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EVALUATION OF PHYTOCHEMICAL PROFILING AND ANTIMICROBIAL ACTIVITY OF *ALLIUM SATIVUM* CLOVES AND *ARTOCARPUS HETEROPHYLLUS* LEAVES

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ABSTRACT

The aim of the present study was to exhibit the quantitative analysis of the phytochemicals extracted from cloves of *Allium sativum* i.e., garlic and leaves of *Artocarpus heterophyllus* i.e., jackfruit. The extraction of these phytochemicals was done using the organic solvents such as ethanol, ethyl acetate and acetone using a Soxhlet apparatus. Few of the bioactive compounds like protein, phenol, carbohydrates, tannin, flavonoids and alkaloids were analysed for their total content in the extracted phytochemicals using UV spectrophotometer. Along with the quantitative estimation the antibacterial and antifungal activity of these extracted phytochemicals were checked against *E. coli, S. aureus, Bacillus subtilis* and fungal strains of *Aspergillus niger and Fusarium* species using the well diffusion method. Acetone extracts were proven effective against both bacterial and fungal isolates.

KEYWORDS: *Allium sativum, Artocarpus heterophyllus,* Soxhlet apparatus, Phytochemicals. Antimicrobial activity.

INTRODUCTION

There is a long history of the medicinal plants which have active compounds and have been proven to cure sickness as well as relieve pain. Traditional remedies and medicinal herbs are mostly used in the emerging countries as health-care providers and have been widely praised.[1] There are evidences associated with the use of medicinal plants that can be dated back up to five thousand years. India is one of the world's major diversity hotspots, with a rich heritage of traditional folk medicinal expertise. As a result, medicinal plants are widely used in several parts of India, both as folk remedies in various indigenous medical systems such as Siddha, Ayurveda, and Unani, and as processed pharmaceutical products.[2] One such medicinal plant is Garlic (*Allium sativum*) it is "aroma" vegetable that is widely utilized as a food ingredient. Jackfruit is another medicinal plant (Artocarpus heterophyllus) which is a mulberry tree species belonging to the Moraceae family. It is endemic to India's Western Ghats and Indonesia, as well as Central and Eastern Africa, Southeast Asia, Florida, Brazil, Australia, and a number of Pacific Islands.[3] Traditional folk medicine in Indonesia has employed jackfruit plants to treat inflammation, malarial fever, stomach aches, ulcers, abscesses, dysentery, diarrhoea, deficient urine discharge, and skin illness. Several studies have found that jack fruit extracts have Antibacterial, anti-diabetic, anti-inflammatory, antioxidant, and anti-helminthic properties.[4,14]

Artocarpus heterophyllus Lam. is a plant from the Moraceae family commonly known as jackfruit or “tree bread” in Latin America, a common name of Artocarpus genus like: *Artocarpus brasiliensis* Gomez., *Artocarpus*

heterophylla Lam., *Artocarpus maxima* Blanco, *Artocarpus philippinensis* Lam., among others. Native to Southeast Asia and is widely cultivated in Malaysia and the Western Ghats of India.[26,27,28]

A diverse range of secondary metabolites, including flavonoids, carotenoids, prenylflavones, and sterols, have been discovered in *A. heterophyllus*.[5,6] The phytochemicals that are extracted from *Allium sativum* comprises of various chemical constituents such as tannin, carbohydrates, saponin, alkaloids, terpenoids, steroids, phenols etc,[6,17,18,20] To precisely extract various chemicals from plants, a variety of solvents with various polarities must be used. With the use of different organic solvents like acetone, ethyl acetate, chloroform, etc., these phytochemicals may be extracted.[15,19] The Aim of the present experiment is to quantitatively analyse the phytochemicals extracted from *Allium sativum* cloves and *Artocarpus heterophyllus* leaves and tested against few bacterial and fungal species.

MATERIALS AND METHODS

Sample Collection

The samples used in the present study are garlic cloves (*Allium sativum*) and jackfruit (*Artocarpus heterophyllus*) leaves. The garlic cloves were bought from Yeshwanthpur market, Bangalore and the jackfruit leaves were collected from a healthy tree in Peenya locality, Bangalore.

1. Phytochemical screening

The samples jackfruit leaves and garlic cloves were thoroughly washed and then dried in hot air oven for 24 and 48 hours respectively. They were then crushed to fine powder. In order to extract the phytochemicals, the Soxhlet apparatus was used. The organic solvents selected were Ethanol, Ethyl Acetate and Acetone. Five grams of powdered samples and 250 ml of the organic solvents were used in extraction separately. In order to make the samples concentrated, the excess solvents were evaporated with the help of rotary evaporator. Now the concentrated samples were used for the further experiment.[7,8,12,15]

1.1. Test for Phenol

Gallic acid was employed as a reference to measure the total phenolic content in plant extract. Gallic acid stock solution was prepared using 10 milligrams dissolved in 100 ml distilled water, five various quantities of gallic acid was employed as a test standard, 0.2, 0.4, 0.6, 0.8 and 1 ml. After dilution, 1 ml of the reagents (0.5 ml FC reagent, 10 ml Na_2CO_3, and 5 ml distilled water) were added. At 760 nm, absorbance was then measured. One millilitre of each standard was obtained, the chemicals were added, and the absorbance recorded by using UV spectrophotometer at 760 nm.

1.2. Test for Flavonoid

The flavonoids were quantified using quercetin as the reference standard. The standard curve was created using different concentrations of quercetin (0.2, 0.4, 0.6, 0.8, and 1 mL). All the samples were made up to 1ml to which 0.5ml of sodium nitrate, 1ml of aluminum chloride and 0.5ml of NaOH. Incubated the reaction mixture at room temperature for 5 minutes and then add 2ml distilled water. After that, the reaction mixture was checked for absorbance at 510nm.

1.3. Test for Carbohydrate

In order to determine the total carbohydrates content glucose considered as a standard, the standard curve was obtained by creating various concentrations of glucose 0.2,0.4, 0.6, 0.8 and 1ml. All the concentrations of glucose were taken in test tubes their volume was made up to 1ml and then the reagents such as 5% phenol and concentrated H_2SO_4 was added and then the absorbance was checked at 488nm. Now repeated with the test samples and then the absorbance was recorded at 488nm.

1.4. Test for Tannin

Total tannin estimation was carrying out by using tannic acid 0.005g/ml as a standard. A standard curve was plotted after making the various concentrations of standards (0.2, 0.4, 0.6, 0.8 and 1.0) and then added the reaction mixtures i.e., 0.5ml FC reagent, 1ml Na2Co3 and 30ml distilled water. Incubated the reaction mixture for half an hour before the estimation of tannin was done. Later the absorbance was observed at 700nm in spectrophotometer. Same procedure carrying out for test samples.[9,10]

1.5. Test for Protein

The total protein estimation was done using Folin-Ciocalteu method. The standard curve, which was obtained by making different concentrations of standard - 0.2, 0.4, 0.6, 0.8 and 1ml and then the standards were made up to 1ml to which the reaction mixtures like alkaline copper reagent, FC reagent and then the absorbance was measured at 660nm in UV spectrophotometer. After the standard curve was obtained, 1ml from each of 6 samples were taken and then the reagents were added and incubated in dark for 30 minutes and then the absorbance was measured at 660nm.[10]

1.6. Test for Alkaloid

The total alkaloids content was determined by taking 1ml from each of the 6 samples to which 40ml of 20% of acetic acid was added and incubated the mixture at room temperature for 4 hours. Later the mixture filtered and evaporated. The filtrate using the steam bath in order to reduce the volume to one fourth of the original quantity followed by cooling down the mixture. To this drop by drop the concentrated ammonium hydroxide added to precipitate the alkaloid extract. Later the precipitate was filtered in a pre-weighed Whatman filter paper and then the precipitate was washed with 9% ammonia solution and was dried in oven at 60^0C for minutes. And then paper was reweighed in order to know the total quantity of alkaloids. the formula that was used to calculate the

total alkaloids content. Percentage of alkaloids= w2-w1/weight of the sample *100 W1 being the initial empty paper weight. W2 is the final filter paper weight after the final filtration.

2. Determination of the Antimicrobial Activity

2.1. Inoculum Preparation

For the preparation of the inoculum, test bacteria and fungi were taken from the stock culture and were grown separately in nutrient broth and potato dextrose broth and incubated at 37 ºC for24 hours on an orbital shaker at 200 rpm respectively. This culture was used for the antibacterial assays.[13,16]

2.2. Antimicrobial activity testing using Agar well diffusion assay

The selected bacterial cultures (*E. coli*, *B. subtilis* and *S. aureus*) and fungal cultures (*A. niger* and *Fusarium sp.*) were inoculated by swab culture technique on nutrient agar and potato dextrose agar plates respectively Agar wells were prepared with the help of sterilized corkborer with 10 mm diameter. Using a micropipette, 100 ul of different extracts were added to the wells in the plate and antibiotic was used as a control. The plates were incubated in an upright position at 37 ºC for 24 hours. The inhibition zones were measured. The reference antimicrobial agents such as Azithromycin and Cefixime were used for the comparison.

RESULTS AND DISCUSSION

Results

The quantitative analysis of the extracted six samples i.e., jackfruit acetone extract, garlic acetone extract, Jackfruit leaves ethyl acetate extract, garlic cloves ethyl acetate extract, jackfruit leaves ethanol extract, garlic cloves ethanol extract. Among all, garlic cloves ethanol extract showed that the phenolic content was highest. The flavonoid content was high in the garlic ethanol extract. The carbohydrates content was highest in garlic acetone extract. The tannin content was highest in garlic ethanol extract, while the protein content was highest in the jackfruit acetone extract and the alkaloids content was highest in garlic ethanol extract (Table 1 and Table 2). Among the six samples the acetone extracts and the ethyl acetate extracts had the effective antibacterial activity on *E. coli*, *S. aureus* and *Bacillus* species (Table 3). While only acetone extract of garlic was found to have effective antifungal activity against the fungal strains that were used in this study i.e., *Fusarium sp.* and *A. niger* (Table 4).

Table 1: Total phenol, flavonoid, Tannin and Alkaloid content in the extracted samples.

Samples	% of phenol.	% of Flavonoid	% of Tannin	% of Alkaloid
Garlic ethanol extract	0.03	0.077	0.15	1.782
Garlic ethyl acetate extract	0.065	0.0010	0.025	0.62
Garlic acetone extract	0.02	0.042	0.050	2.1
Jackfruit ethanol extract	0.0052	0.015	0.099	0.94
Jackfruit ethyl acetate extract	0.0015	0.0085	0.025	0.98
Jackfruit acetone extract	0.0041	0.055	0.1	1.52

Table 2: Total Carbohydrate and Protein content in the extracted samples.

Samples	Percentage %	Percentage %
Garlic ethanol extract	0.00515	0.06
Garlic ethyl acetate extract	0.0027	0.007
Garlic acetone extract	0.0255	0.050
Jackfruit ethanol extract	0.0005	0.075
Jackfruit ethyl acetate extract	0.0249	0.018
Jackfruit acetone extract	0.0025	0.080

Table 3: Antibacterial activity of the extracted samples.

Samples	Diameter of zone of inhibition in *E. coli*	Diameter of zone of inhibition of *S. aureus*	Diameter of zone of inhibition of *Bacillus* species
Garlic ethanol extract	-ve	-ve	1mm
Garlic ethyl acetate extract	-ve	-ve	-ve
Garlic acetone extract	2mm	3mm	2mm
Jackfruit ethanol extract	-ve	3mm	-ve
Jackfruit ethyl acetate extract	7mm	5mm	7mm
Jackfruit acetone extract	-ve	2mm	8mm
Azithromycin	5mm	5mm	1.8cm
Cefixime	6mm	6mm	1.8cm

Table 4: Antifungal activity of extracted samples.

Samples	Diameter of zone of inhibition of *Fusarium*	Diameter of zone of inhibition of *Aspergillus*
Garlic ethanol extract	-ve	-ve
Garlic ethyl acetate extract	-ve	-ve
Garlic acetone extract	7mm	5mm
Jackfruit ethanol extract	-ve	-ve
Jackfruit ethyl acetate extract	-ve	-ve
Jackfruit acetone extract	-ve	-ve
Azithromycin	-ve	-ve
Cefixime	-ve	-ve

DISCUSSION

The beneficial medicinal effects of plant materials is basically important from the secondary products, it is usually not contributed to a single compound but a combination of the metabolites.[18,19] The medicinal effects of plant are unique to a particular plant species or group, consistent with the concept that the combination of secondary products with different taxonomic species of plants. Essential oils and extracts have been used since from many years for food preservation, in pharmaceuticals, and an alternative medicine and also natural therapies. Plant extracts are potential and strong sources of antimicrobial compounds, against bacterial and fungal pathogens. *In vitro* studies in this regard work showed that the plant extracts inhibited bacterial growth but their effectiveness varied with the determination by zone of inhibition.[25] Phenolic compounds are related to defensive response in plants against plant pathogen attack to itself.[23] Some small chemical structure phenolic compounds such as phenolic acids also alter the fungal membranes and adhere and later polymerize fungal wall, thus minimises the growth of the fungus.[23,24,25]

Excessive usage of antibiotics creates problems and also making resistant strains.[22] thus create a problem while treating of infectious diseases, furthermore, antibiotics sometimes associated with side effects.[23] whereas there are some advantages such as side effects will be less and better tolerance, relatively cost effective, long history of use and can be renewable in environment.[24,25] These research work supports the use and knowledge for local users and it is a preliminary, scientific, validation for the use of these plants for antibacterial activity. It also promotes proper preservation of such type of plant bodies in the nature.

CONCLUSION

There is a history of using the herbs and plants as medicines since the dawn of humanity. Decades of research work has been done on utilizing these medicinal herbs for curing hundreds of diseases. The selected samples of garlic and jackfruit have important bioactive elements or contents such as phenols, flavonoids, tannin, carbohydrates, proteins and tannin, the study showed that the garlic ethyl acetate extract had the highest amount of phenol while jackfruit ethyl acetate had the lowest content of phenol. The flavonoid and tannin content were largely found in garlic ethanol extract. The garlic acetone extract was rich in alkaloids and carbohydrates. Protein was one of the least bioactive compounds that was present in these samples. This work demonstrates that the numerous therapeutic purposes given to the organic solvent extracts of *Artocarpus heterophyllus* leaves and *Allium sativum* cloves in folk medicine are justified by the presence of significant and active phytochemical components. Thus, the results of the present study clearly imply that jackfruit and garlic may be beneficial in reducing diabetes problems.

REFERENCES

1. Singh K. Phytochemical Analysis and Antimicrobial Activity of Various Indigenous Plant Species. Int J Res Appl Sci Eng Technol, 2021; 9(2): 241–5.
2. Singh V, Kumar R. Study of Phytochemical Analysis and Antioxidant Activity of Allium sativum of Bundelkhand Region. Int J Life-Sciences Sci Res., 2017; 3(6): 1451–8.
3. Phan ADT, Netzel G, Chhim P, Netzel ME, Sultanbawa Y. Phytochemical characteristics and antimicrobial activity of Australian grown garlic (Allium sativum L.) cultivars. Foods, 2019; 8(9): 358.
4. Tadu, C K., Dutta, T., Ghorai, M., Biswas, P., Samanta, D., Oleksak, P., Jha, N K., Kumar, M., Radha, Prockow, J., Lastra, J M P. and Dey, A. Traditional uses, phytochemistry, pharmacology and toxicology of garlic (*Allium sativum*), a storehouse of diverse phytochemicals: A review of research from the last decade focusing on health and nutritional implications. Frontiers Nutrition, 2022; 9: 949554.
5. Dhawan D, Gupta J. Comparison of Different Solvents for Phytochemical Extraction Potential from Datura metel Plant Leaves. Int J Biol Chem, 2016; 11(1): 17–22.
6. Kaur, M. Ghinta, P and Gupta, R. Phytochemical analysis, antioxidant and antimicrobial activity assessment of Allium sativum (garlic) extract. Research Journal of Biotechnology, 2021; 16(3): 134-140.
7. Chavez-santiago JO, Rodríguez-castillejos GC, Montenegro G, Bridi R, Valdés-gómez H, Alvarado-

reyna S. Phenolic content, antioxidant and antifungal activity of jackfruit extracts (Artocarpus heterophyllus Lam), 2022; 2061: 4–10.
8. Ali M, Ibrahim IS. Phytochemical Screening and Proximate Analysis of Garlic (Allium Sativum), 2019; 478–82.
9. Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants, 2017; 6(4): 42.
10. Prakash O, Kumar R, Mishra A, Gupta R. PHCOG REV : Review Article Artocarpus heterophyllus (Jackfruit): An Overview, 2009; (6): 353–8.
11. Barbu, I A., Ciorita, A., Carpa, R., Mot, A C., Butiuc-keul, A. and Parvu, M. Phytochemical characterization and antimicrobial activity of several Allium Extracts. Molecules, 2023; 28(10): 3980.
12. Yadav S, Trivedi N, Bhatt J. Antimicrobial activity of fresh garlic juice: An in vitro study. AYU (An Int Q J Res Ayurveda), 2015; 36(2): 203.
13. Wickramaratne MN, Punchihewa JC, Wickramaratne DBM. In-vitro alpha amylase inhibitory activity of the leaf extracts of adenanthera pavonina. BMC Complement 44 Altern Med [Internet], 2016; 16(1):1–5.
14. Vadivu R, Lakshmi KS. In vitro and In vivo anti-inflammatory activity of leaves of Symplocos cochinchnensis (Lour) Moore ssp laurina. Bangladesh J Pharmacol, 2008; 3(2).
15. Ahmad, M N., Karim, N U., Normaya, E., Piah, B M., Iqbal, A and Bulat, KH K. *Artocarpus altilis* extracts as a food-borne pathogen and oxidation inhibitors: RSM, COSMO RS, and molecular docking approaches. Scientific reports, 2020; 10: 9566.
16. Devi, P. S. S., Kumar, N S. and Sabu, K K. Phytochemical profiling and antioxidant activities of different parts of *Artocarpus heterophyllus* Lam. (Moraceae): A review on current status of knowledge. Future journal of pharmaceutical sciences, 2021; 7: 30.
17. Joaquin Omar CHAVEZ-SANTIAGO, Guadalupe concepcion RODRIGUEZ-CASTILLEJOS, Gloria MONTENEGRO, Raquel BRIDI, Hector VALDES-GOMEZ, Sofia ALVARADO-REYNA, Octelina CASTILLO-RUIZ and Ruben SANTIAGO-ADAME. Phenolic content, antioxidant and antifungal activity of jackfruit extracts (Artocarpus heterophyllus Lam). Food Sci Technol, 2022; 42.
18. Shafiq, M., Mehmood, S., Yasmeen, A., Khan, S.J., Khan, N. H. and Ali, S. Evaluation of phytochemical, nutritional and antioxidant activity of indigenously grown jackfruit (Artocarpus heterophyllus Lam). J. Sci. Res., 2017; 9(1): 135-143.
19. Pusztahelyi, T., Holb, I. J., & Pócsi, I. Secondary metabolites in fungus-plant interactions. Frontiers in Plant Science, 2015; 6: 573.
20. Joaquín-Ramos, A. D. J., López-Palestina, C. U., Pinedo-Espinoza, J. M., Altamirano-Romo, S. E., Santiago-Saenz, Y. O., Aguirre-Mancilla, C. L., & Gutiérrez-Tlahque, J. Phenolic compounds, antioxidant properties and antifungal activity of jarilla (Barkleyanthus salicifolius ENT# 91; KunthENT# 93; H. Rob & Brettell). Chilean Journal of Agricultural Research, 2020, 80(3), 352-360.
21. Parekh J, Chanda S. Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. African Journal of Biomedical Research, 2005; 10: 175-181.
22. Sydney S, Lacy RW, Bakhtiar M. In: The Betalactam antibiotics Penicillin and Cephalosporin in perspective. Hodder and stongton London, 1980; 224.14.
23. Cunha BA. Antibiotics side effects. Med Clin North Am, 2001; 85:149-185.15.
24. Vermani K, Garg S. Herbal medicine for sexually transmitted diseases and AIDS. J. Ethnopharmacology, 2002; 80: 49-66.
25. Jadon R. and Dixit S. Phytochemical extraction and antimicrobial activity of some medicinal plants on different microbial strains. J. of Medicinal plants studies, 2014; 2(3): 58-26.
26. Prakash, O., Kumar, R., Mishra, A., & Gupta, R. *Artocarpus heterophyllus* (Jackfruit): an overview. *Pharmacognosy Reviews*, 2009; 3(6): 353.
27. Nayak, M., Nagarajan, A., & Majeed, M. Pharmacognostic evaluation of leaf and stem wood extracts of *Artocarpus hirsutus* Lam. *Pharmacognosy Journal*, 2017; 9(6): 887-894. http://dx.doi.org/10.5530/pj.2017.6.139.
28. Vazhacharickal, P. J., Sajeshkumar, N. K., Mathew, J. J., Kuriakose, A. C., Abraham, B., Mathew, R. J., Albin, A. N., Thomson, D., Thomas, R. S., Varghese, N., & Jose, S. Chemistry and medicinal properties of jackfruit (*Artocarpus heterophyllus*): a review on current status of knowledge. *International Journal of Innovative Research and Review*, 2016; 3(2): 83-95.