Physical Education, Health and Social Sciences

https://journal-of-social-education.org

E-ISSN: 2958-5996
P-ISSN: 2958-5988

Structural Characterization, Antioxidant, Antidiabetic and Antimicrobial Activities of Cynodon Dactylon, Arundo Donax, Alstonia Scholaris Linnand Plumeria Rubra Linn.

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DOI: https://doi.org/10.63163/jpehss.v3i2.317

Abstract

Numerous plants were being used in conventional treatment and for cure of many diseases along with maintaining health from ancient times. Cynodon dactylon, Arundo donax, Alstonia scholaris linn and Plumeria rubra linn have great medicinal importance due to their antioxidant, antidiabetic, antimicrobial, antirheumatic or antihyperlipidemic activities. In this study, extract of aerial parts of C. dactylon , Arundo donax, Alstonia scholaris and Plumeria rubra were prepared in three different solvent via ethyl acetate, methanol and aqueous. Extracts were further processed in order to assess antioxidant, antidiabetic and antimicrobial activity of C.dactylon, A.donax, Alstonia scholaris and Plumeria rubra. Antioxidant activity was assessed by TPC, TFC and DPPH scavenging assay while antidiabetic activity was assessed by Alpha amylase inhibition and glycation analysis. Minimum inhibitory concentration was performed for the determination of antimicrobial activity. Structural characterization were done by Fourier transform infrared spectroscopy (FTIR) and High-performance liquid chromatography (HPLC).Highest yield was obtained by the aqueous extract of P. ruba (10.47%) while lowest yield was obtained by ethyl-acetate extract of C.dactylon and A.donax (0.652%).Highest TPC was observed in Arundo donax aqueous extract (681.27±0.40mg GAE\100g dry weight) while lowest was observed by aqueous extract of Cynodon dactylon (270.83±0.40mg GAE\100g DW). Highest TFC was observed in A. scholaris ethyl acetate extract (807.36.69±0.66mg CE\100g dry weight) while lowest was observed by ethyl-acetate extract of Arundo donax (26.96±0.37mg CE\100g DW). Similarly highest DPPH radical scavenging activity was observed in ethyl acetate extract of P.ruba (77.84±0.33%) while lowest in aqueous extract of A.scholaris (16.96±0.22%). Arundo donax showed highest zone of inhibition against Escherichia coli (15.1±0.94mm) while P.ruba showed lowest zone of inhibition against Staphylococcus aureus (6.0±0.40mm). Highest antiglycation activity was observed in methanolic extract of A.donax (70.20±0.76%) while lowest was observed in ethyl- acetate of C.dactylon (28.40 ±0.15%). Best alpha-amylase activity was observed in methanolic extract of A.scholaris (61.14±0.21%).FTIR analysis of selected plants showed the presence of compounds like alcohol, phenol, carboxylic acid, primary and secondary amides and amines, alkanes, sulphonyl , sulphones, nitro groups, alkyl-halides ,aldehydes, methyl and methylene groups, fluorides, chloride ,alkenes, aromatic compounds ,ether ,ester and anhydrides were observed. HPLC analysis showed the presence of gallic acid, quercetin, caffeic acid, vanillic acid, m-coumaric acid, p-coumaric acid, sinapic acid syringic acid, ferulic acid, benzoic acid and chlorogenic acid. Data was analyzed statistically by using one-way ANOVA and hence it was concluded that a significant difference ($p<0.001$) was present among extracts made by using different solvents in term of all biological activities.

Keywords: Total phenolic content (TPC), Total flavonoid content (TFC), DPPH radical scavenging assay, Antiglycation activity, alpha-amylase inhibition, High performance liquid chromatography (HPLC), Fourier-transform infrared spectroscopy (FTIR).

Introduction

From prehistoric time medicinal plants were an essential component of our life. Man has used plants for the fulfilment of basic food requirement and as therapeutic agents. Classical treatment procedures are well-known worldwide. Traditional medication has been applied within Egypt to treat various human and livestock ailments (Jima and Megersa, 2018). According to WHO (World Health Organization), 80% of the world's population largely depend on herbal medication or utilization of plant extracts or their active compounds to cure diseases The use of plants and their products as food and drug sources is increasing as they are cheap specially for people of developing countries (Hussain et al., 2016), easily accessible and contain secure and safe biologically active compounds and have no or less side effects on humans (Duraiswamy et al., 2016).

Cynodon dactylon belongs to family poaceae that is being used by both herbal and traditional system to cure different diseases. It is native to tropical and subtropical regions. C.dactylon comprises numerous phyto-consitituents such as flavonoids, carotenoids, phenolics, phytosterols, glycosides, saponins and volatile oils. For screening of phytochemical compounds different parts of plants are used like roots, stem, leaves and bark (Khatun et al., 2020). Cynodon dactylon roots, stalk or leaves, each has antidiabetic as well as antimicrobial activity (Mishra et al., 2021). Thus, according to preclinical studies leaf extract of C. dactylon affects the quantity of peroxidation that has an oxidizing impact on COLO 320 DM cells, a colon cancer cell line (Savadi et al., 2020). C.dactylon has been used to cure tumor, pneumonia, spasms, cholera, pyrexia, migraine, hypoxia, influenza, hematoma, syphilis or insect bites. In Unani system C.dactylon could be used as cough suppressant and stimulant for grippe children in pain inflammation (Khatun et al., 2020).

Another plant is Arundo donax L. which have important medicinal and economical benefits like bioenergy, paper and cellulose production. In many countries aerial parts of Arundo donax are used for fuel purpose. It is used to treat dermal, bowel, neuromuscular, reproductive, and pulmonary or bladder disorders. Phytochemical studies have identified alkaloids, sterols, terponids, phenolic and lignin. Isolated constituents of A.donax exhibit potent antibacterial and antioxidant activities. It is also used in helminthic infection in cattle's (Kumar et al., 2021). The antibacterial activity of Arundo donax L. extracts were evaluated using disc diffusion assay against yeast C.albicans and Gram-positive (S.aures, B.cereus, and B.subtilis) Gram-negative, E.coli (Pansuksan et al., 2020).

Alstonia scholaris common name "devil tree" has been widely spread and utilised for its medicinal and ethnomedicinal properties since prehistoric days. Alstonia scholaris (L) R Br, a plant from the Apocynaceae family. It is an important medicinal plant in Ayurveda (Arulmozhi et al., 2007). Plant's decoction has been used historically for tonic, reasons. Asthma, bronchitis, cardiopathy, helminthiasis, agalactia, debility, diarrhea, dysentery, dyspepsia, leprosy, tumors, chronic and unpleasant ulcers. Additionally, it helps with tumors, pruritus, and skin issues (Baliga et al., 2012).

The fast-growing huge tree Plumeria rubra is a tropical and subtropical plant native to Central Asia. It's a lovely decorative shrub that grows in cemeteries, gardens, and parks. The plant contains bioactive components like flowers containing resin, quercetin, and kaemforol. Fresh leaves contain plumeride resinic. Bark contains fulvoplumerin. Plumeria rubra Linn used in the treatment of many diseases like itching, wounds, pain, toothache, antifertility, leprosy, acne, inflammation, earache, tongue cleaning and asthma. The milky liquid also has anti-viral activities and very effective against scabies (Khan et al., 2018). Plumeria rubra has antifertility, antioxidant, antimicrobial, anti-inflammatory, antitumor, antiviral and hypoglycemic activities (Bihani, et al 2021).

The objective of current study was to evaluate the difference in the type of bioactive compounds present in the extract obtained using polar and non-polar solvents and their distinguishing roles in activities observed under this study. Structural characterization was done

in order to assess the type of phytochemicals responsible to perform antioxidant, antidiabetic and antimicrobial activities. Comparison of antioxidant, antidiabetic and antimicrobial activities of methanolic, aqueous and ethyl-acetate extract of Cynodon dactylon, Arundo donax, A.scholaris and P.ruba were done for considering the one with better performance.

Materials and Methods

Preparation of plant extracts: Aerial parts of Cynodon dactylon, Arundo donax, A.scholaris and P.ruba were dried and the ground to fine powder. Ethyl-acetate, methanol, and aqueous solvents were used for extraction process. Plant powder and solvent in case of ethyl acetate and methanol were taken in1:5 ratio while 1:10 ratio was used for aqueous extraction. The extraction was taken place at room temperature by using maceration process. Finally the prepared extract was transferred into falcon tubes and were kept for further experiment work (Noreen et al., 2020).plant yield will be calculated as follow:

Percentage Yield = (Extraction gram/ Plant sample (g) × 100 **(1)**

Antioxidant activity**:** The antioxidant activity of selected plants were evaluated by the following described methods. The phenolic content was determined using the Folin-Ciocalteu reagent. Add 250 µL distilled water and 25 µL samples in test tube. Then added Na_2CO_3 solution (100 µL) and incubated for two hours at room temperature. At 750 nm, the absorbance was measured. The results were expressed as mg GAE/100g dry weight. Total flavonoids content was determining by using AlCl3 colorimetric. Sample (38µL each), $NaNO_2$ (9.5 µL) and 156µL distilled water were incubated at room temperature 10 minutes. Then 9.5µL NaNO2, 19µL of 10% $AlCl_3$ were added and again incubated for almost 5 minutes. The absorbance at roughly 510 nm was measured and the results were expressed as mg CE\100g dry weight. The antioxidant activity was measured in term of 2, 2-diphenyl-l-picrylhydrazyl (DPPH) scavenging abilities. 250 µL DPPH solution (prepared by adding 0.004mg of DPPH in 100mL ethanol) was added in 2.5 µL sample were incubated for 30 minutes at room temperature. Took absorbance at 520nm and then used to calculate radical scavenging activity as: IC_{50} (half-maximal inhibitory concentration; %) = (Absorbance blank –Absorbance sample/Absorbance blank) ×100 (Hussain et al., 2021).

% DPPH scavenging = A (control) –A (sample) /A (control) ×100 **(2)**

Well-diffusion method: A well-known agar well diffusion method was used to determine the antibacterial potential of Cynodon dactylon , A.donax, A.scholaris and P.ruba leaves extracts against gram positive and gram negative bacteria by using nutrient agar as culture media. Sample was prepared by taking 5mg of methanolic extract in 1mL DMSO in an Eppendrof tube. Gel for Bacterial growth was prepared. 8.4g Oxide TM agar in 300mL distilled water in two separate flasks. The gel mixture and petri plates were autocalved.The temperature of agar solution was cooled down to 40°C then 100µL of E.coli was inoculated in one flask and S.auerus was inoculated in other flask. Bacteria containing agar solution was then poured into two petri plates in laminar air flow and was allowed to solidify. After the gel was solidified three wells were creating by using tip (1mL) of micropipette .100µL of both samples were poured into two wells in both plates and Central well was taken as positive control and filled with 100µl of ciprofloxacin. Then plates were incubated in incubator for 24 hours at 37°C. Inhibition zone were measured with the help of measuring scale. (Haq et al., 2018; Ahmed et al., 2020).

Minimum inhibitory concentration (MIC): First the methanolic extracts of selected plants were prepared at a ratio of 20 mg/mL in DMSO. Then the each well was loaded with sample and serial dilution was done. After that bacterial strain of E. coli was added in each well except the 7^{th} and 8^{th} well in each row of 96- well micro-titer plate which served as positive and negative control respectively. Incubate the 96- well micro-titer plate in broth microdillution for 20hrs.After that, add 9µL of dye in each well of the plate and incubate at 37°C for 2 hours.

Minimum inhibitory concentration was determined by blue color to purple or pink colour and compared the colorimetric analysis with the visual turbidity. Tests was performed in triplicates and got the average values of MIC (Wiegand et al., 2008).

Antidiabetic activity: Antiglycation potential was determined by taking 150µL of extract, 100 mg of D-glucose, and 10 mg of bovine serum albumin (BSA) in 67Mm sodium phosphate buffer (PH 7.2), was maintained at 37 °C for two days. Then, a spectrophotometer was used to measure the absorbance of 0.2 mL of diluted reaction solution at 370 nm and 440 nm, respectively. The reaction mixture without D-glucose was used as blank solution. Niacinamidewas used as reference compound (Hussain et al., 2021).

Calculation of Percentage inhibition (%) = A (400)/ A (370)-A (440) ×100 **(3)**

In alpha- amylase inhibition assay was performed according to Hussain et al., (2021).In 96 well-plates took 0.01g bacterial alpha amylase in 10mL phosphate buffer. Added 30µL of samples in above solution and incubated it for 10 minutes and then added 40µL starch and again incubated for 30 minutes. 20.0 µL of 1 M HCl and75µL of iodine solution were also added. The absorption was taken at 580nm.Percetage inhibition was estimated as:

Percentage inhibition%= 1-A (control)/ A (sample) ×100 **(4)**

Structural Analysis: HPLC is chromatographic technique with great analytical power and versatility which can be used for compounds having liquid solubility that could be utilized as mobile phase for HPLC. It is also used to quantify, separate and purify macromolecules (Manju et al., 2016). Structure characterization of selected plants leaves were determined by FTIR. This is most suitable technique for structural and functional group identification (Iqbal et al., 2018).

Results

Extraction yield: Preparation of extract was done via maceration process and were transferred into falcon tubes and were kept for use further experiment work (Noreen et al., 2020).Different solvents have different resolving strength towards the both plants constituents which resulted in different yield as shown in table 1. Percentage yield was calculated as follow:

Percentage yield = Extraction grams/plant sample (g) ×100

(5)

Table 1. Calculated percentage yield of the solvents of C.dactylon,A.donax , A.scholaris and P.ruba

Plant name	Plant part	Ethyl-acetate	Methanol	Aqueous
C.dactylon	Leaves	0.652%	2.77%	8.12%
Arundo donax	Leaves	0.652%	4.642%	7.1%
A.scholaris	Leaves	2.103%	6.7%	8.8%
Plumeria rubra	Leaves	3.413%	5.366%	10.47%

From all the extractions aqueous extract of Plumeria rubra produced the highest yield (10.47%) while ethyl acetate extract of Arundo donax produced lowest yield (0.652%).

Table 2. Comparative analysis of TPC, TFC and DPPH between different solvents of C.dactylon, A. donax , A.scholaris and P.ruba

Extracts	Ethyl acetate			Methanol			Aqueous		
plants	TPC (mg GAE /100g DW)	TFC (mg CE\100g DW)	DPPH % inhibition	TPC (mg GAE /100g DW)	TFC (mg CE\100g DW)	DPPH % inhibition	TPC (mg GAE /100g DW)	TFC (mg CE\100g DW)	DPPH % inhibition
C.dactylon	404.86 ±0.52	330.7 ±0.18	38.92± 0.59	491.0±0 .40	659.75 ±0.37	19.96 ±0.62	270.8 ±0.40	472.3±0 .27	46.80±0 .45
A.donax	346.35 ±0.40	269.0 ±0.37	68.16 ±0.75	681.27 ±0.40	664.96 ±0.36	64.87± 0.45	415.04 ±0.40	689.69 ±0.36	66.16 ±0.29
A. scholaris	586.66± 0.36	807.3 ±0.66	60.07± 0.42	512.11± 0.45	651.05 ±0.52	42.11± 0.32	448.35± 0.55	418.59± 0.45	16.96±0 .22
P.ruba	570.2±0 .54	212.7 ±0.92	77.84± 0.33	432.05± 0.55	536.84 ±0.40	70.75± 0.41	343.02± 0.36	418.15± 0.59	63.37±0 .24

According to above mentioned table a large difference was observed in the TPC, TFC and DPPH values of different extract of plants .Highest TPC was observed in A.donax aqueous extract (681.27±0.40mg GAE\100g dry weight).Highest TFC was observed in A. scholaris ethyl acetate extract (807.36±0.66mg CE\100g dry weight).Similarly highest DPPH radical scavenging activity was observed in ethyl acetate extract of P.ruba (77.84±0.33%) while lowest in aqueous extract of A.scholaris (16.96±0.22%).

Table 3. Statistical analysis of TPC, TFC and DPPH of selected plants extract made by using three different solvents

Extract type	TPC	TFC	DPPH
Ethyl –acetate	0.001	0.001	0.001
Methanol	0.001	0.002	0.001
Aqueous	0.001	0.001	0.002
Significant value (P<0.005), Strongly significant P<0.005			

A strong significant difference (p<0.005) was observed in the TPC,TFC and DPPH extracts of different plant made by using different solvents and it was observed that selected plants showed different results with same solvents.

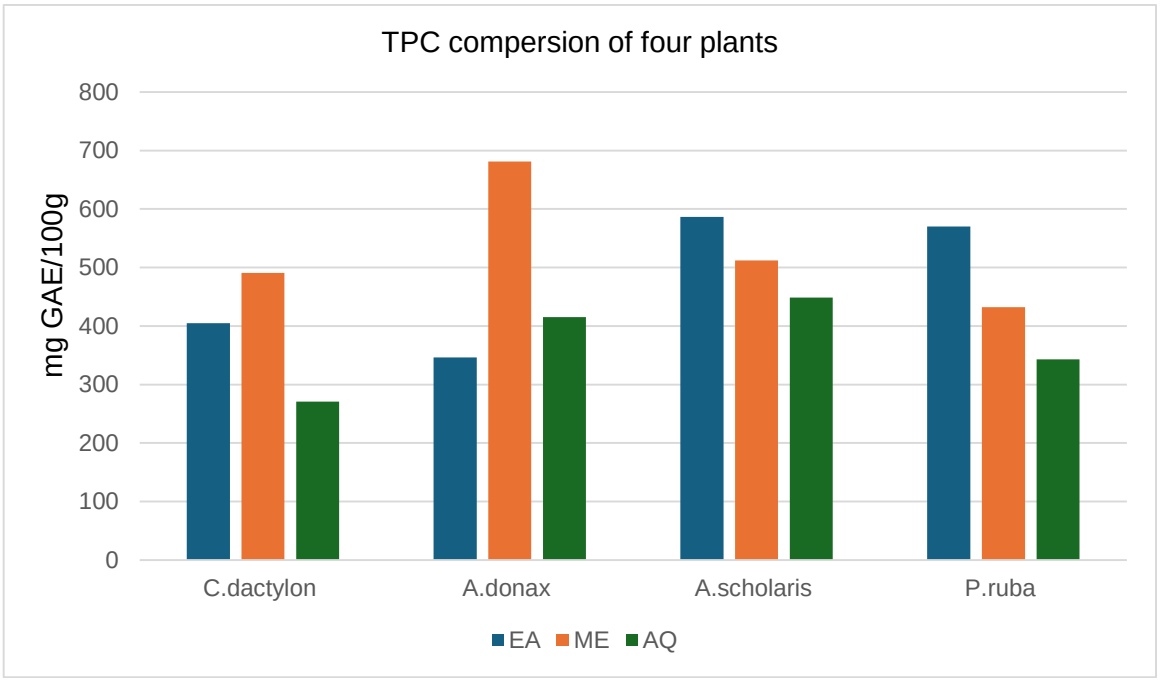


Figure 1. Graphical representation of TPC of C. dactylon, A. donax A. scholaris and P. ruba.

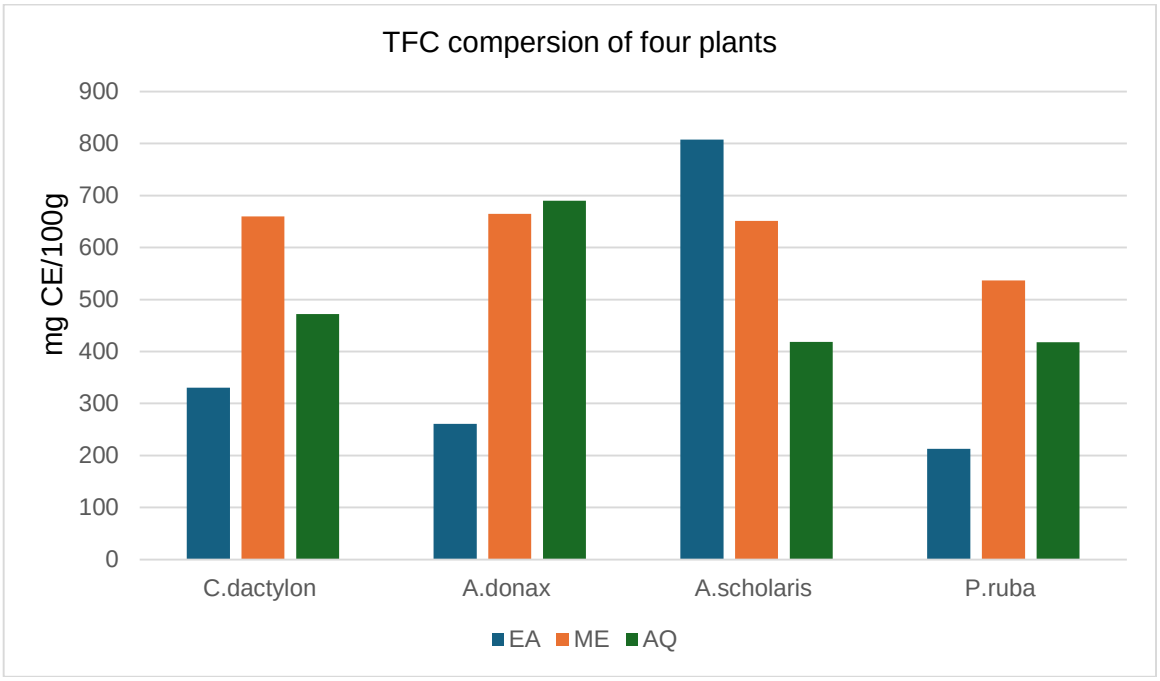


Figure 2. Graphical representation of TFC of C. dactylon, A. donax A. scholaris and P. ruba

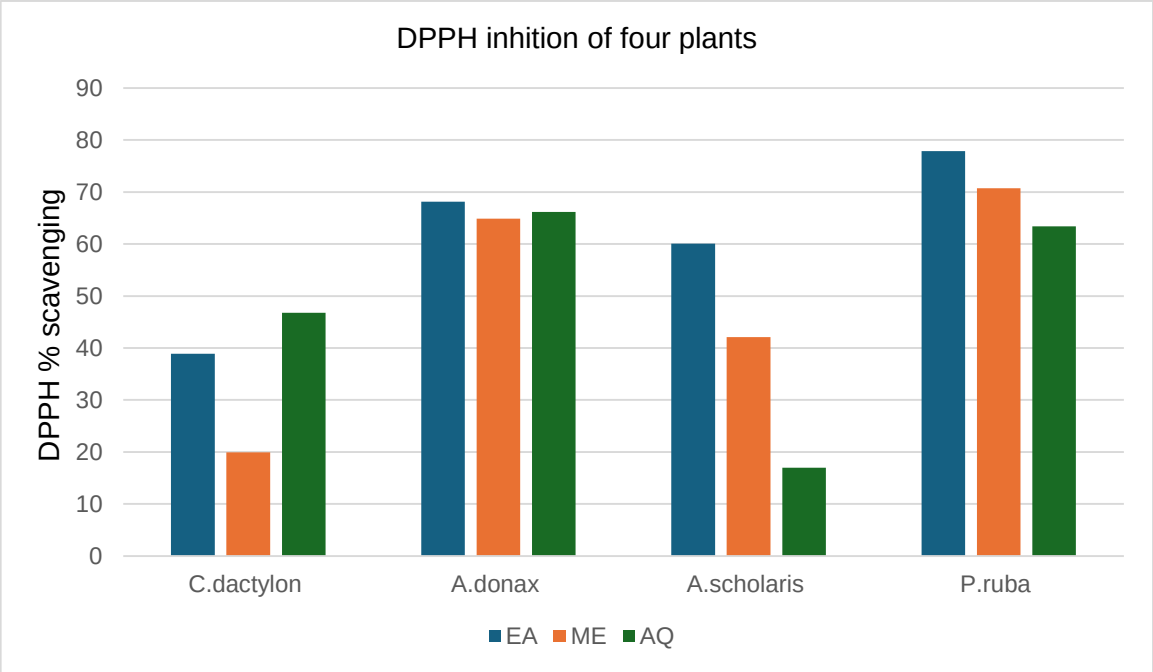


Figure 3. Graphical representation of TFC of C. dactylon, A. donax A. scholaris and P. ruba

Results were analyzed statistically between extracts of different plants made by using same solvent**.**

Table 4. Antibacterial assay of C. dactylon, A.donax, A.scholaris and P.ruba against E. coli and staphylococcus aureus

Plant extract	Bacterial strain	Inhibition zone(mm) E.coli	Inhibition zone(mm) S.aureus
Cynodon dactylon	Escherichia coli and staphylococcus aureus	13.8 ± 0.91	11.07 ± 0.56
Arundo donax		15.1±0.94	10.09 ± 0.54
A.scholaris		10.02± 0.91	11.01± 0.49
P.ruba		6.12± 0.71	6.01± 0.87
Positive control (Ciprofloxacin)		45.56±0.98	46.27 ± 0.97

Growth inhibitory effects on bacterial strains E. coli and S.aureus were shown in table 4. The Arundo donax showed 15.1±0.94 zone of inhibition against E. coli while C.dactylon showed inhibitory zone of 11.07±0.56 against S.aureus, the positive control- Ciprofloxacin showed highest zone of inhibition 45.56±98.

Table 5. MIC of C. dactylon, A. donax, A.scholaris and P.ruba for E. coli

Plant sample	MIC for ATCC E.coli(mg\mL)
Cynodon dactylon	5mg\mL
Arundo donax	5mg\Ml
A.scholaris	5mg\Ml
p.ruba	5mg\Ml

Table 6. Comparative analysis of a-amylase inhibition and Antiglycation between different solvents of C. dactylon, A. donax, A.scholaris and P.ruba

Extracts	Ethyl acetate		Methanol		Aqueous	
plants	Antiglycation %	a-amylase inhibition %	Antiglycation %	a-amylase inhibition %	Antiglycation %	a-amylase inhibition %
C.dactylon	28.40 ±0.15	42.43±0.06	53.64 ±0.17	57.77±0.04	35.79±0.6	30.33±0.03
A.donax	44.99±0.62	42.12±0.08	70.20±0.76	32.02±0.12	38.30±0.26	52.19±0.15
A.scholaris	48.72± 0.29	47.56 ±0.09	61.04± 0.25	61.14± 0.21	58.17 ±0.39	59.06 ±0.01
P.ruba	70.32 ±0.23	40.37± 0.17	53.43± 0.33	50.42± 0.06	66.44± 0.52	23.34 ±0.40

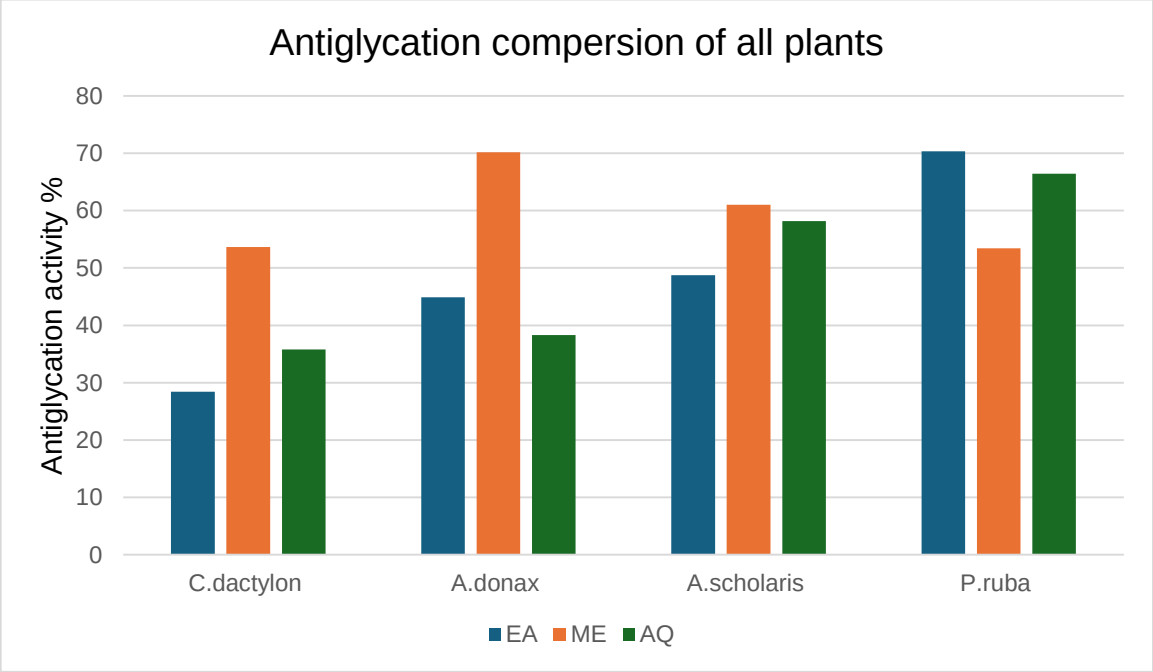


Figure 4. Graphical representation of antiglycation potential of all four plants

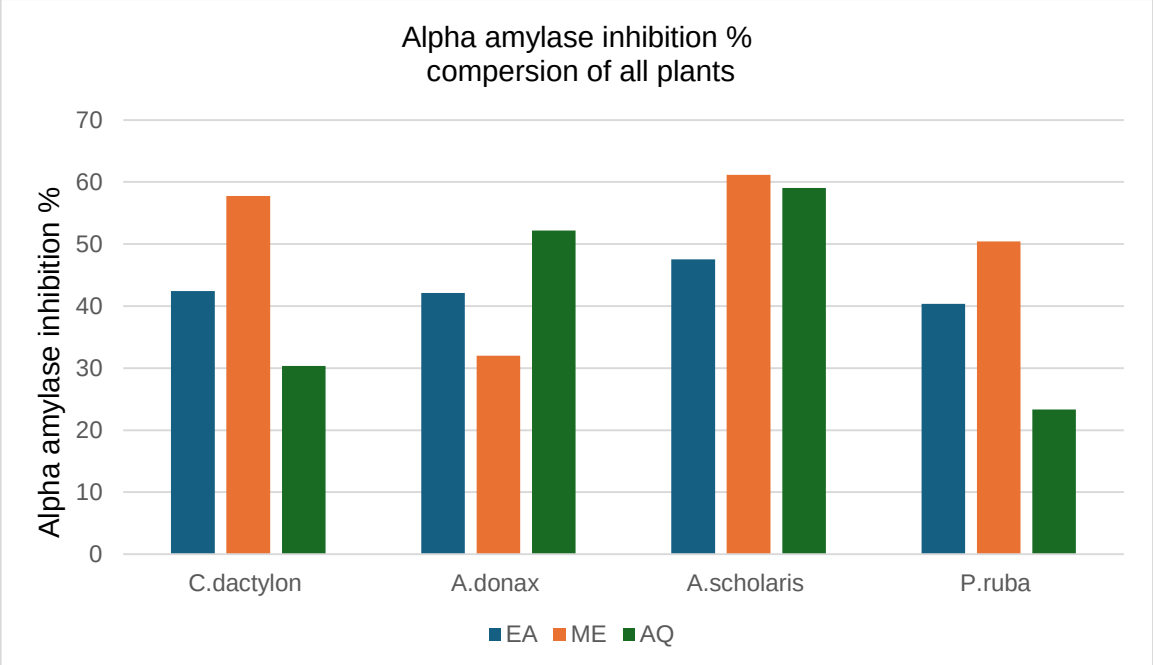


Figure 5. Graphical representation of alpha amylase inhibition of all four plants

Table 7. Statistical analysis of antidiabetic activity of selected plants extract made by using three different solvents

Extract type	Antiglycation %	Alpha –amylase inhibition %
Ethyl –acetate	0.001	0.001
Methanol	0.001	0.001
Aqueous	0.001	0.002
Significant value (P<0.005), Strongly significant P<0.005		

A strong significant difference (p<0.005) was observed in the antidiabetic extracts of different plant made by using different solvents and it was observed that selected plants showed different results with same solvents.

Table 8. Important of compounds with their respective functional groups as antioxidant, antimicrobial and antidiabetic agents.

Sr.No	Compound name				Functional group	Functions
1	C.dactylon	A.donax	A.scholaris	P.ruba		
2	Quercetin	Quercetin	Quercetin	Quercetin	OH,C-H	Antioxidant and antidiabetic agent
3	Gallic acid	Gallic acid	Gallic acid	Gallic acid	OH-,COOH	Antioxidant, antimicrobial and antidiabetic agent
4	Caffiec acid	Caffiec acid	Caffiec acid	-	OH	Antioxidant
5	p-courmaic acid	p-courmaric acid	-	-	OH,COOH	Antidiabetic
6	Sinapic acid	Sinapic acid	Sinapic acid	Sinapic acid	Phenol,COOH	Antioxidant
7	-	-	Vanillic acid	Vanillic acid	Methoxy,OH	Antioxidant
8	-	-	Benzoic acid	-	COOH,benzene ring	Antioxidant
9	-	-	Chlorogenic acid	Chlorogenic acid	C=O,OH	Antidiabetic
10	-	-	Synirgic acid	Synirgic acid	OH,COOH,O-CH_3	Antioxidant and antimicrobial
11	-	-	M-coumaric acid	M-coumaric acid	OH,COOH	Antidiabetic
12	-	-	-	Cinamic acid	C=C,COOH	Antioxidant

According to Baghel et al., (2012) the functional group of quercetin is phenol with the hydrogen bonded O-H and has important role as antioxidant and antidiabetic agent. Gallic acid a phenolic structure including carboxylic acid, high antioxidant capacity due to its three hydroxyl groups. Antioxidant properties were concluded using DPPH. Gulcin et al., (2006) Caffeic acid was consists of both phenolic and acrylic functional groups. In the present study, the antioxidant properties of the caffeic acid were evaluated by using different in vitro

antioxidant assays such as 2-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) and radical scavenging 2, 2-diphenyl-1-picryl-hydrazyl free radical (DPPH). Niciforovic et al., (2014) Sinapic acid was also consist of phenolic group and its antioxidant properties were evaluated using DPPH. Benzoic acid has COOH group and a benzene ring. It has been observed to contain antioxidant properties. Vanilic acid contains methoxy group and a hydroxyl group at C-4 and plays an important role as an antioxidant. Chlorogenic acid (phenolic acid) contain C=O and OH at C-4. It plays an important role as antidiabetic agent. Synirgic acid is composed of OH, COOH and O-CH_3 groups and is composed of antioxidant and antimicrobial properties. M-caumaric acid contains OH group at C-3 and a COOH group. It has been observed to have antidiabetic properties. Ferulic acid is composed of methoxy and hydroxy substitute at C-3 and 4 and have been observed to act as a strong antioxidant Khan et al. (2021)

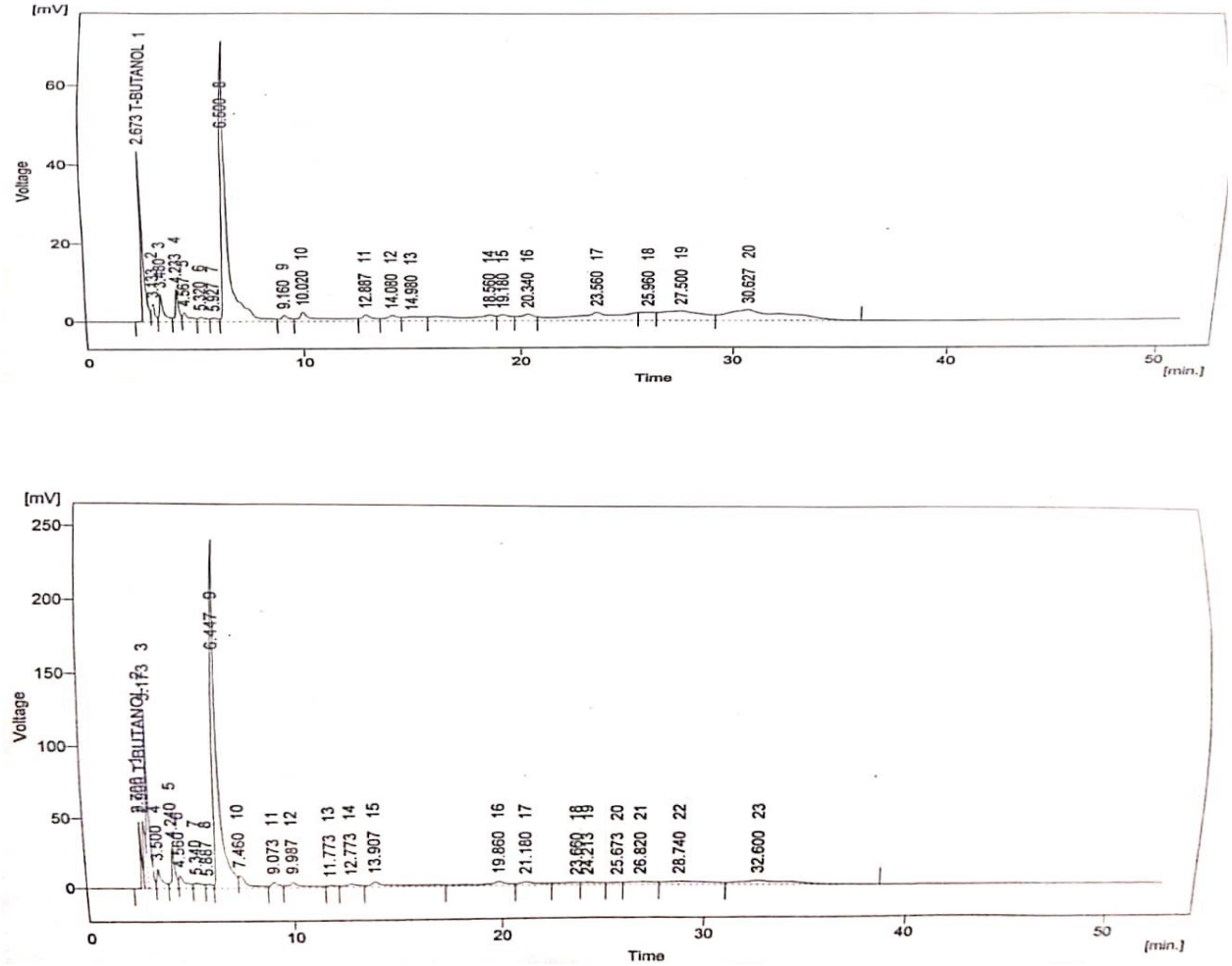
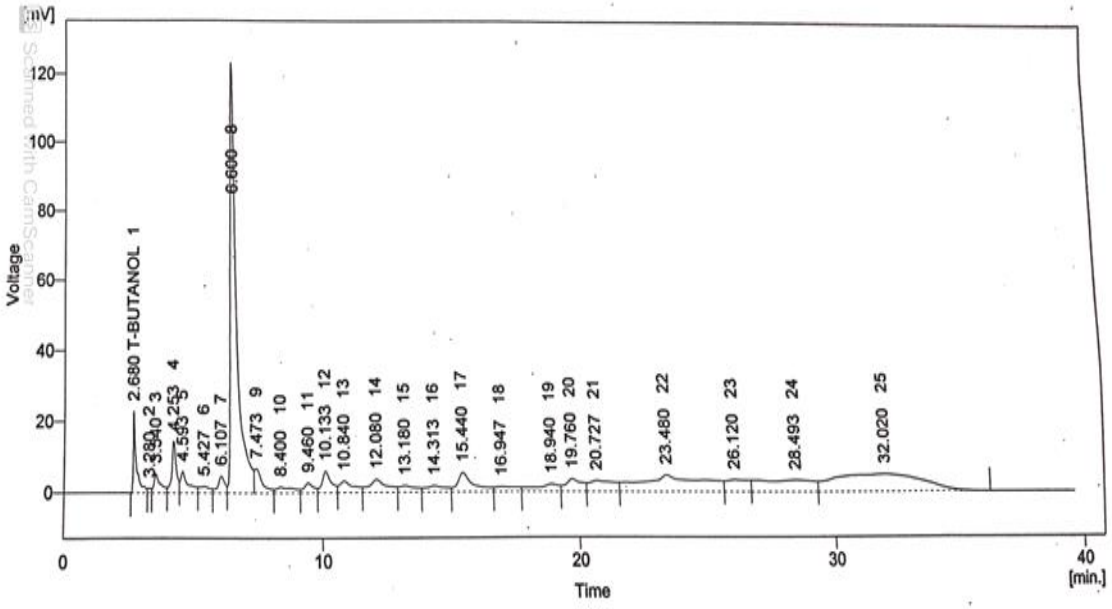


Figure 7. HPLC spectra of C.dactylon and A.donax



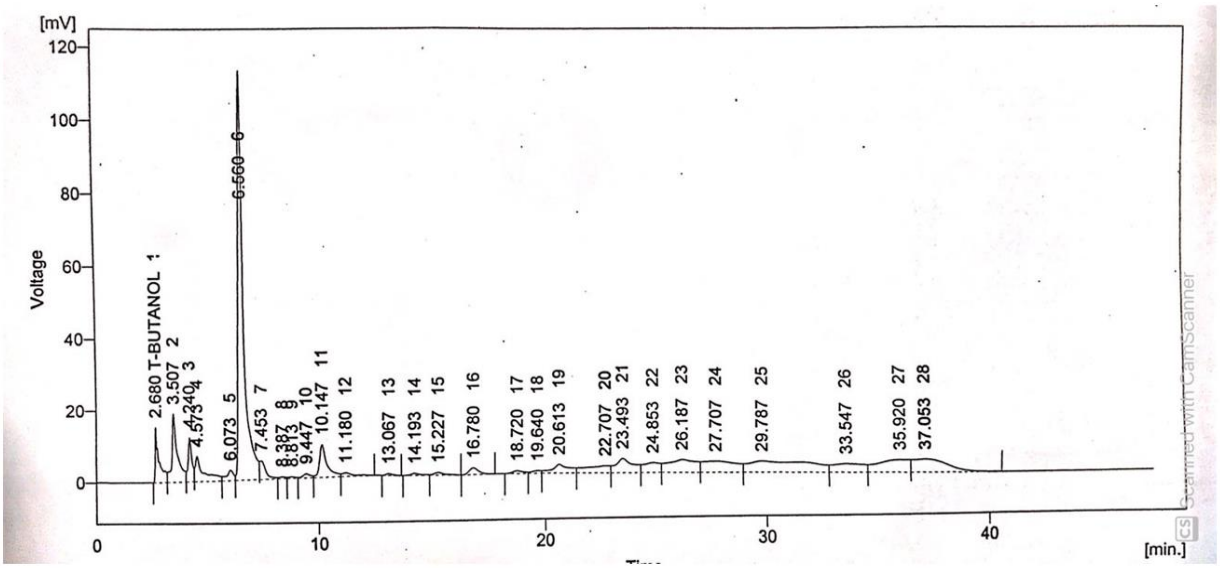


Figure 8. HPLC spectra of A.scholaris and P.ruba

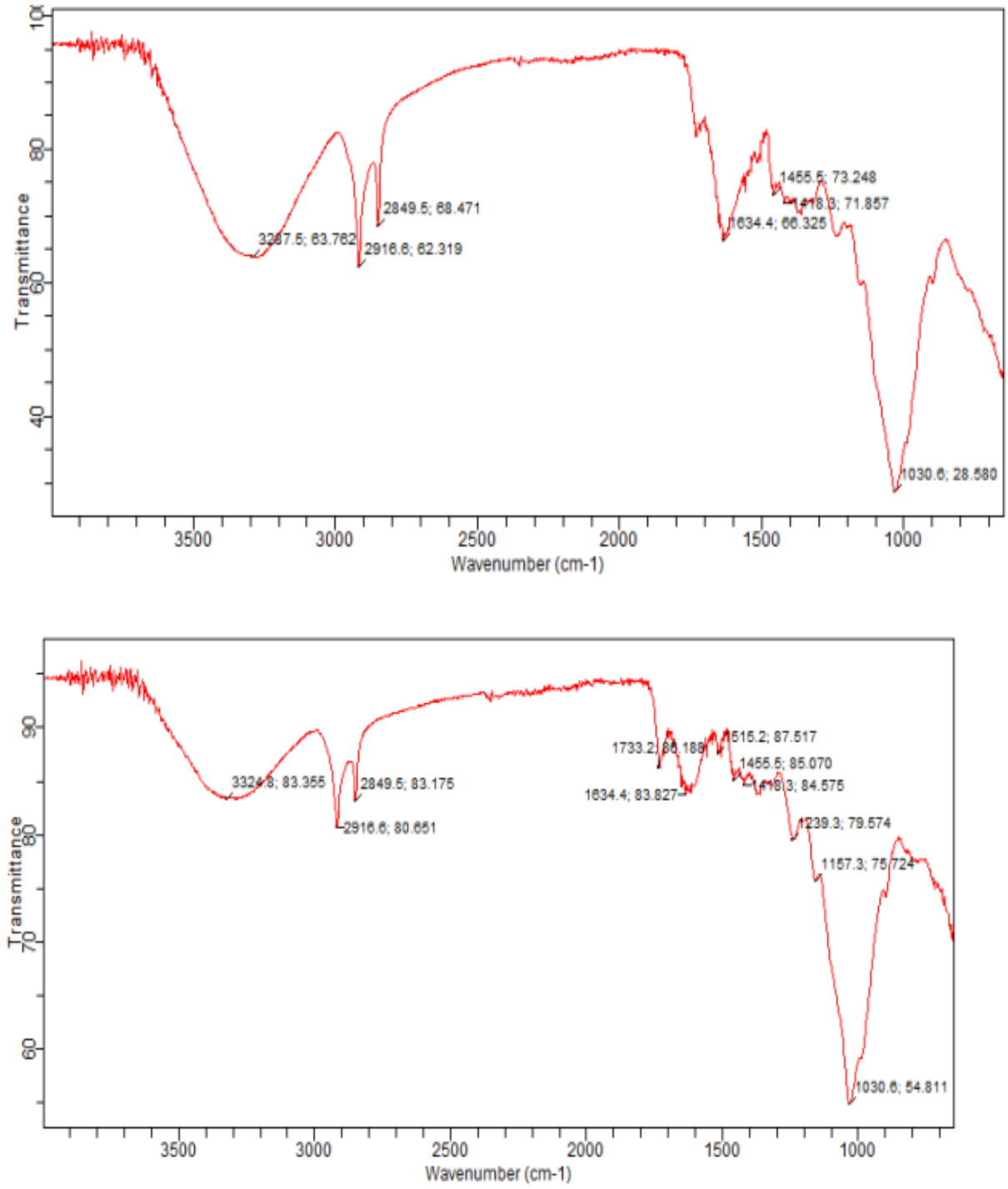


Figure 9. FTIR spectra of C.dactylon and A.donax

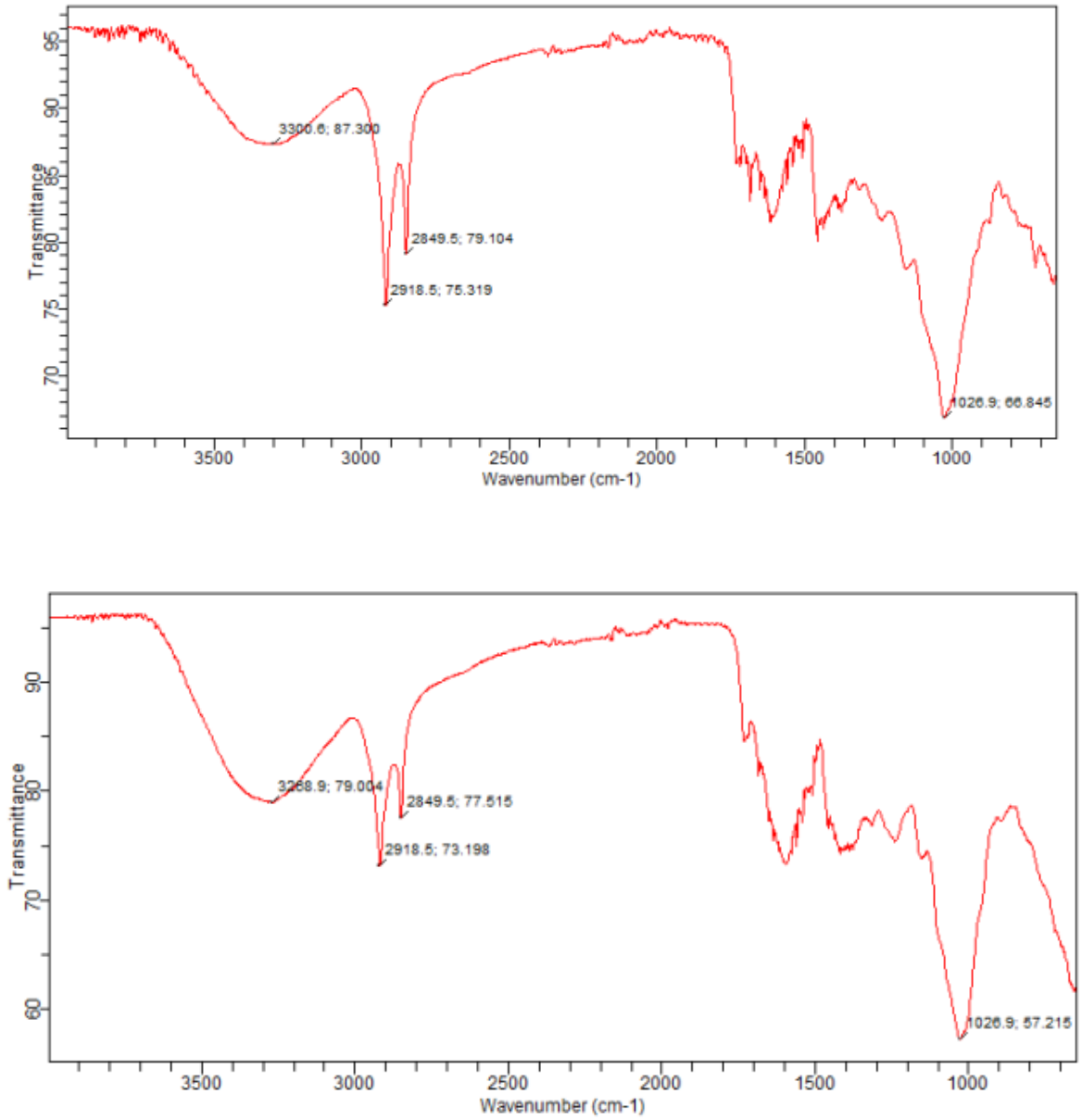


Figure 10. FTIR spectra of A.scholaris and P.ruba

Discussion

Savadi et al. (2020) conducted experiment for evaluating the antioxidant activities of rhizomes of C. dactylon and observed TPC ranging from 917.08 mg GAE/100g dry weight. . Licuris et al., (2018) observed TPC value of 3.16mgGAE/100g dry weight and 565mgGAE/100g dry weight in extract of Arundo donax while current study has similar observed TPC values of 346mgGAE/100g dry weight, 681mg GAE/100g dry weight, 415mg GAE/100g dry weight. Mistry et al. (2016) obtained the yield for methanolic and aqueous extract of Alstonia scholaris leaves was 2.82 ± 0.03% and 3.94 ± 0.12 % respectively while currently extract yield was 6.7% and 8.8% respectively that was contradictory to recent study values.16.6% yield was obtained by Kuete, V. and T. Efferth (2011) of aqueous extract of Plumeria rubra while recently aqueous extract of Plumeria rubra was 10.47% that was contradictory to current values.

Roy et al., (2016) observed TFC value of C. dactylon leaves of dry weight 8.84mg CE/100g and 174.58mg CE/100g while current study showed TFC values of 659mg CE/100g dry weight, 472mg CE/100g dry weight and 330mg CE/100g dry weight. Licuris, et al., (2018) observed TFC value for Arundo donax to be 2.23mg CE/100g dry weight for methanolic extract. Zahid et al. (2010) observed TFC value of 241 mg(CE)/100g and 565 mg(CE)/100g in extract of

Plumeria ruba while current study has similar TFC values of 570.29±0.545 mg(CE)/100g, 432.05±0.55 mg(GAE)/100g and 343.02±0.36 mg(CE)/100g.Patrick et al. (2015) observed TFC value of 382.46mg(CE)/100g and 274.56 mg(CE)/100g in extract of A. scholaris while recent study showed TFC values of 807.36±0.662 mg(CE)/100g, 651.05±0.527 mg(CE)/100g and 418.5965±0.458.

Ahmed et al., (2013) used methanolic extract of dried leaves of Cynodon dactylon in order to determine DPPH percentage inhibition and observed percentage inhibition ranging from 70%-80% while current study showed inhibition between mostly 40%-60%. Jagetia et al. (2013) used methanolic extract of dried leaves of Alstonia scholaris in order to determine DPPH percentage inhibition. He observed percentage inhibition ranging from 70-80% while current study showed inhibition between 40-60%. Gomati et al. (2012) observed DPPH radical scavenging activity of Plumeria ruba and observed 74% inhibition that was almost similar to recent study.

According to study conducted by Sharma et al., (2016) there was 5mm zone of inhibition of Cynodon dactylon extract against both E. coli and S. aureus that found to be contradictory to current study. According to study conducted by Khlifi et al., (2013), inhibitory zone of methanol extract of Arundo donax against E. coli was 13 mm while in current study, inhibitory zone of 15.1mm found to be close to above study. According to study conducted by Mishra et al. (2013) inhibitory zone of methanol extract of Alstonia scholaris against E. coli was 13 mm while in current study inhibitory zone of 10 mm found to be analogous to above study. Another study by . conducted by yaseen et al. (2016) showed that there was 5mm zone of inhibition of plumeria ruba against both E. coli and S. aureus that found to be contradict to current study.

Licuris et al., (2018) observed minimum inhibitory concentration of 5mg/mL of A. donax against E. coli and the current study also showed the same minimum inhibitory concentration of 5 mg/mL against E. coli. Gopi et al. (2011) observed MIC of 12.mg/mL of Alstonia scholaris against E. coli. While the current study showed the MIC of 5 mg/mL against E. coli. Islam et al. (2019) observed MIC of 5.mg/mL of Plumeria rubra against E. coli. While the current study showed the MIC of 5 mg/mL against E. coli. It means our study have shown same MIC results.

Kadu et al., (2019) observed antiglycation values of Cynodon dactylon 0.024% and 0.006% while current study showed antiglycation values of 28.40%, 53.64% and 35.79%. Al-Snafi, (2016) observed antiglycation value of A. donax 200%and 174.58% while current study showed antiglycation values of 70.20%, 38.30% and 44.99%. Cia et al. (2010) observed the antiglycation potential of Alstonia scholaris between 43-80% range while current study showed the 48-61% antiglycation potential of Alstonia scholaris.Dawood et al. (2015) observed the antiglycation potential of Plumeria ruba is between 45-75% range while curreny study showed the 53-76% Antiglycation potential of Plumeria ruba.

Randive et al., (2019) observed 40% alpha amylase inhibition value of Cynodon dactylon that was similar to current studies. Sharma et al., (2019) observed 96.2% alpha amylase inhibition of A. donax while current study showed 70 % inhibition value. Arulmozhi et al.(2007) observed the alpha amylase inhibition of Alstonia scholaris is between 55-77% range while recent study showed 48-61% alpha amylase inhibition of Alstonia scholaris. Patrick et al. (2015) observed the alpha amylase inhibition of Plumeria ruba is between 55-77% range while recent study showed the 40-60% alpha amylase inhibition.

According to Niciforovic et al., (2018) a stretching frequency of C. dactylon 3200-3400cm^{-1}, O-H group was observed that is similar to current study. Peaks indicating the presence of C-H group (stretch) was showed at 2916.6cm^{-1} and 2849.5cm^{-1} that is similar to current results., Mir et al., (2020) observed highest peaks between the range of 3100cm^{-1}-3500cm^{-1} that indicated Amides-H and O-H bending in A. donax that is similar to current results. Peaks between 2900cm^{-1}-3000cm^{-1} showed amide group A. donax while current study observed the presence of C-H (stretch) and O-H group. The functional groups identified in FTIR results were also previously confirmed is a study conducted by the Yaseen et al. (2021) in methanolic leaves extract of Alstonia scholaris showed relatively sharp peak at 3338.2 cm^{-1} owing to presence of

hydroxyl group present in the Alstonia scholaris leave extract. A strong peak at 1547.3 cm^{-1} which confirmed the carbonyl functional group. The presence of C-H was confirmed at 3193.8 cm^{-1}. Peaks at 704.7 cm^{-1} and 588.9 cm^{-1} was also studied which belongs to the Mg–O and Fe–O, respectively. The functional groups identified in these FTIR results were also previously confirmed by the Dawood, et al. (2015) in methanolic leaves extract of Plumeria rubra who identified O-H group study at the peak of 3406 cm^{-1} which shows the presence of hydroxyl (OH) groups. C-H stretching of the alkanes groups was confirmed at 2931cm^{-1}. A strong peak at around 1631cm^{-1} and a weak peak at around 1384cm^{-1} belongs the carbonyl bond C–O of the amide group and the bending vibration of the N–H bond in peptides or proteins.

According to Baghel et al., (2012) the functional group of quercetin is phenol with the hydrogen bonded O-H stretch and also act as antioxidant and antidiabetic agent. Badhani et al., (2015) Gallic acid (GA), a phenolic structure including carboxylic acid, high antioxidant capacity due to its three hydroxyl groups. Niciforovic et al., (2014) Sinapic acid was also consist of phenolic group and its antioxidant properties were evaluated using DPPH. The flavonoids compounds of Alstonia scholaris Linn were also identified using HPLC results conducted by the Feng et al. (2013). In optimum chromatographic conditions, using an RP-C18 column, involved the use of acetonitrile in preference to methanol. Spiking the extract with authentic standards revealed the presence of five major compounds, namely chlorogenic acid, caffeic acid vamillic acid, quercetin, and sinapic acid. The flavonoids compounds of Plumeria rubra were studied by Khan et al. (2021) using HPLC results. HPLC analysis validated many phytoconstituents in varying concentrations. They performed HPLC by Spiking the extract with authentic standards. It revealed the presence of chlorogenic acid, keamoferol, plumeric acid, vamillic acid, m-Coumeric acid, isoquercetin, and Sinapic Acid. The retention time of chlorogenic acid was 14.839min with the amount of 5[M.vs]. but in current sample have some contradict values with retention time of 15.227min with the area of 3.3[Mv.s].

Conclusion:

It was concluded that all four plants processed significantly different ($P<0.005$) antioxidant, antimicrobial and antidiabetic activities. Highest yield was obtained by the aqueous extract of P. ruba while lowest yield was obtained by ethyl-acetate extract of C. dactylon. Highest TPC was observed in Arundo donax methanol extract. Highest TFC was observed in A. scholaris ethyl acetate extract. Similarly, highest DPPH radical scavenging activity was observed in aqueous extract of P. ruba. Arundo donax showed highest zone of inhibition against Escherichia coli in all four plants. Highest antiglycation activity was observed in methanolic extract of A. donax while P. ruba was observed as highest antiglycation in ethyl- acetate and aqueous medium. Best alpha-amylase activity was observed in all types of extract of A. scholaris. Structural analysis of all plants showed the compounds that involved in antioxidant, antimicrobial and antidiabetic activities was due to presence of gallic acid, quercetin, caffeic acid, p-coumaric acid and sinapic acid. Hence research medicinal plants could be used as an antioxidant, antimicrobial and antidiabetic agent. The antiglycation activity of flavonoids has been found to be effective. The plants under consideration are also rich in such phytochemicals so it strengthens the correlation between the phytochemicals and antiglycation activity.

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