Comprehensive Profiling of Indigenous Medicinal Plants: Exploring Biological and Chemical Characteristics for Therapeutic Applications

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ABSTRACT

Therapeutic plants contain natural products known as phytochemicals. Locally available remedial plant leaves such as *Aloe barbadensis* (Aloe vera), *Azadirachta indica* (Neem), *Moringa oleifera* (Horseradish tree), *Psidium guajava* (Guava) and *Syzygium cumini* (jambolana), were selected and extracted by two methods including sonication-assisted extraction and percolation-assisted extraction in ethanol. Phytochemical screening, antioxidant activity, scavenging assays, antimicrobial activity, antidiabetic activity, antihemolytic activity, anti-lipid peroxidation activity was determined using pre-established standard protocols. Chemical properties by Thin layer chromatography and Fourier transform infrared spectroscopy method were determined. In antioxidant activity, *Psidium guajava* showed highest reducing ability (1.6%) whereas, least (0.8%) reducing ability was observed by *Moringa oleifera*. In scavenging assays, *Psidium guajava* showed high absorbance (63.44%) while *Azadirachta indica* was poor scavenger (43.94%). In hydrogen peroxidation assay *Psidium guajava* showed better results than *Aloe barbadensis*. In antimicrobial activity *Syzygium cumini* gave higher zone of inhibition for all selected bacteria compared to *Salmonella typhii* whereas, *Psidium guajava* gave higher zone of inhibition. In antidiabetic activity, higher glucose uptake by yeast cells (36%) was observed in the presence of *Aloe barbadensis* while in the presence of *Psidium guajava* sonicated extracts least glucose uptake capacity was shown by yeast cells. The antihemolytic activity of *Psidium guajava* extract at higher concentration has more % inhibition of RBS lysis (81.76%) while *Moringa oleifera* extract showed least percentage inhibition of lysis of RBC (50.11%). In anti-lipid peroxidation activity *Moringa oleifera* inhibited lipid peroxidation (52.9%) while *Aloe barbadensis* showed poor anti-lipid peroxidation (27.2%). The TLC analysis results were better observed when butanol: acetic acid: distilled water was used as solvent. In FT-IR analysis, *Syzygium cumini* showed greater number of

peaks. All poly extracts gave good biological activities. The presence of the biological activities of these compounds can make these plants an alternative treatment to different medicines.

Key words: Antioxidant activity, Bioactive agents, Anti-diabetic Activity, Anti-hemolytic activity, Antimicrobial activity, Therapeutics.

1. INTRODUCTION

The use of herbal plants as an alternative to medicines is in practice around the world. These therapeutic plants contain several phytochemical compounds that help protecting the innate system from oxidative damage leading to protection from chronic illnesses. These plants contain several compound including alkaloids, carbohydrates, flavonoids, phenols, steroids and tannins. These compounds are produced in plants through primary and secondary metabolism and play a vital role in disease control (Sharma et al., 2019).

The use of phytomedicines is safe compared to allopathic medicines due to their less harmful effects, effectiveness and they are cost effective as well (Balyan et al., 2021). For the treatment of various illnesses and care for human health, different chemicals are obtained from plants that are nonnutritive bioactive compounds known as phytochemicals. Phytochemicals are beneficial in the control of ailments and provide a defensive mechanism to herbs along with imparting color and characteristic odor to the plants (Thakur and Sharma, 2018).

Foods obtained from plant origin have flavonoids (dietary polyphenols) and phenolic acids which are expended in large quantities. These have a major role in the curing of prolonged and degenerative ailments besides many other positive effects. Biologically micro and macronutrients are high as likened to dietary polyphenols. 5-10% of dietary polyphenols are immersed in the small intestine. The pathogenic intestinal microflora is controlled by phytochemicals acting as prebiotics revealing antimicrobial properties. Perennial plants produce a major group of chemicals known as terpenoids. These downregulate cytokine expression, reduce tissue loss and act as antioxidants (Hossenet al., 2020). Less systemic toxicity is caused by Flavonoids which are major group of bioactive substances and have defensive potential against cutaneous inflaming reactions (Adamczak et al., 2020 and Chuang et al., 2017).

The initiation of inflammatory mediators is obstructed by quinones. Cytokine expression is slowed down, oxidation is suppressed and inflammation is reduced by quinones (Hossen et al., 2020). Quinones are very important phytochemicals for metabolic processes. Metabolic processes include the transfer of electrons in the Mitochondria through the respiratory chain in the process of

respiration and photosynthesis. The high content of ammonia is present in the alkaloids which are the largest group of secondary chemical constituents. Amino acid building blocks synthesized the contents of nitrogen bases present in alkaloids. In a peptide ring, one or more hydrogen atoms are replaced with various radicals. Most of the peptide ring is occupied by an Oxygen atom. Nitrogenous compounds play role in guarding plants against herbivores and pathogens. They are widely used as poisons, pharmaceuticals, narcotics and stimulants due to their strong biological activities. They are also used in the preparation of central nervous system stimulants and anesthetics (Mousavi et al., 2018).

Cells have enzymatic and co-enzymatic elements present in a complex antioxidant system which help to avoid oxidative stress. As diabetes is caused by oxidative stress due to increase in reactive oxygen species in the body so it could be treated by plants as they are having antioxidant phytochemicals (Mendoza et al., 2021). Moringa tree has antioxidant activity (scavenging of free radicals). From the therapeutic point of view, It helps to treat skin infections, fever, sores, high blood pressure and diabetes (Sen and Behera, 2019). Diabetes mellitus is effectively cured by *Syzygium cumini* fruit as it increases the level of insulin enzyme by lessening the rate of sugar in the blood (Raza et al., 2017). Neem tree is having various therapeutic values that help to combat microbial diseases, dental problems and problems of the skin (Rupani and Chavez, 2018). Phytochemical flavonoid resides more in the guava fruit which imparts therapeutic properties along with antibacterial activity (Adamu, 2021). Many properties such as antioxidant (free radical and superoxide scavenging activity) and anti-inflammatory activities via inhibition of prostaglandin E2 are linked by Aloe vera (Nirala, 2020). Herbs play a major role by providing us with alimentary and trace elements. Chemicals having medicinal value are also gained through therapeutic plants (Evanjelene and Velu, 2021). Several diseases are treated by many medicinal plants with about 350 herbal preparations Local awareness about the uses of medicinal plants has been widely accepted all over the world (Wangchuk et l., 2017).

The aim of the present study is isolation and preparation of the plant extracts, determination of their phytoconstituents and investigation of their biological and chemical properties. The results from this study will enable us to add new information to existing knowledge about the cost-effective treatment of different ailments with medicinal plants.

2. METHODOLOGY

2.1 Collection and extraction of plant samples

Fresh leaves of therapeutic plants *Aloe barbadensis* (Aloe vera), *Azadirachta indica* (Neem), *Moringa oleifera* (Horseradish tree), *Psidium guajava* (Guava) and *Syzygium cumini* (Jambolana), were collected from Kot Addu, Pakistan. The samples were air dried for 7-14 days and ground to fine powder. Plant extract was prepared in 50% ethanol (v/v) using following methods.

2.1.1 Percolation-assisted extraction technique (PAE)

For percolation-assisted extraction, 10 grams of fine powder of each sample and fresh *Aloe barbadensis* gel was infused in 50% ethanol for 24 hours at 37°C under constant shaking (120 rpm). Using Whatman's Filter paper no. 4, filtrate was collected and concentrated using rotary vacuum at 40°C. Concentrated filtrate was stored in a cool, dry place at 4°C (Yasmeen and Hassnain, 2016).

2.1.2 Sonication-assisted extraction technique (SAE)

For sonication-assisted extraction, fine powder of each sample and fresh *Aloe barbadensis* gel were placed in a sonication machine at 20 KHrtz for 60 minutes at 30°C. The filtrate was collected and concentrated followed by storage at cool and dry place under refrigeration conditions (Yasmeen and Hassnain, 2016).

2.2 Qualitative phytochemical analysis

Extracts were subjected to qualitative phytochemical tests. Change in color or appearance of precipitate was the indication for positive results. These include tests for alkaloids, anthocyanin, carbohydrates, cellulose, flavonoids, protein, quinone, starch, steroids and terpenoids (Ofongo et al., 2021; Ahmad, 2020; Rao and Anisha, 2018; Gayathri and Kiruba, 2014).

2.3 Antioxidant activity

2.3.1 Ferric reducing power assay (FRAP Assay)

Ferric reducing power assay was carried out by mixing 2ml of plant extract solution (20-100mg/ml) with 1ml phosphate buffer (0.2 M, pH 6.6) and 1ml of 1% potassium ferricyanide following incubation for 20 min at 50°C. After incubation, 1ml of 10% trichloroacetic acid was added and centrifuged at 3000 rpm for 10 min. One ml of supernatant was diluted with 1ml of distilled water and 0.5ml of 0.1% $FeCl_3$ was added. After 10 min of incubation, absorbance was measured at 700nm. Standard (ascorbic acid), blank and negative controls were also run along the experiment.

2.3.2 Antioxidant activity by scavenging of DPPH radicals

DPPH radical-scavenging assay was performed using the method outlined by Shabbir et al (2013). Stock solution was prepared by mixing 2.4 mg DPPH in 100 ml absolute methanol and stored at -20°C. Aliquot of 0.01ml of plant extracts from each concentration (20-100µg/ml) was added in 3ml of DPPH working solution. Ascorbic acid and trolox were used as reference compounds (positive controls) while negative control (not having extract) was also taken. After 15min of incubation in dark, absorbance was determined at 517 nm. Radical scavenging activity was calculated by the formula: (control absorbance – sample absorbance) / control absorbance x100).

2.3.3 Hydrogen peroxide scavenging assay

Hydrogen peroxide scavenging assay was performed according to Shabbir et al (2013). For hydrogen peroxide scavenging assay, working solution of hydrogen peroxide of 2mM was prepared by mixing phosphate buffer (5mM) pH 7.4. Different concentrations of plant extracts (20-100 µg/ml) were prepared and aliquot of 0.1ml of plant extract from each concentration was added along with 0.4ml of phosphate buffer (50mM) followed by 0.6ml of hydrogen peroxide (50mM). After 10 minutes, absorbance was calculated at 230 nm using the formula for % of H_20_2 scavenging activity= (1-Absorbance of sample /Absorbance of control x100).

2.4 BIOLOGICAL PROFILING

2.4.1 Determination of Glucose Uptake Capacity by Yeast Cells

The assay was performed according to Rehman et al (2018). Instant yeast 0.2g was dissolved in 20ml distilled water to prepare 1% of yeast suspension (incubated overnight at 37°C). The yeast suspension was centrifuged at 4200 rpm (5 minutes), pallet was suspended in sterile distilled water and 10% yeast cell suspension was obtained. 1-5mg/ml w/v ethanolic plant extract was prepared and mixture was supplemented with (5mM, 10mM, and 25mM) of glucose concentrations (incubated at room temperature for 10 minutes).

To initiate the reaction, 100µl of yeast cell suspension was added to each tube having glucose solution followed by extract and incubated at 37^0C for 1 hour. After 60 min of incubation, centrifuged at 3800rpm for 5 min. The amount of glucose was estimated at 520nm. Negative control having all chemicals except extract absorbance was recorded at the same wavelength (520nm). The formula used for percentage increase in glucose acceptance by yeast cells= (Absorbance of control – Absorbance of the sample) / Absorbance of control) x100.

2.4.2 Anti-hemolytic assay

The assay was performed with some modifications in method described by (Yasmeen and Hassnain, 2016; Bhat et al., 2019; Shabbir et al., 2013). 3ml of a healthy person's blood was

collected in EDTA tube, centrifuged at 1500 rpm for 3 min. Pellet was washed 3-4 times with sterile phosphate buffer saline (PH 7.4) until light pinkish supernatant obtained. Pellet was suspended in a normal 0.5% saline solution. 0.5ml volume of plant extract were added in 0.5ml of cell suspension. Mixture incubated at 37°C for half an hour. After incubation of 30min, mixture was centrifuged at 1500rpm for 10min. Control made by addition of 1ml of the cell suspension to 1ml of distilled water. Ascorbic acid was used as reference compound. The absorbance was recorded at 540nm. Inhibitory activity was calculated by formula: % age hemolysis = (Absorbance of control − Absorbance of the sample) /Absorbance of control x 100).

2.4.3 In vitro anti-lipid peroxidation Assay

Anti-lipid peroxidation assay was performed with some modifications in method of Shabbir et al (2013). Freshly excised liver of chicken 10x homogenous was prepared in cold phosphate buffer saline having PH 7.4. Different concentrations of plant extracts (20-100µg/ml) were made from stock solution (1 mg/ml) of the ethanolic extract. Extract and each fraction were added in 100ul of ferrous sulfate (15mM) followed by the addition of 3ml of the mixture (homogenate). The mixture was incubated for 30mins. 0.1ml of the reaction mixture was added to 1.5ml of 10% trichloroacetic acid, mixture was incubated for 10minutes. Filtrate was centrifuged, supernatant was collected and 1.5ml of 0.67% of thiobarbituric acid (TBA) made in 50% acetic acid. Mixture was incubated for 30 mins. Change in color of the mixture indicated the peroxidation of lipids. Absorbance was recorded at 535nm. The formula used for anti-lipid peroxidation was calculated as % inhibition = (Absorbance of Control-Absorbance of test/Absorbance of control x100).

2.4.4 Sugar estimation test

Freshly prepared plant extracts were homogenized and treated with a few drops of concentrated sulphuric acid. Incubated at room temperature (4hours). Absorbance checked at 420nm (Sadat et al., 2021).

2.4.5 Antimicrobial Assay of therapeutic plants

Antimicrobial activity was performed with some modifications in the protocol of (Sadat et al., 2021; Preethi et al., 2010). Activity of plant extracts was checked for *Escherichia coli*, *Pseudomonas aeroginosa*, *Salmonella typhi* and *Staphylococcus aureus*. Standardized culture of bacteria was inoculated by swabbing and 50µl of various extracts were added to the wells. The results were checked for growth inhibition after 24 hours of incubation at 37^{o}C.

2.5 CHEMICAL PROFILING

2.5.1 Thin layer chromatography

Thin-layer chromatography was performed following the method of Algfri et al., 2021 and Cavin et al., 1998. TLC of ethanolic extracts was performed with two separate solvent systems namely chloroform-methanol-formic acid (22:1.8:1.2) and butanol-acetic acid-distilled water (20:25:5). 20µl of plant extract was applied to the TLC plate. Ascorbic acid was used as a positive control. TLC spots were observed at 254nm and 366nm and results were recorded.

$$\mathrm{RF} = \frac{\text{Distance travelled by solute}}{\text{distance travelled by solvent}} \text{ X } 100$$

2.5.2 FT-IR Analysis of therapeutic plant extracts

Fourier-transform infrared spectroscopy was used for the conformation of the functional groups in *Aloe barbadensis*, *Azadirachta indica*, *Moringa oleifera*, *Psidium guajava* and *Syzygium cumini*. The range of wavenumber was calculated from 4100 to 500 cm^{-1}.

3. RESULTS

3.1. Qualitative analysis of phytochemicals

Phytochemical study of ethanolic extract of therapeutic plants was done which revealed the presence of different phytoconstituents such as alkaloids, flavonoids, steroids, terpenoids, quinone, starch, anthocyanin, protein, carbohydrates, and cellulose. Anthocyanin was absent in all plant extracts while terpenoids, steroids, quinone and alkaloids were fully absent in some therapeutic plant extracts.

3.2 Antioxidant Activity

3.2.1 Ferric reducing power Assay (FRAP Assay)

The maximum activity was revealed by *Psidium guajava* leave extract and the least reducing power was shown by *Moringa oleifera* leave extract at all concentrations from 100-20(µg/ml) for sonication assisted extraction but results were slightly different for percolated extracts as *Syzygium cumini* showed higher reducing power than all other extracts but *Moringa oleifera* in both sonicated and percolated extracts revealed less reducing power. Reducing power of *Psidium guajava* and *Syzygium cumini* was more than ascorbic acid reducing power at all concentrations. *Moringa oleifera* have less reducing power than standard reducing power values. Extracts obtained through percolation-assisted extraction revealed less reducing power than ascorbic acid reducing power when compared at all concentrations (Figure 1).

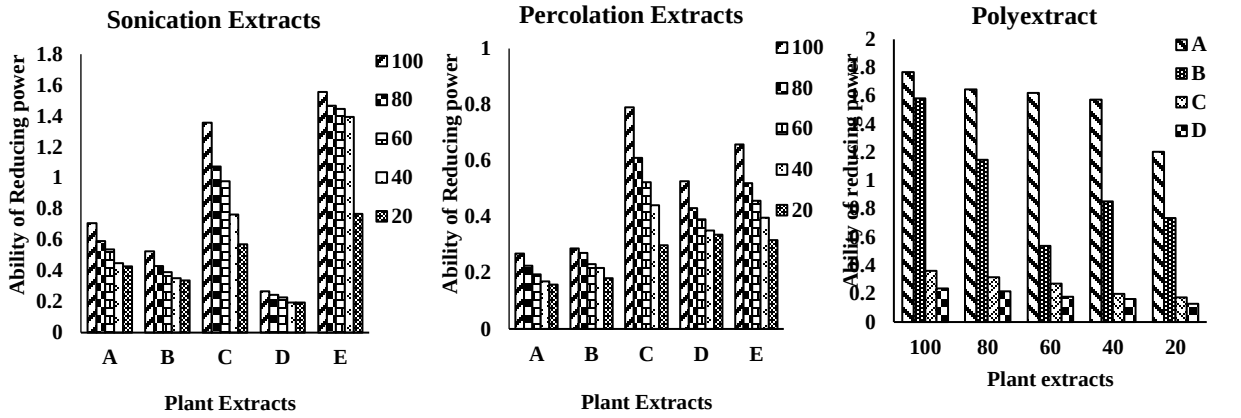


Figure 1 FRAP assay of therapeutic plant extracts.

3.3. Biological Activities

3.3.1 Antimicrobial Assay

The antimicrobial activity was checked against *Staphylococcus aureus* and others *Escherichia coli, Pseudomonas aeroginosa, and Salmonella typhi*. Percolated extracts showed a higher diameter of zone of inhibition than sonicated plant extracts for *Staphylococcus aureus*. While sonicated plant extracts showed a higher diameter of zone of inhibition for *Escherichia coli, Pseudomonas aeroginosa, and Salmonella Typhi*. Higher zones of inhibition were observed in case of *Syzygium cumini* sonicated extracts compared to other sonicated extracts as indicated in figure 2

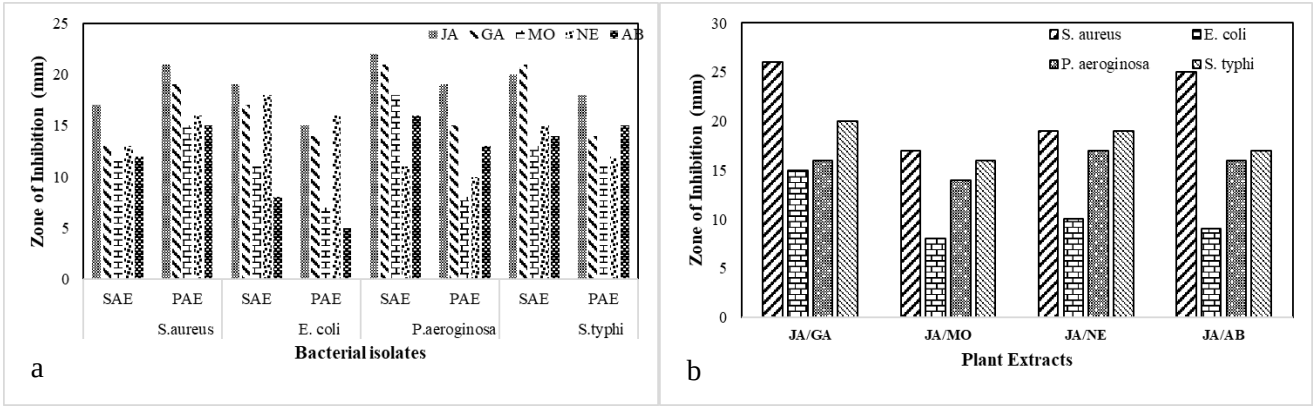


Figure 2: A) The antimicrobial potential of sonication assisted extracts (SAE) and Percolated (PAE) extracts of plants on selected clinical bacterial strains. B) Effect of polyextracts on clinical bacterial isolates.

3.3.2 Anti-hemolytic Assay

Syzygium cumini extract has higher percentage inhibition of lysis of red blood cells at all concentrations than other sonicated plant extracts except guava extract at 500µg/ml displayed 81.76% higher than the standard value 37.19% and *Syzygium cumini* extract value which was recorded as 72.86%. *Moringa oleifera* extracts has the least percentage 50.11% inhibition of lysis

of red blood cells at higher concentration. The polyextract result of *Syzygium cumini + Azadirachta indica* was higher than all other sonicated poly extract extracts at all concentrations. *Moringa oleifera + Syzygium cumini* combined result showed the least percentage inhibition as indicated in figure 3.

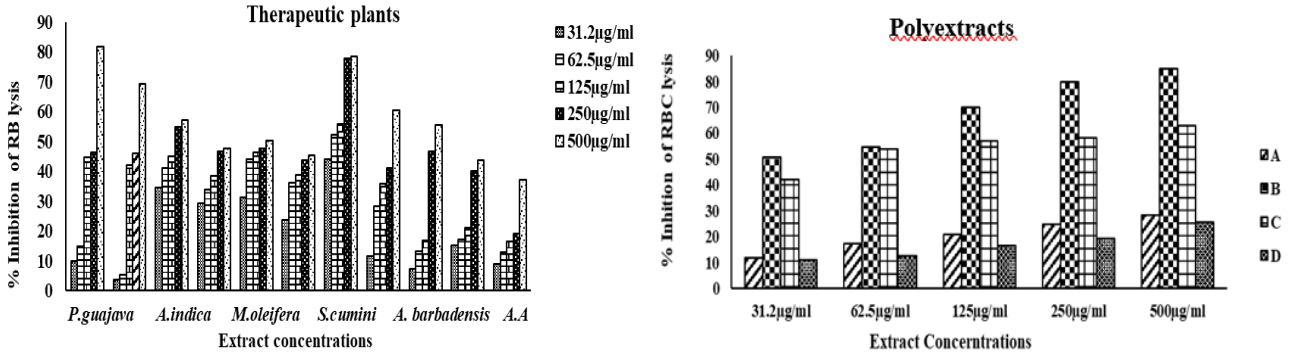


Figure 3. Antihemolytic Assay revealed by plant extracts.

3.3.3 Antidiabetic Assay

Glucose uptake by yeast cells at 5mM concentration of glucose in the presence of sonication assisted extracts showed a higher value of glucose uptake compared to 10Mm and 25mM. Sonication-assisted extracts of therapeutic plants had more glucose uptake capacity by yeast cells than percolated extracts. Sonicated extract of *Aloe barbadensis* at all concentrations showed higher glucose uptake. Higher glucose uptake capacity by yeast cells was observed in the presence of sonicated poly extracts of *Aloe barbadensis +Moringa oleifera* > *Aloe barbadensis + Psidium guajava* > *Aloe barbadensis + Syzygium cumini* > *Aloe barbadensis + Azadirachta*.

3.3.4 *Invitro* anti lipid peroxidation assay

Moringa oleifera inhibited higher lipid peroxidation at 100µg/ml as 52.9% while percolated extract value was 50.1% recorded. At 20µg/ml its value was 23.7% higher than percolated extract value 17.5%. While *Aloe barbadensis* showed poor anti-lipid peroxidation (4.3%). All selected therapeutic plant extracts proved to be significant in anti-lipid peroxidation as shown in figure 4.

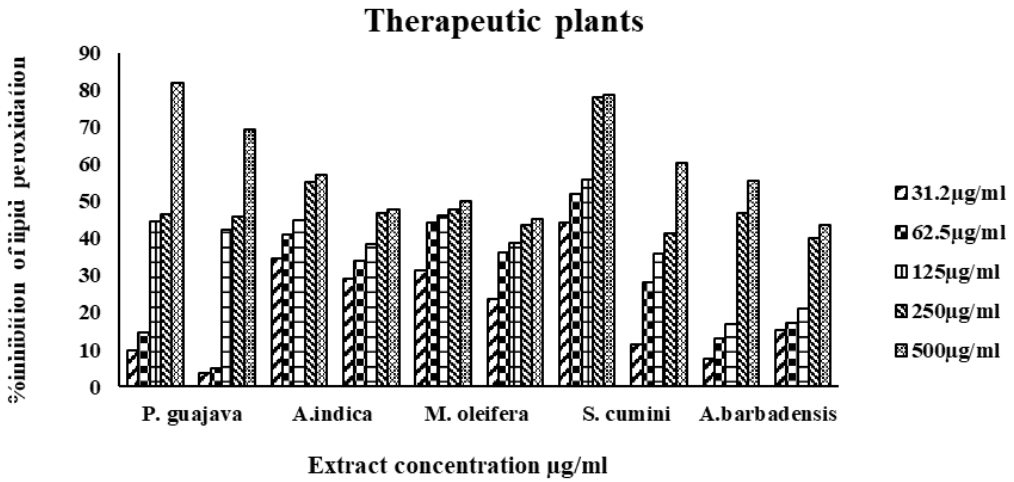


Figure 4. The *In vitro* lipid peroxidation activity by therapeutic plant extracts.

3.3.5 DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate) assay

The highest activity was observed by the *Psidium guajava* extract. The least scavenging activity was shown by the *Azadirachta indica* extract. When sonicated extracts scavenging activity was compared with standards scavenging activity, *Psidium guajava* sonicated extract gives similar DPPH radical scavenging activity as Trolox at 0.97µg/ml while all other sonicated extracts at the same concentration give least effective radical scavenging activity than Trolox. Results are indicated in the figure 5.

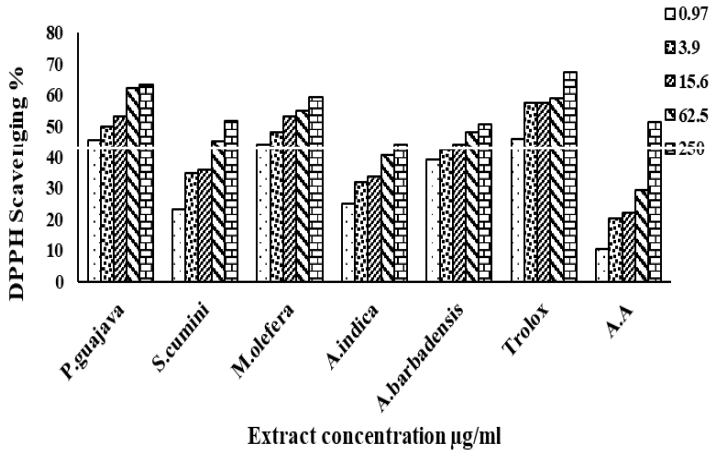


Figure 5. DPPH activity shown by therapeutic plant extracts

3.3.6 Hydrogen peroxide scavenging activity

Sonicated extracts of all medicinal plants showed higher hydrogen peroxide scavenging activity than all percolated plant extracts. *Psidium guajava* extract at 100µg/L has 0.959% while at 20µg/L has 0.866%. *Aloe barbadensis* proved poor hydrogen scavenger at at 100µg/L has a value of 0.486% and at 20µg/L has 0.298% (table 1).

Table.1. H_2O_2 scavenging activity of Sonicated (SAE) and Percolated (PAE) plant extracts

Concentration µg/L	H_2O_2 Scavenging activity (230nm)											
	Aloe barbadensis		*Azadirachta indica*		*Psidium guajava*		*Syzygium cumini*		*Moringa oleifera*		Ascorbic acid	
	SAE	PAE	SAE	PAE	SAE	PAE	SAE	PAE	SAE	PAE	SAE	PAE
100	0.486	0.593	0.692	0.747	0.899	0.954	0.714	0.728	0.799	0.845	0.552	0.592
80	0.431	0.544	0.688	0.792	0.875	0.984	0.701	0.726	0.632	0.756	0.5391	0.596
60	0.379	0.473	0.672	0.712	0.813	0.957	0.483	0.584	0.611	0.693	0.4997	0.657
40	0.344	0.468	0.611	0.640	0.796	0.874	0.439	0.499	0.589	0.645	0.4763	0.613
20	0.298	0.398	0.569	0.639	0.755	0.866	0.395	0.476	0.487	0.612	0.3652	0.452

3.4.1 Thin Layer Chromatography (TLC)

TLC shows separation and indication of phytochemicals. The best solvent system was butanol: acetic acid: distilled water as more clear bands of extracts are formed with more bright spots in this system at 366nm while black spots at 254nm than other chloroform: methanol: formic acid solvent system. Sonicated extracts extensively showed more RF values and colored spots than percolated extracts in figure 6.

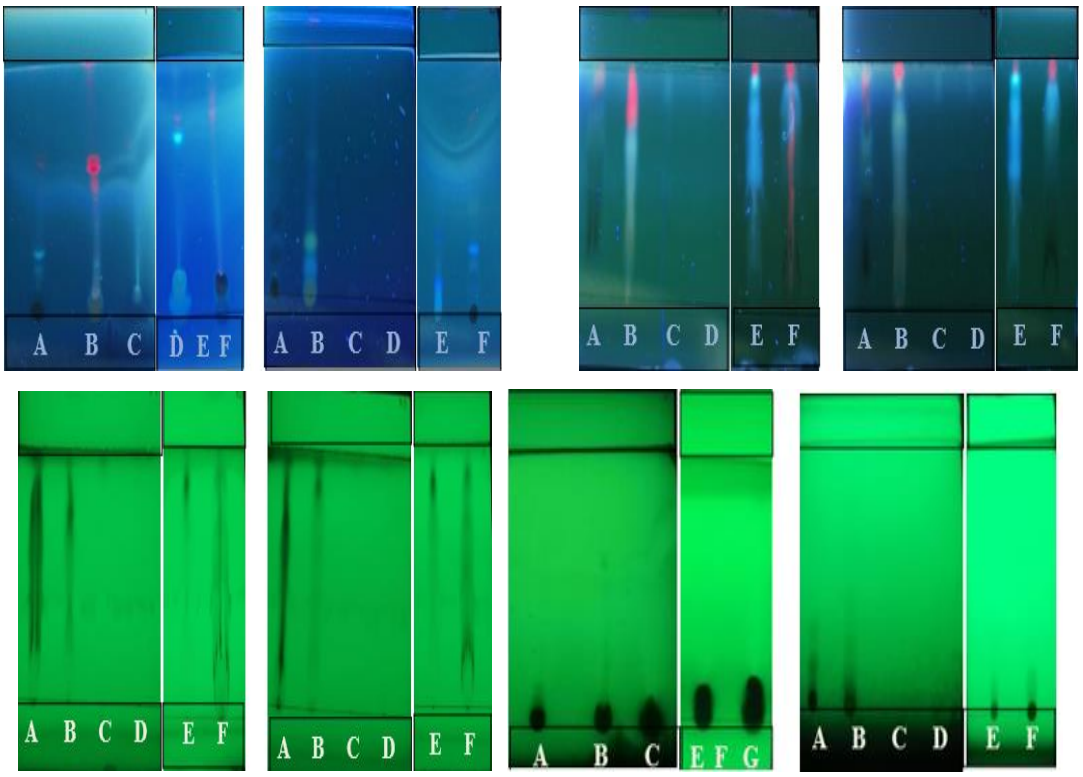
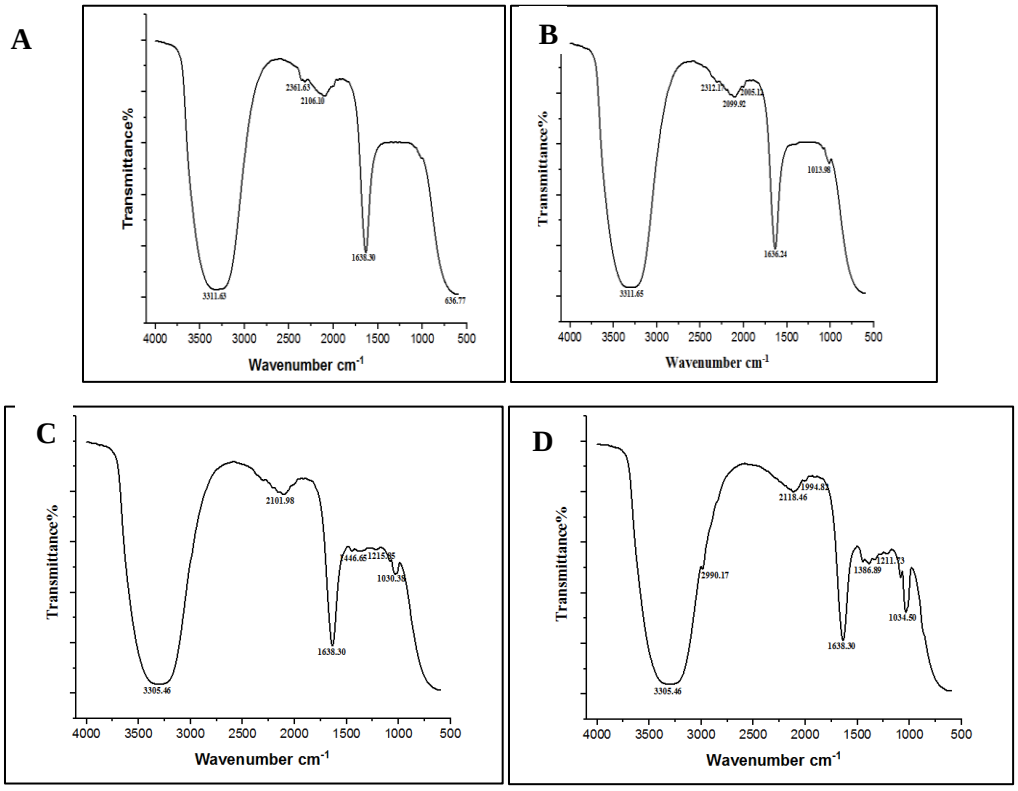


Figure 6: Thin-layer chromatography of selected therapeutic plant extracts along with standard (Trolox) under 365nm UV light and 258nm UV light. The color of the bands indicates the presence of different phytochemicals present in plant extracts. Upper left box sonicated extracts (PG, MO, AB, AI, T, JA) and (PG, MO, AB, AI, T, JA), upper right box percolated plant extracts (PG, MO, T, AB, AI, JA) and (PG, MO, T, AB, AI, JA) and (PG, MO, AB, T, AI, JA) with Chloroform, Methanol, and Formic acid, as a solvent in system. While lower left box (PG, MO, AB, T, AI, JA) and lower right box percolated plant extract (PG, MO, AB, AI, T, JA) and (PG, MO, AB, AI, T, JA) with Butanol: Acetic acid: Distilled water as the solvent system used.

3.4.2 FTIR Analysis

FTIR spectroscopy was used to identify different phytochemicals present in the plant extracts. Amides, Amines, Carboxylic acid, Alcohols, Ethers, Phosphorylated proteins, Aldehydes, Alkanes functional groups yield bands at wavelength 3305, 2105, 1225, 2990 and 1211cm^{-1}. The absorbance peaks in the region 1000-1500 show stretching of C-OH, C-O-C groups. Whereas, those around 2000-2500 due to stretching of –O-H groups. Absorbance peaks at region 1500-2000 were attributed to the -N-C-O groups. Absorbance peaks around 2500-3000 due to stretching of C=O. All peaks absorbed at different wavelengths are shown in figure 8



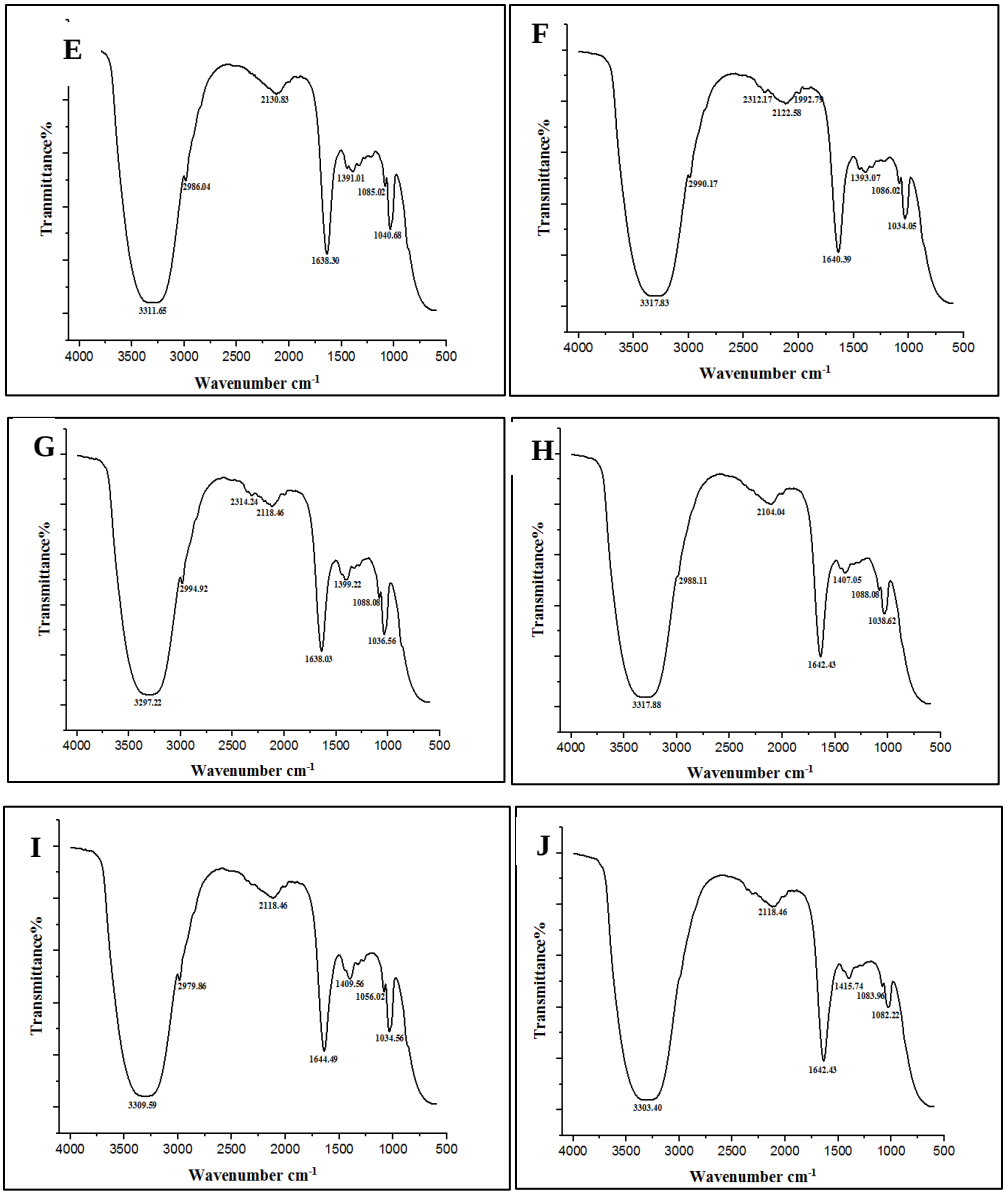


Fig.10. Represents FTIR Spectrum of Therapeutic plant extracts

A= *Aloe barbadensis* sonicated extract, B= *Aloe barbadensis* percolated extract, C= *Psidium guajava* sonicated extract, D= *Psidium guajava* percolated extract, E= *Syzygium cumini* sonicated extract, F= *Syzygium cumini* percolated extract, G= *Moringa oleifera* sonicated extract, *H=Moringa oleifera* percolated extract, I= *Azadirachta indica* sonicated extract, *J=Azadirachta indica* percolated extract.

4. DISCUSSION

Secondary compounds have been used as medicinal agents which impart medicinal properties to the plant. Leaves are the largest organ for the assembly of bioactive compounds. Flavonoids, Tannins, Terpenoids, Glycosides, Steroids and Carotenoids are a few phytoconstituents present in therapeutic plants (Choudhary et al., 2021). The aim of the present study is isolation and

preparation of the plant extracts, determination of their phytoconstituents and investigation of their biological and chemical properties. Locally available remedial plant such as *Aloe barbadensis*, *Azadirachta indica*, *Moringa oleifera*, *Psidium guajava* and *Syzygium cumini* were selected and extracted by two methods Sonication-assisted extraction and Percolation-assisted extraction method in ethanol. Phytochemical screening, antioxidant activity (FRAP), scavenging assays (DPPH, H_2O_2), antimicrobial activity, antidiabetic activity, antihemolytic activity, anti-lipid peroxidation activity was determined using pre-established standard protocols. Chemical properties by Thin layer chromatography and Fourier transform infrared spectroscopy method were determined.

Phytochemicals such are proteins, alkaloids, carbohydrates and starch were present in the leave extract of *Psidium guajava* in lesser quantity than flavonoids, cellulose and quinone but steroids were absent in the leave extracts of *Psidium guajava*, *Syzygium cumini* and *Aloe barbadensis* while extract of *Azadirachta indica* and *Moringa oleifera* showed the presence of steroids in greater quantities. The results of *Azadirachta indica* in this study correlate with (Ogidi et al., 2021) another study which reported the presence of alkaloids and steroids in *Azadirachta indica* leaf extract. Maybe a change of solvent or extraction method may help the isolation of other phytochemicals from extracts. The present study revealed the presence of alkaloids whereas, according to (Gayathrii and Kiruba, 2014) alkaloids were absent while many results are in accordance to (Gayathri and Kiruba, 2014) where proteins and quinones were present on ethanolic solvent and anthocyanins were absent same as in this study. A reducing power assay (FRAP) was employed to determine the antioxidant activity of therapeutic plants. In this activity *Psidium guajava* showed highest reducing power (1.6%) whereas least (0.8%) was revealed by *Moringa oleifera*.

Almost all extract gave antimicrobial activity but for *Staphylococcus aureus*, percolated extract of *Syzygium cumini* showed higher inhibitory activity but the least was shown by *Moringa oleifera* extract. For *Pseudomonas aeroginosa* sonicated extract of *Syzygium cumini* gave a high zone of inhibition while the least by *Azadirachta indica* sonicated extract. For *Escherichia coli* maximum zone of inhibition was given by Syzygium cumini sonicated extract but least by *Aloe barbadensis* extract. *Psidium guajava* sonicated extract gave the highest zone of inhibition of all the extracts for *Salmonella typhii* while the least was by *Moringa oleifera* extract. Higher zones of inhibition were given by *Syzygium cumini* sonicated extracts than all other sonicated extracts. When plant extracts were applied gram-positive bacteria *Staphylococcus aureus* was more susceptible and gave more zone of inhibition than gram-negative bacteria *Escherichia coli*, *Pseudomonas*

aeroginosa and *Salmonella typhi*. As gram-positive bacteria have a single layer as compared to gram-negative bacteria which have a multi-layered structure covered by an outer membrane. The cell wall of gram-positive is also thicker than gram-negative bacteria. All plant extracts used in this study have many secondary compounds which also play role in inhibiting the growth of bacteria. The same condition is reported earlier (Choudhary et al., 2021). In this study as antimicrobial activity was checked against *Escherichia coli*, *Pseudomonas aeroginosa* and *Staphylococcus aureus* by therapeutic plants. The same bacteria were susceptible to neem extract according to previously reported results (Ali et al., 2021).

In antihemolytic activity *Psidium guajava* extract at higher concentration had higher inhibition of RBS lysis (81.76%) while *Moringa oleifera* extract has least (50.11%). The synergistic results came as *Syzygium cumini* + *Azadirachta indica* > *Syzygium cumini* + *Aloe barbadensis* > *Syzygium cumini* + *Psidium guajava* > *Syzygium cumini* + *Moringa oleifera* at all plant extract concentrations. This combination proved good anti-hemolytic inhibitors. Phytochemicals present in the plant extracts include flavonoids, tannins especially polyphenols which destroy and scavenge the reactive oxygen species present in the abnormal cells to inhibit the damage by restoring the normal function of the cells (Li et al., 2016).

Glucose uptake by yeast cells at 5mM concentration of glucose in the presence of sonication assisted extracts showed a higher value than at 10Mm and 25mM. In antidiabetic activity higher glucose uptake by yeast cells (36%) was observed in the presence of *Aloe barbadensis*. While in the presence of *Psidium guajava* sonicated extracts least glucose uptake capacity was shown by yeast cells. More glucose uptake by yeast cells was seen by synergistic sonicated extracts of *Aloe barbadensis* + *Moringa oleifera* > *Aloe barbadensis* + *Psidium guajava* > *Aloe barbadensis* + *Syzygium cumini* > *Aloe barbadensis* + *Azadirachta indica* at all concentrations of plant extracts and glucose (Ali et al., 2021).

Many diseases are caused due to lipid peroxidation including cancer, heart diseases and many other infections in the body. Diseases due to oxidative stress can be controlled by taking antioxidant supplements. Polyphenolic antioxidants which have a positive effect on the human body are present in significant amounts in fruits and vegetables (Kedlaya and Vasudevan 2004). In anti-lipid peroxidation activity *Moringa oleifera* inhibited lipid peroxidation (52.9%) while *Aloe barbadensis* showed poor anti-lipid peroxidation (27.2%).

In scavenging assay (DPPH) *Psidium guajava* revealed absorbance of (63.44%) while *Azadirachta indica* was poor scavenger (43.94). The results of the assay greatly depend upon the amount of

phenol present in the plant extracts. As phenol has a free hydroxyl group that scavenges free radicals. When polyphenol and tannin content increase then DPPH scavenging activity becomes effective. The antioxidant property is directly related to phenol. The results are following the results of (Rubavathi et al., 2020) because DPPH activity is dose-dependent.

Hydrogen peroxide when generates hydroxyl radicals then it becomes harmful for cells because hydroxyl radicals act as free ROS which damage the cellular system. Although hydrogen peroxide itself is not considered much injurious to the cells in the human body (Shabbir et al., 2013). In hydrogen peroxidation assay Psidium guajava proved more scavenger (0.959%) while *Aloe barbadensis* proved poor hydrogen scavenger (0.486%).

Sonicated extracts extensively showed more RF values and colored spots than percolated extracts. Colored spots formed indicate different phytochemicals which are according to (Algfri et al., 2021). In their study chloroform: methanol: formic acid was considered the best solvent system while in our study butanol: acetic acid: distilled water proved to be the best solvent system same as reported earlier by (Yasmeen and Hassnain, 2015).

FTIR spectroscopy was used to identify different phytochemicals present in the plant extracts. Amides, Amines , Carboxylic acid, Alcohols, Ethers, Phosphorylated proteins, Aldehydes, Alkanes functional groups yield bands at wavelength (3305,2105,1225,2990,1211) cm^{-1}. Many phytochemicals were analyzed by FT-IR analysis almost same phytoconstituents are present in sonicated and percolated plant extracts. In the extract of *Syzygium cumini* more peaks developed as compared to other plant extracts. This shows *Syzygium cumini* leaves are bucket of phytoconstituents (Li et al., 2016).

In vitro research revealed many phytochemicals present in these therapeutic plants which are having anti-diabetic, anti-hemolytic, antibacterial and antioxidant properties. The synergistic effect of plant extracts was also studied. All synergistic extracts gave more antidiabetic, anti-hemolytic and antimicrobial activity so to obtain more clear and best results one should use a combination of therapeutic plant extracts. Many human diseases are treated with the help of newly formed natural drugs.

5. CONCLUSION

Proper extraction and purification procedures help to access active molecules from plants that are used in drug formation. In the study leaf extract of all plants showed good antioxidant properties and medicinal properties so clinical trials should be conducted for utilization of them in herbal

products formulation. The present study revealed that *Aloe barbadensis*, *Azadirachta indica*, *Moringa oleifera*, *Psidium guajava* and *Syzygium cumini* can be likely candidates to be explored in the future.

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