INTERNATIONAL ASSOCIATION OF BIOLOGICALS AND COMPUTATIONAL DIGEST
International & Peer-Reviewed Journal
E-ISSN: 2583-3995

EVALUATION OF ANTIOXIDANT POTENTIAL AND PHYTOCHEMICAL CHARACTERISATION OF PEEL EXTRACTS OF LUFFA ACUTANGULA AND LUFFA CYLINDRICA

Smit Bhavsar[1], Nainesh Modi[2]*, Mukeshkumar Thakor[3]

[1,2,3]Department of Botany, Bioinformatics, Climate Change Impacts Management, School of Sciences, Gujarat University, Ahmedabad – 380 009
Corresponding author: nrmodi@gujaratuniversity.ac.in

ABSTRACT

Luffa acutangula and Luffa cylindrica are popular vines belonging to the Cucurbitaceae family. They are extensively used as vegetables across the globe. Cucurbitaceae plants have played a major role in Indian medicinal systems and ethnopharmacology. Various plant parts have pharmacological properties viz. antioxidant, antiinflammation, antidiarrheal, anticancer, antiulcer and so on. These properties are due to phytochemicals present in the plants. Phytochemistry is the study of plant chemicals, particularly secondary metabolites. These metabolites are typically produced as a self-defence mechanism against insects, pests, pathogens, herbivores, ultraviolet exposure, and environmental hazards. The present study is aimed to identify and quantify certain phytochemicals which are known to be responsible for bioactivities. Peels are generally thrown away in culinary practice, but they are highly rich in phytochemicals. Peels of both plants have taken for the study and qualitative analysis of phytochemicals such as alkaloids, phenols, flavonoids, sterols, tannins, saponins, etc. were carried out. Plant extracts of different solvents were compared for antioxidant potential as well as total phenols and total flavonoids. Quantification of phenols and flavonoids was carried out as they are reported to have antioxidant potential. FRAP assay was performed to check the capacity of plant extract to scavenge free radicals. The correlation of phenols and flavonoids was carried out with antioxidant results to establish the fact. The plant extracts' total phenolic content was 66-357 mg gallic acid equivalent. The highest flavonoid content was found in methanol extracts of L. acutangula and L. cylindrica with values of 133.450 and 126.132 mg quercetin equivalent. FRAP assay concluded that methanol extracts of both plants have highest reducing power. TPC and TFC have shown strong positive correlation with FRAP assay. The phytochemicals and antioxidant potential of L. acutangula and L. cylindrica state that they possess valuable nutraceuticals. The pharmacological potential of the plants should be explored to maximum potential.

Keywords: Antioxidants, Cucurbitaceae, Luffa acutangula, Luffa cylindrica, FRAP, TPC, TFC.

1. INTRODUCTION

Vegetables have been used as medicine for centuries by people across the world, and they have become an integral part of daily diets. (Shrivastava et al., 1996.) Vegetables are rich sources of phytochemicals and medicinal properties (Fallah et al.,2005). Herbal medicines have little side effects on humans and less expensive compared to synthetic medicines, so their popularity is growing considerably (Tijani et al., 2008). The plants of Cucurbitaceae family are widely known as cucurbits and some people recognise as member of gourd family. It is one of the biggest families which includes popular vegetables and fruits. The family comprises of more than 120 genera and 900 species. The plants are cultivated globally, and majority of the plants are found in natural habitats across the globe (Zhou et al., 2016). India is one of the major consumers of cucurbits (Rolnik et al., 2020). Most essential nutrients required for human health are present in cucurbits, including carbohydrates, proteins, vitamins, minerals, sugar, and others. (Rahman, 2003; Duke,1999). The plants from Cucurbitaceae family have been used in culinary practices and in ancient medicines for long time (Mukherjee, 2022). They are widely used as medicinal plants for treating variety of ailments throughout the world, especially in Indian and Chinese medicinal plants (Rajasree

et al., 2016). Numerous plants from the families have listed in Ayurveda, Unani, and folk medicines. The plants are used to treat jaundice, respiratory diseases, syphilis, scabies, constipation, piles, ulcers, skin infections, diabetes, obesity, gonorrhoea, liver and kidney diseases, cancer and so on (Saboo et al.,2013). In addition to improving human health, cucurbit fruits are helpful in cleansing the blood, purifying harmful substances, and aiding in digestion. (Phillips et al.,2005). Luffa acutangula (Ridge gourd) and Luffa cylindrica (Sponge gourd) are majorly used plants from the family.

Luffa acutangula contains trace amounts of vitamins K and E. Many minerals are present in the plants of this family, which are beneficial for nutrient absorption. (Avinash and Rai,2017). In Sanskrit, the Ridge gourd is called 'Kosataki' and in Bengali, it is known as Zinga. Many diseases can be treated with the whole plant, like 'Kustha' (skin-related disorders), 'Pandu' (anaemia), 'Arsa' (piles), 'Sopha' (inflammation), 'Garavisa' (poisoning) 'Gulma' (tumor growth), 'Adhmana' (indigestion. Acidity), 'Pliharoga' (splenomegaly), used in the traditional Ayurvedic formulation of Abhaya Lavana (Mukherjee et al., 2022). Several phytochemical compounds are reported from various parts of Luffa acutangula which are bioactive in nature (Shendge et al., 2018). Some of them are Luffangulin, luffaculin, saponin glycosides, gallic acid, p-coumaric acid, ferulic acid, protocatechuic acid, Acutoside C; Acutoside D, Unsaturated aliphatic alcohols, Carboxylic acids, fatty acids and their esters (Nagarajaiah and Prakash, 2015; Suryanti et al., 2015). Luffa cylindrica (Sponge gourd) fruit is known to treat jaundice, leaf juice is consumed to treat conjunctivitis, roots act as an excellent laxative and various skin diseases have been used with the help of seed oil of the plant (Chen et al., 2014). Phytochemicals from various parts of Luffa cylindrica are Lucyin A, lucyoside, maslinic acid; ginsenosides, luffin P1; luffin S, luffacylin, apigenin-7-O-D-glucuronidemethyl ester; luteolin-7- O–D-glucuronide, methyl ester, p-coumaric acid, chlorogenic acid, caffeic acid (Vellapandian et al., 2022).

Antioxidant-rich plants are gaining importance around the world. In developing countries, approximately 80% of the world's population uses herbal medicine as their primary source of healthcare. Oxidative stress, which is caused by oxygen radicals, is thought to be a primary cause of many degenerative diseases, including cancer, atherosclerosis, and stomach ulcers (Mamelona et al., 2007). Antioxidants are thought to be potential protective agents for the human body and preventing oxidative damage. As a result, there is a rising interest in antioxidant-containing chemicals that are fed to humans and animals as dietary components or as particular pharmaceutics. Natural antioxidants have recently emerged as a key focus of scientific investigation (Irshad et al., 2014). Oxidative and reductive process can be easily run by flavonoids, this process is run in both outer and inner side of the cell. Flavonoids' antioxidant power is based on their ability to interact with free radicals that initiate or are produced during chain reactions, on the inhibition of oxidation processes, which reduces the activity of oxidase enzymes, or on the complexation of transition metal ions that catalyse oxidation reactions (Chand et al., 2017).

The chief objective of the study is to identify effectiveness of the peel of Luffa acutangula and Luffa cylindrica. Moreover, comparative antioxidant potential in different solvents systems i.e., methanol, petroleum ether and distilled water. In addition, comparative analysis of phytochemicals qualitatively as well as quantitatively. As these phytochemicals are known to be responsible for performing antioxidant activity, statistical analysis was also carried out to find out correlation amongst different entities.

2. MATERIAL AND METHODS:

2.1 Collection of plant material

Fresh fruits were purchased from local market during august 2021. The plant specimens were identified and verified by Dr. Nainesh R. Modi. Voucher specimens were submitted as herbarium in department of Botany, Gujarat University. Fruits were cleaned with distilled water and separated into pulp and peel.

2.2 Chemical required:

Standard chemicals were bought to carry out various experiments. All chemicals were checked for authenticity and expiry. Following chemicals were used in the experiments. 99% Methanol (v/v) %, 99% Petroleum ether (v/v) %, Mayer's Reagent, Wagner's Reagent, Dragondroff's reagent, Hager's Reagent, Lead acetate, Molisch's Reagent, Fehling A Reagent, Fehling B Reagent, Barfoed's Reagent, Benedict's Reagent, Million's Reagent, Folin-Coicalteau Reagent, Chloroform ($CHCl_3$), Pyridine (C_5H_5N), Gallic Acid, 0.1%,3% & 10% $FeCl_3$ (Ferric

chloride) ,10% $Pb(C_2H_3O_2)_2$ (Lead acetate), 10% Na_2 Cr_2O_7 $2H_2O$ (Sodium dichromate Solution), 1% & 95% HCl (Hydrochloric acid), NaOH (Sodium hydroxide), Zinc dust, $CHCl_3$ (Chloroform), 100% Conc. H_2SO_4 (Sulfuric acid), 20% & 35% Na_2CO_3 (Sodium carbonate), Quercetin, 5% $NaNO_2$ (Sodium nitrate), 10% $AlCl_3$ (Aluminium chloride), 1M NaOH (Sodium hydroxide), DPPH , TPTZ, Acetate buffer, Sodium trihydrate, Glacial acetic acid.

2.3 Preparation of plant extracts

Fruits were separated into pulp and peel. Peels were dried in hot air oven at moderate temperature for 6 hours to remove moisture content. Dried peels were ground to powdered form in electronic mixer. The powder was subsequently sieved to obtain fine powder. The dried powders were used to prepare the plant extract for which three solvents were selected with respect to polarity. Methanol, petroleum ether and distilled water were chosen as solvents. Plant extracts were prepared using cold extraction method. In this method, 10 gram of plant powder was taken in 100ml solvent. The solution was kept in rotatory shaker at 120rpm for 24 hours. The solution was filtered by the Whatman filter paper No 1. The filtrates were collected in petri-dishes. They were kept open to evaporate excessive solvents and dried extracts were stored in refrigerator for further use (Pandey et al., 2014; Mohamed et al., 2020).

2.4 Preliminary phytochemical screening of plant extracts

The freshly prepared crude extracts were qualitatively tested for preliminary phytochemical screening. The tests were carried out to detect the presence of phytochemicals such as alkaloids, flavonoids, phenols, sugars, proteins, tannins, saponins, steroids by using standard methods (Harborne, 1998; Kadia et al., 2022).

2.5 Antioxidant Activity by FRAP method

Antioxidant potential of the plant extracts were determined by ferric iron reducing antioxidant power (FRAP) assay. In FRAP assay, ferrous sulphate was taken as standard and distilled water was taken as blank. FRAP solution is required for the assay. It was freshly prepared prior to follow procedure. FRAP solution was prepared as following; 300mM acetate buffer (pH 3.6), a 20mM ferric chloride solution and a 10mM TPTZ solution were mixed in 10:1:1 ratio. The prepared solution was mixed with 40mM HCl solution. Plant extracts were prepared in 1mg/1ml ratio with respective solvents. 1ml of plant extract or blank or standard (ferrous sulphate) were taken for analysis. They were diluted with 10ml of distilled water. Then, 4ml of FRAP reagent was added to each test tubes. The solutions were shaken well and allowed to react for 30 mins. After incubation, absorbance was measured at 593 nm using spectrophotometer (Lasany(r) microprocessor single beam UV-VIS spectrophotometer model: LI-294). The similar procedure was followed for standard ferrous sulphate (200-1000 µg/ml) to obtain calibration curve. The results were expressed as µmol ferrous sulphate equivalents in 1g of dried sample (µmol $FeSO_4$/g) (Yadav et al., 2007).

2.6 Determination of Total Phenolic Content

Quantification of total phenolic content was determined by Folin-Ciocalteu reagent method with minor modification. Gallic acid was taken as standard and distilled water was taken as blank. Plant extracts were prepared in 1mg/1ml ratio with respective solvents.1ml plant extract was diluted with 10 ml H_2O. 1ml of Folin-Ciocalteu reagent was added to the solution and allowed to react with the reagent for 5 minutes. 4 ml of 20% sodium carbonate was added to the mixture. Final volume was made up to 25 ml with distilled water. The final solution was shaken properly and incubated for 30 mins at room temperature. After incubation, absorbance was measured at 765 nm using spectrophotometer (Lasany(r) microprocessor single beam UV-VIS spectrophotometer model: LI-294). The similar procedure was followed for standard gallic acid (200µg/ml – 1000 µg/ml) to obtain calibration curve. The results were expressed as mg gallic acid equivalents in 1g of dried sample (mg GAE/g) using following equation (Swetha et al., 2016; Bhati et al.,2021).

$GAE = C \times V/M$

Where, C = Concentration of gallic acid acquire from the calibration curve,
V = Volume of plant extract in a solution (in mL),
M = weight of the plant extract (in gram).

2.7 Determination of Total Flavonoid Content

Quantification of total flavonoid content was determined by aluminium chloride method with minor modification. Quercetin was taken as standard and distilled water was taken as blank. Plant extracts were prepared in 1mg/1ml ratio with respective solvents. 100 µl of 10% $AlCl_3$ solution was added to the solution and allowed to react with the reagent for 5 minutes. 100

µl of 1M potassium acetate solution was added to the mixture. Final volume was made up with 4.8ml with distilled water. The final solution was shaken properly and incubated for 30 mins at room temperature. After incubation, absorbance was measured at 415 nm using spectrophotometer (Lasany(r) microprocessor single beam UV-VIS spectrophotometer model: LI-294). The similar procedure was followed for standard quercetin (200µg/ml – 1000 µg/ml) to obtain calibration curve. The results were expressed as mg quercetin equivalents in 1g of dried sample (mg QE/g) using following equation (Kadu et. al., 2021; Shah et al., 2021).

$QE = C \times V/M$

Where, C = Concentration of quercetin acquire from the calibration curve,
V = Volume of plant extract in a solution (in mL),
M = weight of the plant extract (in gram).

2.8 Statistical Analysis

All the results of quantitative phytochemical analysis are conducted in triplicates to minimize error, by taking an average and have been denoted in the form of Mean ± Standard Deviation (S.D.). The calculations for statistical analysis were conducted in Microsoft Office 2019 Excel application. From the three graphs, different equations are obtained in the form of regression equation for the straight line, y = mx + c, where y= Absorbance of extract, m = Slope of the graph, x = Concentration of extract and c = Intercept of the graph (Parimi & Pravallika, 2017), for further calculation of TPC, TFC and FRAP Assay. Correlation between TPC, TFC and antioxidant capacity of Luffa acutangula and Luffa cylindrica were carried out using SPSS software. Obtained data of total phenolic content, total flavonoid content and FRAP assay results in different solvents were correlated with each individual values using Pearson Correlation coefficient (r) (Singh et al., 2016).

3. RESULTS AND DISCUSSIONS:

3.1 Phytochemical screening of Luffa acutangula and Luffa cylindrica:

Phytochemical screening of Luffa acutangula and Luffa cylindrica was performed by standard methods with minor modifications. Presence of alkaloids, phenols, sugars, proteins, tannins, saponins, steroids and flavonoids were checked. The phytochemicals are known to possess various pharmacological properties such as antibacterial, antioxidant, antimalarial, antiviral, antitumor, anticancer, anti-inflammatory and so on. Thus, presence of certain phytochemicals may be responsible for antioxidant activity performed in the present study. Based on preliminary phytochemical screening of both plants, it is concluded that maximum number of phytochemicals were detected in polar solvents (methanol and distilled water) compared to non-polar solvent (petroleum ether). Alkaloids were only found in methanol extract of L. cylindrica. Phenolic compounds, sugar, proteins, tannins, saponins, steroids and flavonoids were detected in methanol and distilled water extracts of both plants. Only steroids and flavonoids showed presence in petroleum ether extracts. The phytochemicals detected in different solvents and results are denoted in following table. Presence of phytochemical is denoted by '+' sign and absence of phytochemical is denoted by '-' sign.

Table:1 Phytochemical screening of Luffa acutangula and Luffa cylindrica:

Secondary metabolites	**Tests**	**Luffa acutangula**			**Luffa cylindrica**		
Phytochemicals	**Test**	**MeOH**	**Pet-Ether**	**D.W.**	**MeOH**	**Pet-Ether**	**D.W.**
Alkaloids	Mayer's test	-	-	-	+	-	-
	Hager's test	-	-	-	+	-	-
	Wagner's test	-	-	-	+	-	-
Phenols	Ferric chloride test	+	-	+	+	-	+
	Potassium Dichromate test	+	-	+	+	-	+
Sugars	Molisch's test	+	-	+	+	-	+
	Fehling's test	+	-	+	+	-	+
Protein	Millon's test	+	-	+	+	-	+
	Xantho-protein test	+	-	+	+	-	+
Tannins	Lead acetate test	+	-	+	+	-	+
Saponins	Frothing test	+	-	+	+	-	+
	Liberman Buchard	-	+	+	+	+	+

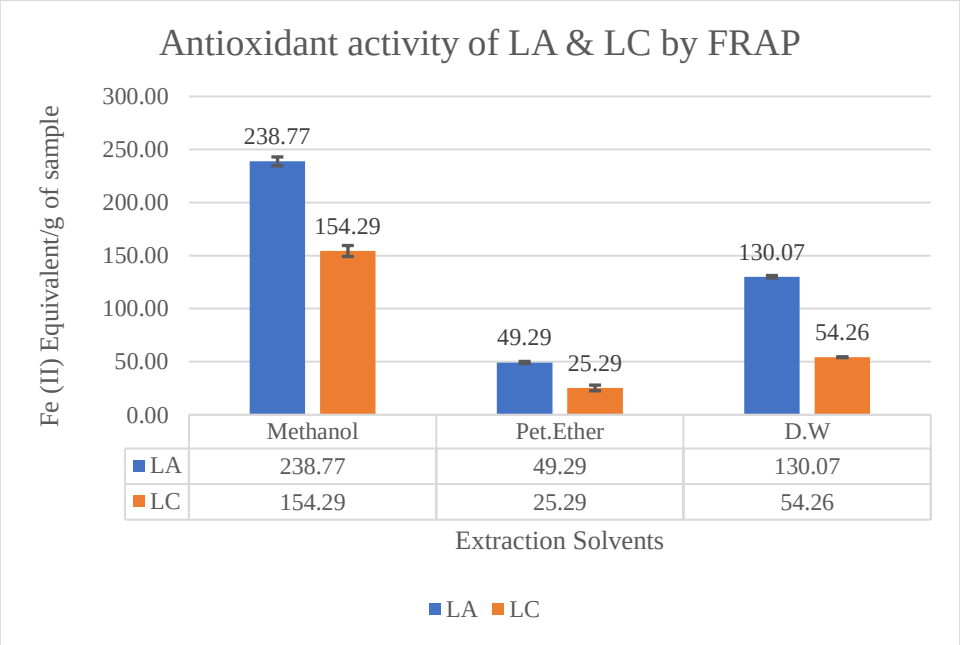
Steroids	Liberman sterol	-	+	+	+	+	+
Flavonoids	Alkaline reagent	+	-	+	+	-	+
	Zinc hydrochloride reduction test	+	+	+	+	+	+

3.2 Evaluation of antioxidant potential by FRAP assay:

The antioxidant potential of the plant extracts was determined by Ferric Reducing Antioxidant Power (FRAP) assay. FRAP assay is used to evaluate antioxidant potential of an extract based on its reducing ability. It is the only assay which can determine amount of reductants or antioxidants in sample. It is based on the principle of reducing ability of a sample antioxidant to reduce ferric tripyridyltriazine (Fe^{+3} – TPTZ) to blue coloured complex ferrous tripyridyltriazine (Fe^{+2} – TPTZ) at acidic pH (Ulewicz-Magulska et al., 2019; Du et al., 2006).

The reducing ability of plant extracts were in order of L. acutangula methanol > L. cylindrica methanol > L. acutangula D.W. > L. cylindrica D.W. > L. acutangula pet-ether > L. cylindrica pet-ether. The results showed the methanol plant extracts have the highest reducing ability compared to D.W. and pet-ether. The standard calibration equation was obtained as y = 0.009x + 0.1887 (R^2 = 0.9977). Equivalent weight $FeSO_4$/g of sample was calculated using the equation.

Table: 2 Comparison of plant extracts of FRAP assay

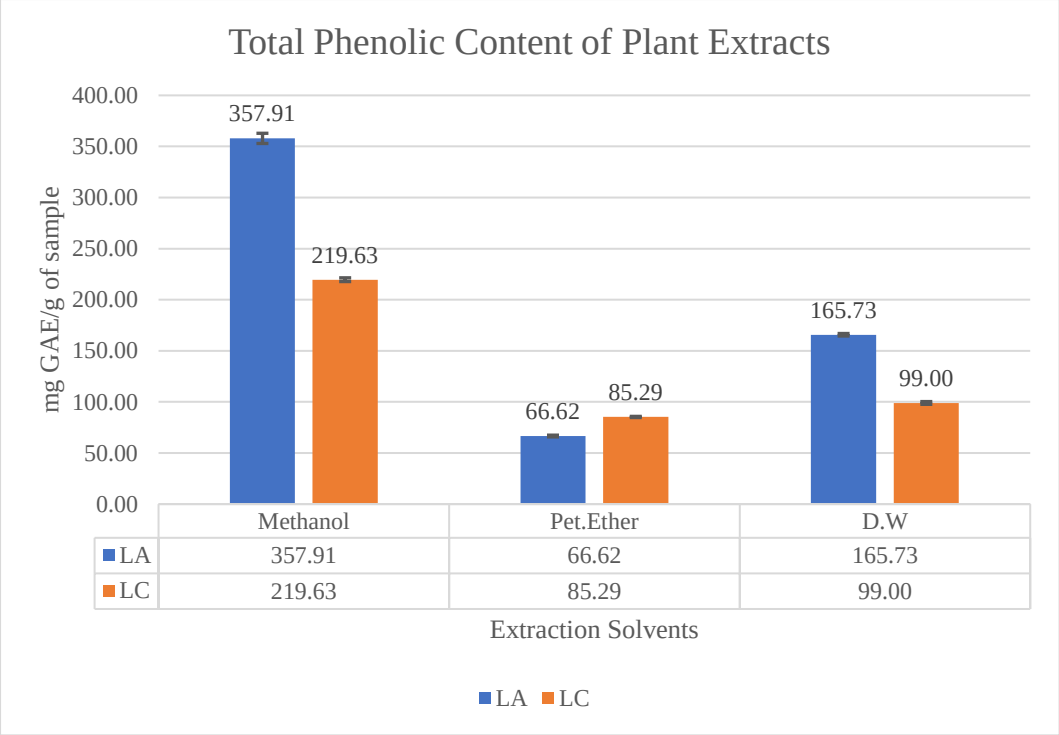


3.3 Determination of Total Phenolic Content:

Total phenolic content in plants extracts was determined by Folin-Ciocalteu method. Folin-Ciocalteu reagent is yellow coloured acidic solution composed of complex polymeric ions formed from phosphomolybdic and phosphotungstic acids. Phenolic content present in the plant extracts is oxidised by the reagent and lead to formation of molybdenum-tungsten blue coloured complex which is detected spectrophotometrically at 765nm (Blainski et al., 2013; adnan et al., 2017).

From the results obtained, the phenolic content was observed highest in methanolic extracts as 357.911 and 219.632 mg GAE/g from L. acutangula and L. cylindrica respectively. In same order, petroleum ether extracts showed comparatively less amount of phenolic content with values 66.619 and 85.285 mg GAE/g. Phenolic contents obtained from distilled water extracts showed moderate values compared to both solvents with values 165.734 and 99 mg GAE/g for L. acutangula and L. cylindrica respectively. Phenolic compounds present in plants are responsible for various pharmacological activities owned by plants. Thus, estimation of phenolic content from the plant extracts helps us to determine the potential of the activities. The study of total phenolic content revealed standard calibration equation as y = 0.0049x – 0.0971 (R^2 = 0.9892).

Table: 3 Comparison of total phenolic content of plant extracts

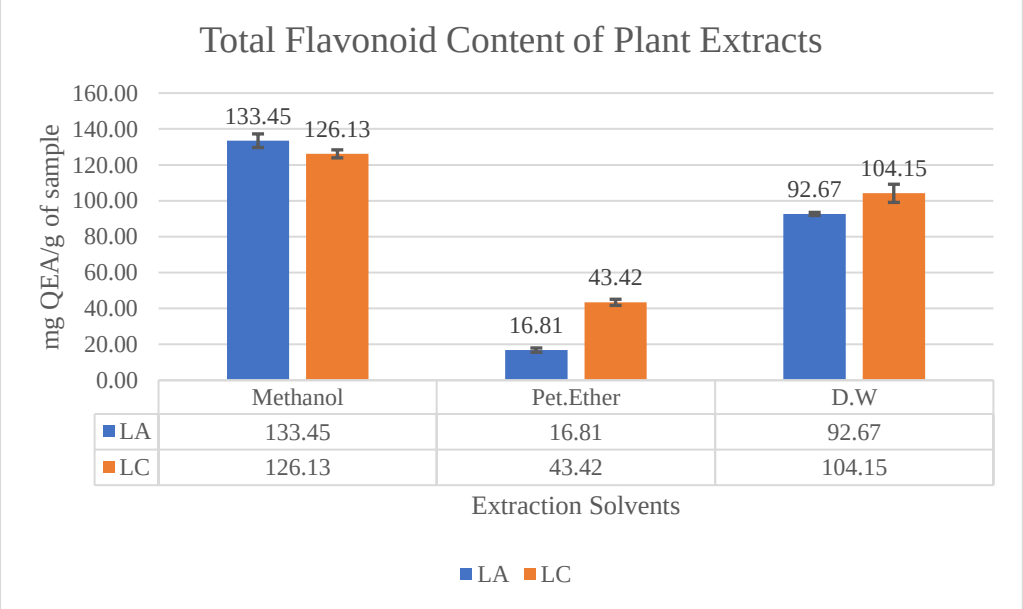


3.4 Determination of Total Flavonoid Content:

Total phenolic content in plants extracts was determined by aluminium chloride method. Total flavonoid content can be determined by Aluminium chloride as it form coloured complex with C4 keto group or C3/C5 hydroxyl group of flavonoids present in the plant extracts. This solution can be analysed using spectrophotometer at 415 nm (Shah et al., 2021).

The results of total flavonoid content in L. acutangula state the flavonoid content was observed highest in methanol extract as 133.450 mg QE/g followed by distilled water extract 92.669 and 16.809 mg QE/g in petroleum ether extract. In case of L. cylindrica methanol extracts showed highest amount of flavonoids in them with values 126.132 mg QE/g and least amount in petroleum ether extract 43.424 mg QE/g. Flavonoid content in distilled water extracts showed moderate values compared to both solvents as 104.153 mg QE/g. The study of total phenolic content revealed standard calibration equation as $y = 0.0128x + 0.1165$ ($R^2 = 0.9915$).

Table: 4 Comparison of total flavonoid content of plant extracts



3.5 Correlation between TPC, TFC and Antioxidant Capacity:

Many researchers have reported phenolic compounds are responsible for exhibiting antioxidant activity. Phenolic compounds are proved to be potential in scavenging free radicals due to presence of certain functional groups in their molecular structures (Singh et al., 2016). Phenolic compounds possess large number of hydroxyl groups and conjugated double bonds in their structures which lead to scavenge free radicals and prevent damage caused by them.

The extracts of the plants in different solvents were correlated amongst parameters tested namely TPC, TFC and FRAP. The results have shown strong positive correlation between phenolic and flavonoid contents of the plants extracts with FRAP assay. Correlation results were obtained significant at the 0.05 level (2-tailed) in methanolic and distilled water extracts of Luffa acutangula and methanolic extract of Luffa cylindrica. In Luffa acutangula, the correlation between TPC and FRAP, and TFC and FRAP have revealed highest correlation in methanolic extracts and water extracts with correlation coefficient (r = > 0.998*) and lower in pet-ether extract (r = 0.891) between TPC and TFC. Though, positive correlation was found between FRAP and TFC (r = 0.995). In Luffa cylindrica, Significant positive correlation was obtained. In methanol extracts, TPC have shown highest values (r = 1.000*) with FRAP. TPC and TFC, and TFC and FRAP have shown correlation as (r = 0.931 and r = 0.921) respectively. In pet-ether and distilled water extracts, highest positive correlation found between TFC and FRAP with values (r = 0.986 and r = 0.955) respectively. Slightly lower values were obtained between TPC and FRAP (r = 0.950 and 0.948) in same order. Lowest correlation was found amongst all data was found between TFC and TPC pet-ether and distilled water extracts (r = 0.885 and r = 0.811) respectively.

Table: 5 Pearson correlation coefficient (r) between TPC, TFC and FRAP of Luffa acutangula

Luffa acutangula											
Methanol				Petroleum ether				Distilled water			
(r)	TPC	TFC	FRAP	(r)	TPC	TFC	FRAP	(r)	TPC	TFC	FRAP
TPC	1	1.000*	.998*	TPC	1	0.891	0.839	TPC	1	.999*	1.000*
TFC	1.000*	1	.999*	TFC	0.891	1	0.995	TFC	.999*	1	1.000*
FRAP	.998*	.999*	1	FRAP	0.839	0.995	1	FRAP	1.000*	1.000*	1

*. Correlation is significant at the 0.05 level (2-tailed).

Table: 6 Pearson correlation coefficient (r) between TPC, TFC and FRAP of Luffa cylindrica

Luffa cylindrica											
Methanol				Petroleum ether				Distilled water			
(r)	TPC	TFC	FRAP	(r)	TPC	TFC	FRAP	(r)	TPC	TFC	FRAP
TPC	1	0.931	1.000*	TPC	1	0.885	0.950	TPC	1	0.811	0.948
TFC	0.931	1	0.921	TFC	0.885	1	0.986	TFC	0.811	1	0.955
FRAP	1.000*	0.921	1	FRAP	0.950	0.986	1	FRAP	0.948	0.955	1

*. Correlation is significant at the 0.05 level (2-tailed).

4. CONCLUSION

The findings of the study revealed that both plants of Cucurbitaceae have shown significant number of phytochemicals. Importantly, significant results were obtained from the peel extracts of the plants. Thus, it has made us to believe that peels have significant amount of phytochemicals and antioxidant potential in them. The maximum number of phytochemicals were obtained from the methanol extracts of both plants. Antioxidant potential of the plant extracts were also found to be highest in methanol extracts. Thus, it is found to be better solvent compared to distilled water and petroleum ether in the present study. Luffa acutangula peel extracts have shown maximum amount of phenolic and flavonoid contents. Moreover, it has also shown maximum potential to scavenge free radicals. Luffa cylindrica

has also shown significant quantity of phenols and flavonoids in them. Their ability to scavenge free radicals make them important in medicinal plants. Luffa cylindrica and Luffa acutangula peel extracts contain certain phytochemicals which are responsible for various pharmacological activities reported. These properties of the plants help us to conclude that they can be used to form various medicines in upcoming future.

5. REFERENCES

1) Adnan, M.; Gul, S.; Batool, S.; Fatima, B.; Rehman, A.; Yaqoob, S.; Shabir, H.; Yousaf, T.; Mussarat, S.; Ali, N.; et al. A review on the ethnobotany, phytochemistry, pharmacology and nutritional composition of Cucurbita pepo L. J. Phytopharm. 2017, 6, 133–139.
2) Avinash, T., & Rai, V. (2017). An ethanobotanical investigation of cucurbitaceae from South India: A review. Journal of Medicinal Plants Studies, 5(3), 250-253.
3) Bellur Nagarajaiah, S., & Prakash, J. (2015). Chemical composition and bioactive potential of dehydrated peels of Benincasa hispida, Luffa acutangula, and Sechium edule. Journal of Herbs, Spices & Medicinal Plants, 21(2), 193-202.
4) Bhati, R., Desai, K., & Modi, N. R. The preliminary phytochemical screening, quantification of phenols and flavonoids and antioxidant potential analysis of leaf sample of Ethnomedicinal plant Actinoscirpus grossus var. kysoor (Roxb.) Noltie (Family: Cyperaceae).
5) Blainski, A., Lopes, G. C., & De Mello, J. C. P. (2013). Application and analysis of the folin ciocalteu method for the determination of the total phenolic content from Limonium brasiliense L. Molecules, 18(6), 6852-6865.
6) Chand, K., Hiremathad, A., Singh, M., Santos, M. A., & Keri, R. S. (2017). A review on antioxidant potential of bioactive heterocycle benzofuran: Natural and synthetic derivatives. Pharmacological Reports, 69(2), 281-295.
7) Chen, Q., Shi, Q., Gorb, S. N., & Li, Z. (2014). A multiscale study on the structural and mechanical properties of the luffa sponge from Luffa cylindrica plant. Journal of Biomechanics, 47(6), 1332-1339.
8) Du, Q., Xu, Y., Li, L., Zhao, Y., Jerz, G., & Winterhalter, P. (2006). Antioxidant constituents in the fruits of Luffa cylindrica (L.) Roem. Journal of Agricultural and Food Chemistry, 54(12), 4186-4190.
9) Du, Q., Xu, Y., Li, L., Zhao, Y., Jerz, G., & Winterhalter, P. (2006). Antioxidant constituents in the fruits of Luffa cylindrica (L.) Roem. Journal of Agricultural and Food Chemistry, 54(12), 4186-4190.
10) Duke J.A., Handbook of Phytochemical and Constituents of Grass Herbs and other Economic Plants, CRC Press, Boco Raton, 98-119 (1999)
11) Fallah H.S.M., Alavian H.R., Heydari M.R. and Abolmaali K., The efficacy of liv-0 on liver cirrhotic patients: A randomised double-blind placebo-controlled first approach, Phytomedicine, 12(9), 619–625 (2005)
12) Harborne, A. J. (1998). Phytochemical methods a guide to modern techniques of plant analysis. springer science & business media.
13) Irshad, M., Ahmad, I., Mehdi, S. J., Goel, H. C., & Rizvi, M. M. A. (2014). Antioxidant capacity and phenolic content of the aqueous extract of commonly consumed cucurbits. International Journal of Food Properties, 17(1), 179-186.
14) Kadia, R., Bhavsar, S., Sapra, P., Pandya, H., Mankad, A., & Modi, N. Phytochemical Investigation and Antioxidant Activity of Ethnomedicinal Plants from Ratanmahal and Udalmahuda Forest, Dahod.
15) Kadu, S., Attarde, D., Kulkarni, D., Shelke, S., & Arsul, V. (2021). Comparative Evaluation of multiple extracts of Pericarp of Luffa acutangula (L.) for its significant Antioxidant, Antibacterial and Antifungal activity. Research Journal of Pharmacy and Technology, 14(4), 1847-1853.
16) Mamelona, J., Pelletier, E., Girard-Lalancette, K., Legault, J., Karboune, S., & Kermasha, S. (2007). Quantification of phenolic contents and antioxidant capacity of Atlantic sea cucumber, Cucumaria frondosa. Food Chemistry, 104(3), 1040-1047.
17) Mohamed, E. A. A., Muddathir, A. M., & Osman, M. A. (2020). Antimicrobial activity, phytochemical screening of crude extracts, and essential oils constituents of two Pulicaria spp. growing in Sudan. Scientific reports, 10(1), 1-8.

18) Mukherjee, P.K., 2019. Ethnopharmacology and ethnomedicine-inspired drug development. In: Mukherjee, P.K. (Ed.), Quality Control and Evaluation of Herbal Drugs. Elsevier, pp. 29–51. https://doi.org/10.1016/B978-0-12-813374-3.00002-8.
19) Pandey, A., & Tripathi, S. (2014). Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. Journal of Pharmacognosy and Phytochemistry, 2(5).
20) Parimi, R., & Pravallika, K. E. (2017). Studies on phytochemical screening, total phenolic content and in vitro antioxidant activity of different extracts of Euphorbia thymifolia roots. Research Journal of Pharmacy and Technology, 10(2), 551-556.
21) Phillips, K.M.; Ruggio, D.M.; Ashraf-Khorassani, M. Phytosterol composition of nuts and seeds commonly consumed in the United States. J. Agric. Food Chem. 2005, 53, 9436–9445.
22) Rahman A.S.H., Bottle gourd (Lagenaria siceraria) a vegetable for good health, National Product Radiance, 2, 249-250 (2003)
23) Rajasree, R. S., Sibi, P. I., Francis, F., & William, H. (2016). Phytochemicals of Cucurbitaceae family—A review. International Journal of Pharmacognosy and Phytochemical Research, 8(1), 113-123.
24) Rolnik, A., & Olas, B. (2020). Vegetables from the Cucurbitaceae family and their products: Positive effect on human health. Nutrition, 78, 110788.
25) Saboo, S. S., Thorat, P. K., Tapadiya, G. G., & Khadabadi, S. S. (2013). Ancient and recent medicinal uses of cucurbitaceae family. International Journal of Therapeutic Applications, 9, 11-19.
26) Shah, R., Sharma, P., & Modi, N. (2021). Preliminary phytochemical analysis and assessment of total phenol and total flavonoid content of Haworthiopsis limifolia Marloth. and its antioxidant potential. International Journal of Botany Studies, 6(3), 902-909.
27) Shendge, P. N., & Belemkar, S. (2018). Therapeutic potential of Luffa acutangula: A review on its traditional uses, phytochemistry, pharmacology and toxicological aspects. Frontiers in pharmacology, 9, 1177.
28) Shrivastava J., Lambart J. and Vietmeyer N., Medicinal Plants: An expanding role in development, World Bank Technical paper, 320 (1996)
29) Singh, J., Singh, V., Shukla, S., & Rai, A. K. (2016). Phenolic content and antioxidant capacity of selected cucurbit fruits extracted with different solvents. J. Nutr. Food Sci, 6(6), 1-8.
30) Suryanti, V., Marliyana, S. D., & Putri, H. E. (2016). Effect of germination on antioxidant activity, total phenolics,[Beta]-carotene, ascorbic acid and [alpha]-tocopherol contents of lead tree sprouts (Leucaena leucocephala (lmk.) de Wit). International Food Research Journal, 23(1), 167.
31) Swetha, M. P., & Muthukumar, S. P. (2016). Characterization of nutrients, amino acids, polyphenols and antioxidant activity of Ridge gourd (Luffa acutangula) peel. Journal of food science and technology, 53(7), 3122-3128.
32) Tijani Y., Uguru M.O. and Salawu O.A., Anti-pyretic, anti-inflamatory and anti-diarrhoel properties of Faidherbia albida in rats, African J. Biotech., 7(6), 696- 700 (2008)
33) Ulewicz-Magulska, B., & Wesolowski, M. (2019). Total phenolic contents and antioxidant potential of herbs used for medical and culinary purposes. Plant Foods for Human Nutrition, 74(1), 61-67.
34) Vellapandian, C. (2022). Phytochemical Studies, Antioxidant Potential, and Identification of Bioactive Compounds Using GC–MS of the Ethanolic Extract of Luffa cylindrica (L.) Fruit. Applied Biochemistry and Biotechnology, 1-15.
35) Yadav, A. S., & Bhatnagar, D. (2007). Free radical scavenging activity, metal chelation and antioxidant power of some of the Indian spices. Biofactors, 31(3-4), 219-227.
36) Zhou, Y., Ma, Y., Zeng, J., Duan, L., Xue, X., Wang, H., ... & Huang, S. (2016). Convergence and divergence of bitterness biosynthesis and regulation in Cucurbitaceae. Nature Plants, 2(12), 1-8.