

Metabolomic Profiling and Bioactivity Evaluation of Plant Resins

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Citation | Rafaquat, Hassan. U, Shahzad. A, Ahmed. S, Naveed. K, Shoukat. M. F, Hussain. R, Noor. M, Shahzadi. T, Khan. S. U, Mehmood. A, “The Metabolic Profiling and Bioactivity Evaluation of Plant Resins”, IJASD, Vol. 07 Issue. 01 pp 101-112, March 2025

Received | Feb 15, 2025 **Revised |** Mar 15, 2025 **Accepted |** Mar 17, 2025 **Published |** Mar 19, 2025.

The present study was conducted to evaluate the phytochemical and antimicrobial potential of local plant resins including Kiker (*Acacianilotica*), Phulai (*Acacia modesta*), Gond babool (*Acacia arabica*), and Aloo bukhara (*Prunus domestica*) collected from Haripur district. Ten-gram powder of the sample is mixed with 50 mL of 70% methanol and extracted by using ultrasonication for 30 minutes. The mixture was filtered and concentrated in a rotary evaporator till 2 mL remained. Samples were diluted with methanol (70%) and were analyzed in triplicates. The results indicated that phenolic compounds increase by heating as compared to raw resin extract. Aloo Bukhara resin showed a higher amount of 8.33 mg/g while Gond babool had 7.21 mg/g, Phulai had 5.73 mg/g and Kiker had 4.61 mg/g total phenolics respectively. Total flavonoid content decreases in microwave heating. Resin extracts have higher radical scavenging activity when it is not heated. Kiker has the highest radical scavenging activity i. e. 80% while Phulai has 69.99%, Gond babool has 74% and Plum has 75%. Moreover, heating results in a decrease in antioxidant activity with the time difference. In microwave heating, Gond babool showed higher activity i. e. 73% for 1 min., 71% for 2 min., and 57% for 3 min. as compared to other resins. Antimicrobial activity results showed that Kiker resin has a greater zone diameter against *E. coli* and *S. aureus* as compared to the rest of the varieties. Heat treatments increased the activity in Phulai and Aloo bukhara resins while decreasing in Babool resin against different bacterial isolates. All the selected resins have a good number of phytochemicals as well as antimicrobial activity. Industries use these plant resin extracts as antioxidants and antimicrobials in many food and other products because of their natural source and easy availability having health-beneficial attributes as well as their potential to improve product structure as a gum base.

Keywords: Acacia Nilotica, Acacia Modestica, Phytochemicals and Antimicrobial

Introduction:

Plants have an immense number of compounds, called 'phytochemicals' [1]. These chemicals can protect plants against all kinds of environmental threats including noise, stress, drought, UV exposure, and pathogen attacks [2]. However, recent findings on various aspects of phytochemicals have shown that certain plant chemicals have important functions in maintaining human health as they have significant dietary consumption [3]. It is understood

that a wide number of pharmacologically active ingredients transmit the miraculous therapeutic consequences of medicinal herbals (chemicals or metabolites). Phytochemical therapeutical benefits include: antioxidant function, reduced platelet aggregation, anti-cancer properties, enzyme regulation, and immune enhancement. They also offer anti-inflammatory and antimicrobial roles [4]. Phytochemicals are not important nutrients and do not yet have significant properties to prevent or battle certain common illnesses and infections. Approximately five secondary metabolites in plants worldwide are predicted to be produced [5]. Thousands of phytochemicals found in plants have been identified so far.

Plants have been used since ancient times to treat medical conditions. Historical evidence from Mesopotamian and Egyptian civilizations suggests their use in trade and medicine, with additional confirmation from early Chinese, Egyptian, African, and Indus Valley cultures [6]. Over the last few years, the popularity of herbal medicines has increased significantly. Plant extracts possess remarkable therapeutic potential and are often considered the first choice in treatment due to their associated benefits. These benefits include easy availability, the perception of minimal side effects, cost-effectiveness, and the absence of advanced healthcare services in rural areas. Studies have shown that approximately 25% of prescription medicines in developing regions are derived from herbal plants [7].

Resins are final products of oxidation, which are just an amorphous blend of essential oil, terpenes oxygen compounds, and plant exudates from carboxylic acids. Since ancient times, humans have explored nature in search of new medicines, relying extensively on plants for therapeutic purposes. Currently, approximately 80% of the global population depends on traditional medicinal remedies, the majority of which are derived from herbal extracts. Almost 90% of the prescriptions in India were based on conventional schemes including unani and homeopathy [8].

Gums are water-soluble polysaccharides that, even at low concentrations, form viscous aqueous structures, including modified polysaccharides. Plants produce varying amounts of pathological products when they are damaged, diseased, or exposed to severe weather. However, gums are not typical plant metabolic products of plants [9]. Plant gum granules primarily contain galactose, arabinose, uronic acid, galacturonic acid, protein, calcium, and magnesium. Additionally, glucose, xylose, mannose, protein, and fat are present as minor components. Additionally, glucose, xylose, mannose, protein, and fat are present as minor components [10].

Pakistan's floral diversity is vast, yet many of the resin-production plants still do not completely recognize and acknowledge their position and ability. The production of different plant resins depends on weather, atmosphere, soil, and water [11]. Their yield fluctuates each year from season to season. The raw resin holds minimal value in foreign markets, leading the government to prioritize imports rather than extraction. Additionally, synthetic materials have largely replaced natural resin. This study aims to explore the nutraceutical potential of various plant resins and their applications in natural and cost-effective food and pharmaceutical products [12].

The causative elements in certain infections of human beings are microorganisms. Antibiotics have been widely used worldwide to combat microbial infections. However, treatment becomes highly challenging when most bacteria develop resistance to antibiotics. The control of a variety of microbial infections is generally performed with antibiotics. However, over the last several years, several research on the growing resistance of biological pathogens such as bacteria and fungi have been published from various parts of the world [13].

A current study was carried out to study and identify the potential importance of plant resin extracts. The health-promoting potential of plant resins is being explored to develop recommendations for their potential use in industrial products. This research examines how

heat treatments affect the bioactive compounds in plant resins and identifies which resins contain the highest concentrations of bioactive compounds and exhibit strong antimicrobial activity.

Material and Methods:

This study was conducted in the Department of Food Science and Technology and the Department of Microbiology at the University of Haripur, Khyber Pakhtunkhwa, Pakistan.

Collection of Plant Resins:

For this study, four different plant resins were sourced from the local market in District Haripur. The names of these resins are listed in table 1. Plant resins were freeze-dried to remove moisture, ground into powder using an electric grinder, and stored at room temperature until further analysis.

Table 1. List of Plant Resin with common and botanical names

S. No.	Common Name	Botanical Name
1	Kiker	Acacia nilotica
2	Phulai	Acacia modesta
3	Gond Babool	Acacia arabica
4	Aloo Bukhara	Prunus domestica

Extract Preparation:

A total of ten-gram powder of the sample was mixed with 50 ml of 70% methanol and extracted by using ultrasonication for 30 minutes. The mixture was filtered and concentrated in a rotary evaporator till 2 ml remained. The sample was kept in a falcon tube, diluted with methanol (70%), and was analyzed for the following parameters in triplicates.

Heat Treatments of Plant Resins:

Resins were heated in the microwave for 1, 2, and 3 minutes (taken as treatments) then ground to powder and extracts were prepared as mentioned above.

Phytochemical Analysis:

Total Phenolic Content (TPC):

For TPC, 1 ml of already prepared aliquot was oxidized with 2.5 mL of Folin–Ciocalteu's reagent (10 %). This oxidized sample was then neutralized with 2 ml Na₂CO₃ (7.5 %). This mixture was kept in a dark place for ¾ hours and then its absorbance was measured at 765 nm wavelength using a spectrophotometer with Gallic acid as standard [14].

Total Flavonoid Content (TFC):

For TFC, 1 mL of already prepared aliquot was mixed with 0.3 ml NaNO₃ (5%) and left for 5 minutes. Then add 0.6 mL of AlCl₃ (10%) and mix it. After 5 minutes, add 2 mL of 1 M NaOH to the mixture. In the end, the absorbance was measured at 510 nm wavelength by using a spectrophotometer taking quercetin as standard [15].

Antioxidant Activity:

To find antioxidant activity (free radical scavenger activity of the aliquot 1, 1-diphenyl 1-2-picrylhydrazyl) (DPPH) was used [16]. The aliquot was added at an equal volume, to methanolic solution of DPPH (0.2 mM). The absorbance was noted at 517 nm at room temperature after 30 minutes. Radical scavenging activity is expressed as the inhibition percentage and calculated using:

$$\text{Radical scavenging activity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Where A_{control} and A_{sample} are the absorbances of the control and sample respectively.

Antimicrobial Activity:

Sample Preparation:

The sample was prepared by soaking fifty grams of powder in 200 ml methanol (70%) and placed in an ultrasonicator for mixing (to dissolve the sample in methanol). After ultrasonication, the extract was filtered by filter paper and concentrated in a rotary evaporator.

The concentrated extracts were then diluted with Dimethyl sulfoxide (DMSO) and stored in the dark at room temperature for analysis of anti-bacterial activity.

Agar-well plate method was used for the analysis of anti-bacterial activity. Firstly, media was prepared by using nutrient agar and poured into the petri plates. The plates were left for 30 minutes to solidify. Clinical isolates were spread on the plate using a spreader one by one. Create wells on the media plate through a borer. Add samples to these wells. Place these plates in incubators at 37 °C 24 hrs. Take reading the next day.

Statistical Analysis:

Analysis of variance was performed using the computer-based software Statistics 8.1. The differences amongst treatments were separated using the least significant difference at 0.01 [17].

Results and Discussion:

This study was conducted to screen phytochemicals in various plant resins available in the local market of District Haripur. These resins were Phulai, Kiker, Gond babool, and Aloo bukhara. The extracts in the study were prepared and phytochemical screening was carried out for the detection of antioxidant activity, total phenolic content, and total flavonoid content. Experiments were performed without heating (control) and microwave heating following 1, 2, and 3 minutes. The phytochemical tests were performed by following proper standard methods and guidelines.

Phytochemical Screening:

Total Phenolic Content:

The phenolic compound is shown in the figure 1. The results showed that phenolic contents increased by heating as compared to raw resin extract. During the analysis of total phenolic content (TPC), plum resin exhibited the highest amount at 8.33 mg, followed by Gond Babool at 7.21 mg, Phulai at 5.73 mg, and Kiker at 4.61 mg. Upon heating, Kiker exhibited a significant increase in Total Phenolic Content (TPC), measuring 38.08 mg, 28.41 mg, and 6.84 mg at time intervals of 1, 2, and 3 minutes, respectively. Many herbal plants contain several biological properties, and non-nutritional compounds such as phytochemicals, which have health benefits [18]. Total phenolic content is useful in the fields of food, pharmaceuticals, and health. Phenols are chemical compounds that have a hydroxyl group (-OH) bonded with an aromatic hydrocarbon group. Plants and animals can produce phenolic compounds under specific conditions. Those plants that produce phenolic compounds can be used as medicinal plants. Although some phenolic molecules are soluble, others are volatile. The extract with more total phenolic exhibits excellent antiulcer effects, which demonstrates that the phenolic content of the extracts is responsible for the extract's activity [19].

Total Flavonoid Content:

Figure 2, showed the results of TFC for resins in mg QE/g of extract. Total flavonoid content decreases in the microwave heating process as compared to without heating. Results showed that all the resins had almost the same amount of TFC for control analysis i.e. Kiker had 44.19 mg, Phulai had 45.96 mg, Gond babool had 44.35 mg and Plum had 44.02 mg. In microwave heating, Phulai and Kiker had a higher amount when heated for 2 minutes while Gond babool and Aloo Bukhara had a higher amount of TFC on heating for 1 minute. The results also indicated that extracts rich in phenolic and flavonoid contents had potent antioxidant activity and were significant in comparison with all the positive controls used in this study. Flavonoids are primarily plant-synthesized secondary metabolites. They possess a 15-carbon skeleton of 2 benzene rings linked by a three-carbon link chain and have an overall structure of C6-C3-C6. Flavonoids can be categorized into classes such as anthocyanidins, flavones, flavonols, flavanones, flavanone 3-ols, flavanonols, and isoflavonoids according to the chemical composition, the degree of oxidation and the binding chain unsaturation (C3).

In comparison, glycoside-like and free aglyconic flavonoids can be found in plants. The type containing glycosides is the most popular form of flavone and flavonol in the diet [20][21].

Flavonoids are the naturally occurring plant compounds responsible for the colors and pigments in flowers. These also have the ability to inhibit some disease-causing microorganisms. These also have anti-allergic, anti-inflammatory, and antioxidant properties. Flavonoids are responsible for the activation of antioxidant enzyme radicals. These are important components of diet but are not considered as nutrients [22][23].

Antioxidant Activity:

Figure 3 shows the results of the radical scavenging activity of resin extracts. Results revealed that resin extracts had higher radical scavenging activity when it is not heated. Among all resins, Kiker had the highest radical scavenging activity i.e. 80% while Phulai had 69.99%, Gond babool had 74% and Aloo bukhara had 75%. Moreover, heating results in a decrease in antioxidant activity with the time difference. In microwave heating, Gond babool showed higher activity i. e. 73% for 1 minute, 71% for 2 minutes, and 57% for 3 minutes as compared to other resins. Antioxidants have the ability to protect the lipids and oils in food from oxidative degradation [24]. When these are added to the food these control rancidity developments and also stop the toxic oxidation product development. As well as these help to maintain the nutritional quality of food hence increasing the shelf life of the products.

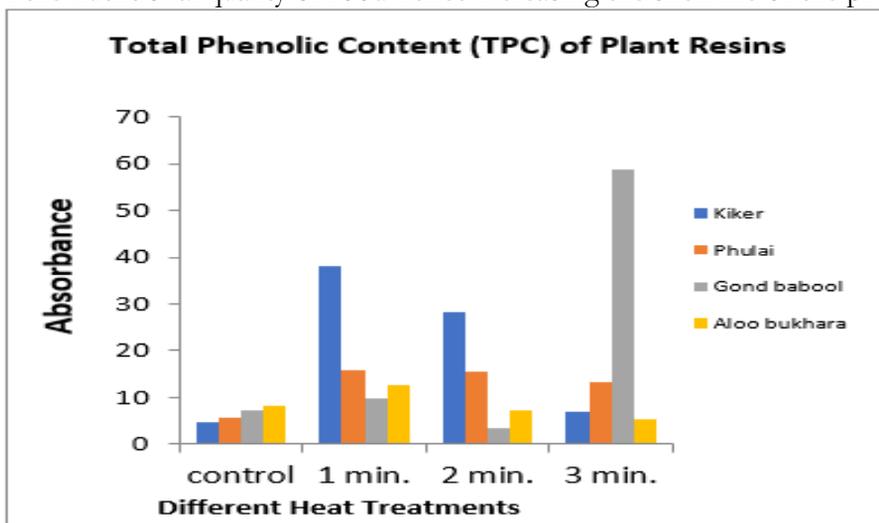


Figure 1. Total phenolic content of plant resins

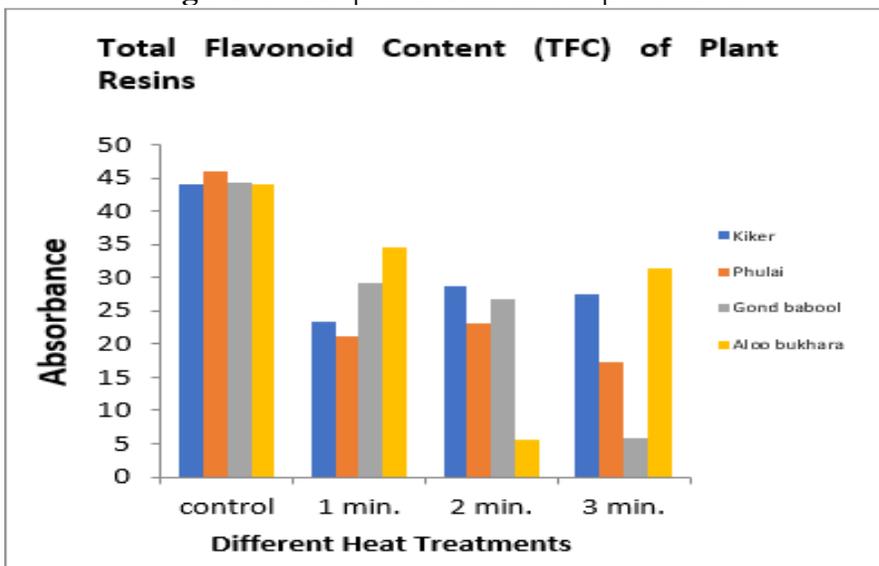


Figure 2. Total flavonoid content of plant resins

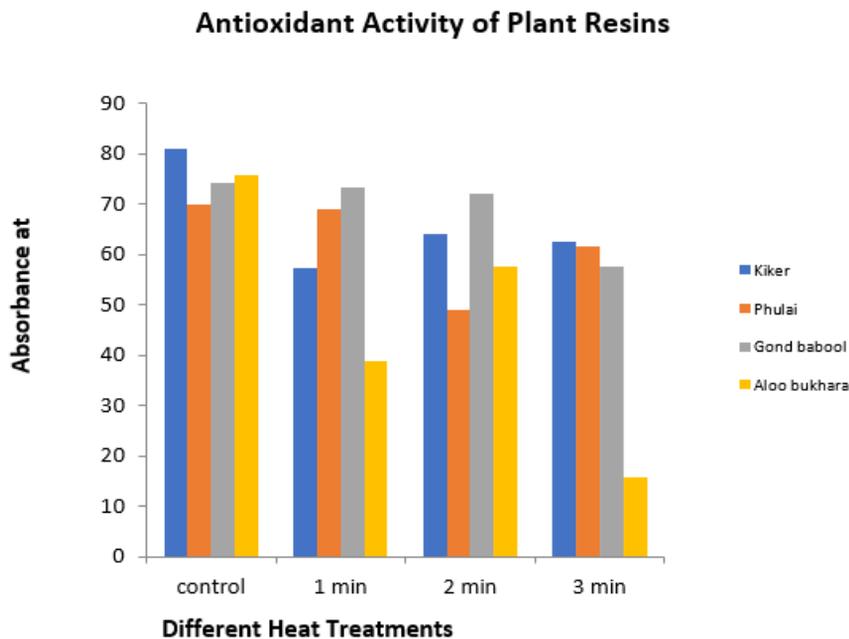


Figure 3. Antioxidant activity of plant resins

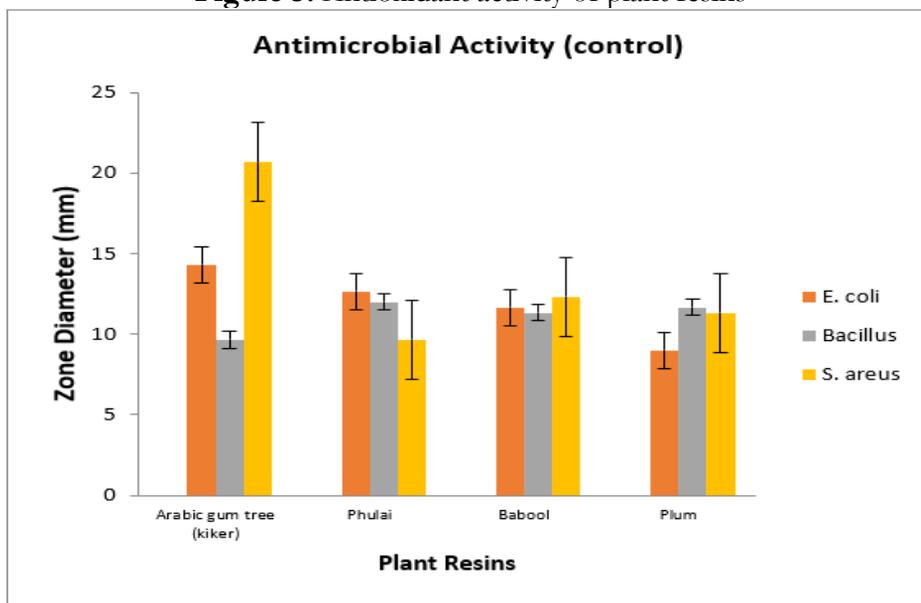


Figure 4. Antimicrobial activity of plant resins (control)

Antimicrobial Activity:

The antimicrobial activity of plant resins was evaluated using the agar well diffusion method against various bacterial strains. Each sample extract was prepared at a concentration of 20µg/2mL.

Bacterial Isolates:

Staphylococcus aureus and Bacillus were employed as gram-positive bacteria, whereas Escherichia coli was used as gram-negative bacteria.

Antimicrobial Activity (Control):

Figure 4 shows the four different resin varieties showing the zone of inhibition against three bacterial isolates. Kiker resin showed the largest inhibition zone against *E. coli* and *S. aureus* i.e. 14 mm and 21 mm respectively as compared to other resin varieties. Among all plant resins Phulai resin showed the most effective activity against *Bacillus* isolate which is 12.5 mm.

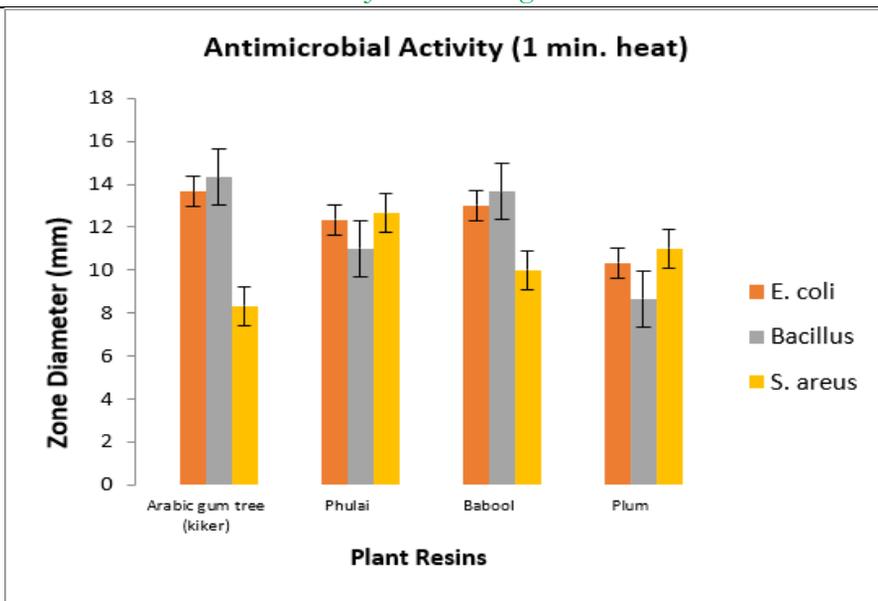


Figure 5. Antimicrobial Activity of plant resins (1 min. heat)

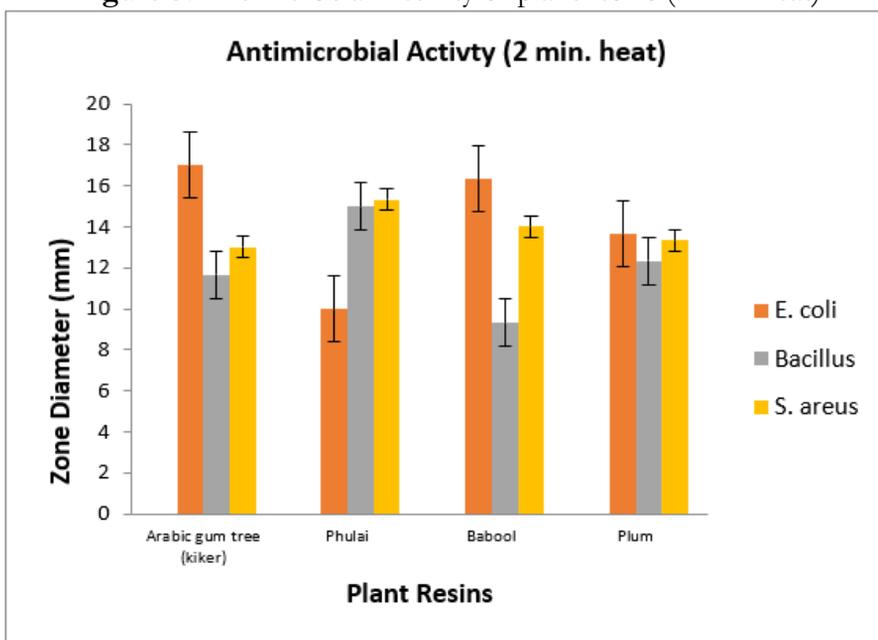


Figure 6. Antimicrobial Activity of plant resins (2 min. heat)

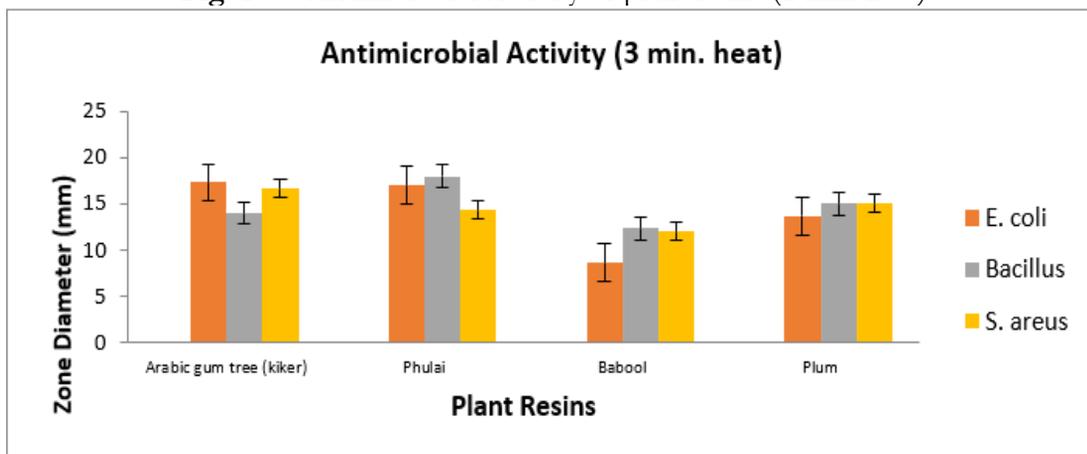
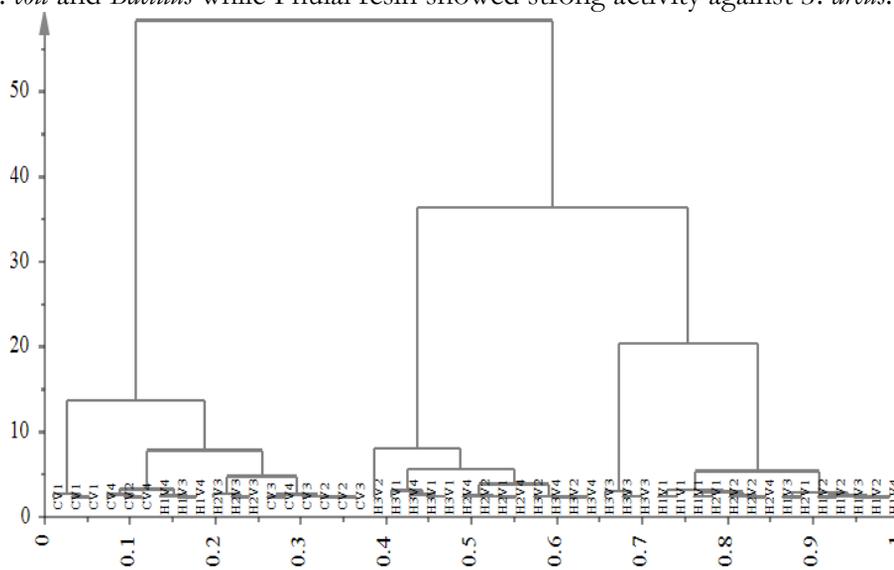


Figure 7. Antimicrobial Activity of plant resins (3 min. heat)

Figure 5 showed that Kiker and Babool resin showed a significant inhibition zone against *E. coli* and *Bacillus* while Phulai resin showed strong activity against *S. aureus*.



Hierarchical analysis based on PCA

Figure 8. PCA for data results

Heat for Two Minutes:

Figure 6, showed when plant resins were heated for 2 minutes Kiker and Babool had the same inhibition zone against *E. coli* but for *Bacillus*, it decreased. Phulai showed a significant zone of inhibition against *Bacillus* and *S. aureus*.

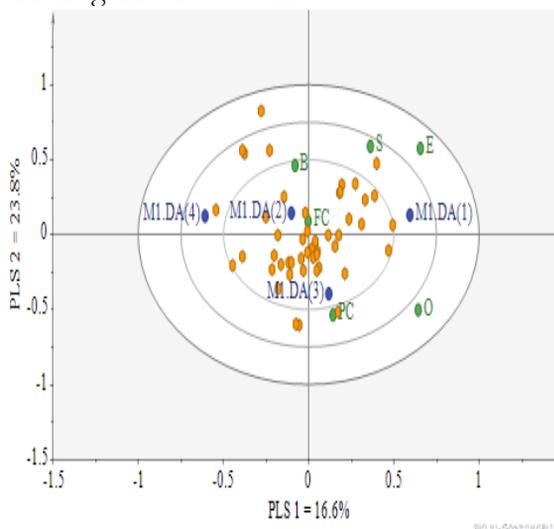


Figure 9. PLS-DA: Groups based on varieties

Heat for Three Minutes:

Plant resins were heated for 3 minutes in the microwave and the results showed that Kiker and Phulai resins exhibited the largest zone diameter as compared to other resins, while Babool resin had a very small inhibition zone against 3 bacterial isolates. The results showed in the above figures that Arabic gum trees have good antimicrobial activity as compared to the other resins. Heat treatments increased the activity in Phulai and Plum resins while decreasing in Babool resin against different bacterial isolates. The results revealed that the methanol extract showed the highest antibacterial activity with a zone of inhibition ranging from 10 mm to 18 mm at 1 mg/ml concentration [25][26].

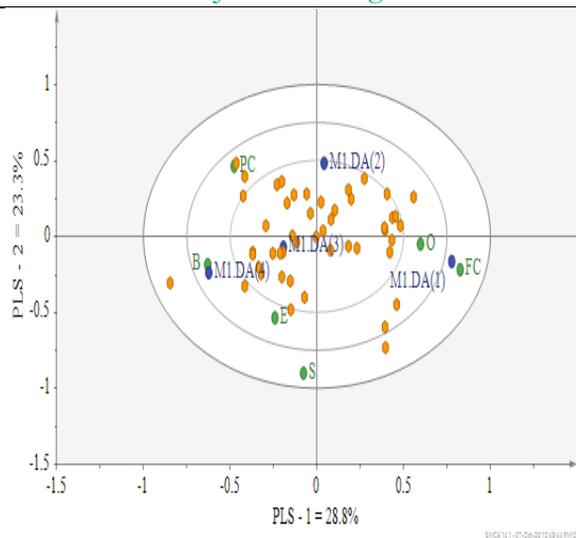


Figure 10. PLS-DA: Groups based on heat treatments

Principal Component Analysis (PCA):

PCA was used to analyze data to examine the relationship between all treatments of plant seeds and the qualitative parameters studied. The scores were generated from principal component analysis of all samples as shown in Figure 8 and the distribution of parameters in space defined by the first and second PCA dimensions is shown in Figure 9 and Figure 10 respectively.

The combined contribution of principal components PC1 and PC2 accounted for 55% of the variations among the treated samples. PC1, the first component contributed to 24.8% of the total variation and the second component accounted for 30.2% of the total variation. The sum of principal components PC1 and PC2 accounted for 40.4% of variations among treated samples. PC1, the first component contributed to 16.6% of the total variation and the second component accounted for 23.8% of the total variation. PC1 was positively correlated with TFC, AE, AS, and AB while negatively correlated with TPC and AO. The sum of principal components PC1 and PC2 accounted for 52.1% of variations among treated samples. PC1, the first component contributed to 28.8% of the total variation and the second component accounted for 23.3% of the total variation. PC1 was positively correlated with TPC while negatively correlated with TFC, AB, AE, AS, and AO. Principal Component Analysis (PCA) is the process of computing the principal components and using them to perform a change of basis on the data, sometimes using only the first few principal components and ignoring the rest [27][28].

Conclusion:

The present study helps to evaluate the phytochemical and antimicrobial potential of plant resins. All the selected resins have a good number of phytochemicals as well as antimicrobial activity. Industries will use this plant resin extracts as antioxidants and antimicrobials in many food and other products because of their natural source and easy availability having health-beneficial attributes thus indirectly contributing to the national economy and alternatively reducing the risk of many diseases in our community.

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