each solution for further experimental use

2.4 Qualitative Analysis of Phytochemicals



The crude water extracts of two different species were subjected to qualitative analysis for presence of chemical constituents by performing various chemical tests. Test for steroids by Salkowski Test; test for terpenoids by Salkowski Test; test for flavonoids by lead acetate test; test for tannins by lead acetate test; test for glycosides by keller- kiliani test; test for saponins by Froth Test; test for alkaloids-Marme's Test; test for proteins-Xanthoproteic Test.









2.5 Determination of the Anti-Bacterial Activity







For determining the antibacterial activity media was prepared by Mueller Hinton Agar and distilled water in required proportions. The media is autoclaved and poured into the dishes and left for solidification. After solidification of the media two particular bacteria *Staphylococcus aureus* and *Klebsiella pneumoniae* from other cultured sources were spreading by the help of swabs stick over the dishes. Paper disk were placed on to the solid media plate. Then 10 micro litre of filtered extract (1mg/ml) of different species were poured on that paper disk with respect to known antibiotic disk. The dishes are then kept for overnight. The plates are then observed regularly after 24 hrs, 48 hrs respectively and the diameter of zone of inhibition was then measured.

3. Results and Discussion

Table 1: For the representation of the phytochemical test results of *Kalanchoe pinnata* and *Pogostemon benghalensis*.

Phytochemicals		Crude Water Extract	
		<i>Pogostemon benghalensis</i>	<i>Kalanchoe pinnata</i>
Steroids	Salkowski test		
		The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence which showed the positive result for steroids.	The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence which showed the positive result for steroids.

<p>TERPENOIDS</p>	<p>Salkowski test</p>	 <p>Pink or pinkish brown colour which showed Positive result for terpenoids</p>	 <p>No pink or pinkish brown colour which showed negative result for terpenoids</p>
<p>FLAVONOIDS</p>	<p>H₂SO₄ test</p>	 <p>Formation of orange colour which showed the Positive result for flavonoids</p>	 <p>Formation of orange colour which showed the Positive result for flavonoids</p>
<p>GLYCOSIDES</p>	<p>Keller-Killiani test</p>	 <p>No formation of a brown ring at the interface which showed the Negative result for glycosides.</p>	 <p>Formation of a brown ring at the interface which showed the positive result for glycosides.</p>
<p>SAPONINS</p>	<p>Froth test</p>	 <p>An emulsion is formed which showed the Positive result for saponins.</p>	 <p>No emulsion is formed which showed the negative result for saponins.</p>



<p>ALKALOIDS</p>	<p>Marme's test</p>	 <p>White or yellow precipitate is not formed which showed the Negative result for alkaloids.</p>	 <p>White or yellow precipitate is formed which showed the positive result for alkaloids.</p>
<p>TANNINS</p>	<p>Lead-acetate test</p>	 <p>NO formation of yellow precipitate which showed Negative result for tannins.</p>	 <p>Formation of yellow precipitate which showed positive result for tannins.</p>
<p>PROTEINS</p>	<p>Xanthoproteic test</p>	 <p>Formation of yellow precipitate showed which showed the Positive result for protein.</p>	 <p>Formation of yellow precipitate showed which showed the Positive result for protein.</p>

The table shows us that the Salkowksi test conducted for the determination of steroids present in both the plants. Terpenoids were present in *P. benghalensis* and absent in *K. pinnata*. H_2SO_4 test revealed the presence of flavonoids in *P. benghalensis* and absence in *K.pinnata*. Glycosides were found to be absent in *P. benghalensis* and present in *K. pinnata* by Keller-Killiani test. Saponins present in *P. benghalensis* and absent in *K. pinnata* by Froth test. By

Marme's test alkaloids absent in *P. benghalensis* and present in *K. pinnata*. Tannins by Lead-acetate test showed its presence in *K. pinnata* and absence in *P. benghalensis*. Proteins are present in both the plants revealed by Xanthoproteic test.

3.2 Antibacterial Activity of Water Extracts of *Pogostemon Benghalensis* and *Kalanchoe Pinnata*

Water extract	Diameter of zone of inhibition	Diameter of zone of inhibition
	<i>Klebsiella pneumoniae</i>	<i>Staphylococcus aureas</i>

		
	<p>In Mueller Hinton Agar For <i>Klebsiella pneumoniae</i> the leaf extract of <i>Pogostemon benghalensis</i> (sample 1) showed a zone of inhibition was 9 mm (including diameter of disk is 6mm) and for <i>Kalanchoe pinnata</i> (sample 2) was 8 mm (including diameter of disk is 6mm).</p>	<p>In Mueller Hinton Agar the zone of inhibition for the culture of <i>Staphylococcus aureus</i> of <i>Pogostemon benghalensis</i> (sample 1) was 8 mm (including diameter of disk is 6mm) and that for <i>Kalanchoe pinnata</i> (sample 2) was 9 mm (including diameter of disk is 6mm).</p>

3.3 Diameter of zone of inhibition of *P. benghalensis* and *K. pinnata*

The results found from the disk diffusion method clarifies that both the plants are having anti-microbial property. In Mueller Hinton Agar for *Klebsiella pneumoniae* the leaf extract of *Pogostemon benghalensis* (sample 1) showed a zone of inhibition was 9 mm (including diameter of disk is 6mm) and for *Kalanchoe pinnata* (sample 2) was 8 mm (including diameter of disk is 6mm). Similarly the zone of inhibition for the culture of *Staphylococcus aureus* of *Pogostemon benghalensis* (sample 1) was 8 mm (including diameter of disk is 6mm) and that for *Kalanchoe pinnata* (sample 2) was 9 mm (including diameter of disk is 6mm).

4. Conclusion

From the above experiments, we got some comparative phytochemical results of two different plant species. Some of the phytochemical were present in both species and some phytochemical present in only one species. In case of anti-microbial activity both sample have resistance against known microorganism (*Klebsiella pneumoniae* and *Staphylococcus aureus*). But *Pogostemon Benghalensis* (sample 1) has the greater resistance than *Kalanchoe Pinnata* against microorganism *Klebsiella pneumonia* (gram -ve bacteria). And *Kalanchoe Pinnata* (sample 2) has slightly greater resistance than *Pogostemon Benghalensis* (sample 1) against microorganism *Staphylococcus aureus* (gram +ve bacteria). We can take both sample as anti-microbial medicine. Future studies can be done by extracting out bioactive components from these plants and characterise the specific compounds against micobes.

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6. References

1. Kirtikar KR, Basu BD. Indian Medicinal Plants with illustrations. Volume-5, second edition, Oriental enterprises, 2003, 1394-1396.
2. Nandkarni AK. Indian Materia Medica, Volume-1, Third edition, Popular Prakashan, Bombay, 2005, 1402-221.
3. Samim Sofika Begum, Rajib Jogoi. Herbal recipe prepared during Bohag or Rongali Bihu in Assam, Indian Journal of Traditional Knowledge. 2007; 6(3):417-422.
4. Huissain S, Hore DK. Collectiopn and conservation of major medicinal plants of Darjeeling and Sikkim, Indian Journal of Traditional Knowledge. 2007; 6(2):352-357.
5. Prabal Sen, Mihin Dollo, Manabendra Dutta Chaudhury, Dhruvad Choudhury, Documentation and Traditional Herbal Knowledge of Khamptis of Arunachal Pardesh. 2008; 7(3):438-442.
6. Cherly A Lans, Ethanomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus, Journal of Ethnobiology and Ethnomedicine, 2006; 2:45.
7. MJ Bhandary, KR Chandrashekar, KM Kaveriappa, Medical Ethanoibotany of Siddis of Uttara Kannada district, Karnataka, India, Journal of Ethanopharmacology, 1995, 47:149-158.
8. Mai Thanh Thi Nguyen, Suresh Awale, Yasuhiro Tezuka, Quan Le Tran, Hiroshi Watanabe and Shigetoshi Kadota, Xanthin Oxidase Inhibitory Activity of Vietnamese Medicinal Plants, Biol. Pharm. Bull. 2004; 27(9):1414-1521.
9. Shiddamallaya N, Azra Yasmeen and Gopakumar K, Medico-Botanical survey of Kumar Parvatha Kukke Subramanya, Manglore, Karnataka, Indian Journal of Traditional Knowledge. 2010; 9(1):96-99.
10. Seema V Pattewar. *KALANCHOE PINNATA*: PHYTOCHEMICAL AND PHARMACOLOGICAL PROFILE, Sanjivani Institute of Pharmacy and Research, Kopargaon- 423603, Maharashtra, India, 26 March, 2012.
11. Shivananda Nayak B, Julien Marshall R, Godwin Isitor. Wound healing potential of ethanolic extract of *Kalanchoe pinnata* Lam. Leaf ---- A preliminary study, Department of Preclinical Sciences, Faculty of Medical

- Sciences. The University of West Indies, St. Augustine, Trinidad, 2010.
12. Sharopov FS, Kukaniev MA, Thompson RM, Satyal P, Setzer WN. Composition and antimicrobial activity of the essential oil of *Hyssopus seravschanicus* growing wild in Tajikistan. *Der Pharm. Chem*, 2012; 4:961-966.
 13. Zotov EP, Goryaev MI, Sharipova FS, Khazanovich RL, Vandysheva VI. Investigation of the essential oil of *Hyssopus zeravshanicus*. *Chem. Nat. Comp*, 1974; 10:110-111. doi: 10.1007/BF00568256. [Cross Ref]
 14. Dzhusmaev KHK, Zenkevich IG, Tkachenko KG, Tsiburskaya IA. Essential oil of the leaves of *Hyssopus seravschanicus* from South Uzbekistan. *Chem. Nat. Comp*, 1990; 36:101-102. doi: 10.1007/BF00605221. [Cross Ref]
 15. Huang Z, Zhu ZX, Li YT, Pang DR, Zheng J, Zhang Q, *et al.* Anti-inflammatory labdane diterpenoids from *Leonurus macranthus*. *J Nat. Prod*, 2015; 78:2276-2285. doi: 10.1021/acs.jnatprod.5b00635. [PubMed] [Cross Ref]
 16. Vvedenskiy A. Flora Uzbekistana (Flora of Uzbekistan) Volume 5. Akad. Nauk UzSSR; Tashkent, Uzbekistan, 1961, pp. 266-410.
 17. Eisenman SW, Zaurov DE, Struwe L. Medicinal Plants of Central Asia: Uzbekistan and Kyrgyzstan. Springer; New York, NY, USA, 2013.
 18. Mamadaliyeva NZ, Bobakulov KM, Vinciguerra V, Tiezzi A, Abdullaev ND, Nahar L, *et al.* GC-MS and q-NMR based chemotaxonomic evaluation of two *Leonurus* species. *Phytochem. Anal*, 2016; 27:284-289. doi: 10.1002/pca.2629. [PubMed] [Cross Ref]
 19. Mamedov N, Mamadaliyeva N. Herbal Medicine in Depression: Traditional Medicine to Innovative Drug Delivery. Springer International Publishing; New York, NY, USA. Medicinal Plants from Countries of Former USSR Used for Treatment of Depression, 2016.