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Research Article

IN-VITRO ANTI-OXIDANT ACTIVITY OF CITRULLUS LANATUS SEED EXTRACTS

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ABSTRACT

Objective: The objective of present work is to study the In-vitro anti-oxidant activities of n-Hexane, Chloroform and Ethanol extract of Citrullus lanatus seeds to find out the extract having highest anti-oxidant activity. **Material and Methods:** The In-vitro anti-oxidant activity of the extracts were studied using 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity, Ferric reducing power activity, Hyderogen peroxide(H_2O_2) scavenging activity and Nitric Oxide(NO) scavenging activity. The total Phenolic contents and Flavanoid contents were estimated taking Gallic Acid and Quercetin calibration curve respectably. **Results:** In In-vitro anti-oxidant studies it was found that all the extracts posses In-vitro anti-oxidant activities. But the order of possessing activities were n-hexane>ethanol>chloroform extracts of Citrullus lanatus seeds. The total Phenolic content was highest in n-hexane extract and Total Flavanoid content was highest in ethanol extract. **Conclusion:** It can be concluded that Citrullus lanatusseed extracts possess anti-oxidant activities and the potency of anti-oxidant activities depends on the type of extract. The n-hexane extract of Citrullus lanatus seeds possess highest anti-oxidant activity in-vitro.

Keywords: Citrullus lanatus, In-vitro anti-oxidant, 1,1-diphenyl-2-picryl hydrazyl (DPPH), Total Phenolic Content,

INTRODUCTION

Oxidative damage to cellular biomolecules such as lipids, proteins and DNA is thought to play a crucial role in the incidence of several chronic diseases.[1–5] Flavonoids are a group of polyphenolic compounds found abundantly in the plant kingdom. Interest in the possible health benefits of flavonoids and other polyphenolic compounds has increased in recent years owing to their potent antioxidant and free-radical scavenging activities. [6–12]

The effects of free radicals on human beings are closely related to toxicity, disease and aging.[1] Most living species have an efficient defense system to protect themselves against the oxidative stress induced by Reactive Oxygen Species (ROS) [2]. Recent investigations have shown that the antioxidant properties of plants could be correlated with oxidative stress defense and different human diseases including cancer, atherosclerosis and the aging process.[3-5] The antioxidants can interfere with the oxidation process by reacting with free radicals, chelating free catalytic metals and also by acting as oxygen scavengers.

Citrullus lanatus of family Cucurbitaceae is commonly known as water melon and in local name Tarmuz (Hindi), Puchakaya (Telugu). The ripe fruits are edible and largely used for making confectionary. Its nutritive values are also useful to the human health. Fruit is used in cooling, strengthening, aphrodisiac, astringent to the bowels, indigestible, expectorant, diuretic, and stomachic, purifies the blood, allays thirst, cures biliousness, good for sore eyes, scabies and itches and as brain tonic to the brain [13]. It also reported having analgesic and anti-inflammatory of seeds [14], anti-ulcerative activity [15-16], antimicrobial activity [17], laxative activity of fruit [18], antioxidant of fruit [19], hepatoprotective [20] and anti-hyperlipidemic [21].

Furthermore, literature survey reveals the uses Citrullus lanatus fruit and have various biological activities including anti-oxidants. But seeds are not in generally given importance and hence the present investigation was conducted to study In-vitro antioxidant activities of various seed extracts so as to make researcher to route for other pharmacological activities.

MATERIAL AND METHODS

Plant material and extraction procedures

Citrullus lanatus fruit were purchased local market from Kodad, Andhra Pradesh and seeds were collected from fruits and was authenticated by Prof. Dr.K.Madhava chetty ,Taxonomist, ,SVU University, Chithoor, Andhra Pradesh (India).The air dried seeds were made into coarse powder and extracted with Ethanol, Choloform and n-Hexane and percentage yield were calculated.

Preliminary Phytochemical Analysis

The various extracts of Citrullus lanatus were tested for different phytoconsituents like alkaloids, glycosides, saponinins, tannins, terpinoids, phenolic compounds, protein, carbohydrates using standard procedures .[22]

In-vitro Anti-oxidant activity

DPPH radical scavenging activity

The ability of the plant extract to scavenge 1,1-diphenyl-2-picryhydrazyl (DPPH) free radicals was assessed by the standard method. [23] The stock solution of extracts were prepared in ethanol to achieve the concentration of 1 mg/ml. Dilutions were made to obtain concentrations of 50, 100, 250, 500 µg/ml. Diluted solutions (1 ml each) were mixed with 3 ml of ethanolic solution of DPPH (DPPH, 0.004%). After 30 min of incubation at room temperature the reduction of the DPPH free radical was measured by reading the absorbance at 517nm using UV-Visible Spectrophotometer. Initially, absorption of blank sample containing the same amount of ethanol and DPPH solution was prepared and measured as control. Ascorbic acid was used as standard. The experiment was carried out in triplicate. Percentage inhibition was calculated using equation (1), whilst IC_{50} values were estimated from the % inhibition versus concentration plot, using a non-linear regression algorithm. The data were presented as mean values ± standard deviation (n = 3).

$$\% \text{ inhibition} = \frac{(\text{Absorbance of control} - \text{absorbance of sample})}{\text{Absorbance of control}} \times 100 \quad \text{equation (1)}$$

Ferric Reducing Power Activity

The reducing powers of the extracts were determined by the method. [24] Various concentration (50, 100, 250, 500 µg /ml) of

extracts were prepared in 1ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN_6]$ (2.5 ml, 1%). The mixture was incubated at 50◦C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 RPM for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl3(0.5 ml, 0.1%) and the absorbance was measured at700 nm. Ascorbic acid was used as the reference material. All the tests were performed in triplicate and the graph was plotted with the average of three observations.

Hydrogen peroxide scavenging activity

Scavenging activity of Hydrogen peroxide (H_2O_2) by the plant extract was determined by the method [26]. Plant extract (4 ml) prepared in distilled water at various concentration(50, 100, 250, 500 µg/ml) was mixed with 0.6 ml of 4 mM H_2O_2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm. Ascorbic acid was used as a positive control compound. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples using Eq. (1). IC_{50} values were estimated from the % inhibition versus concentration plot, using a non-linear regression algorithm.

Nitric oxide scavenging activity

Nitric oxide radical scavenging activity was determined according to the method. [27] 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract at various concentrations prepared in ethanol and the mixture incubated at 25◦C for 30 min. Thereafter, 1.5ml of Griess reagent (1% sulphanilamide, 0.1% naphthylethylenediamine dichloride and 3% phosphoric acid) was added to each test tube. The absorbance was measured, immediately, at 546 nm and percentage of scavenging activity was measured with reference to ascorbic acid as standard. The nitric oxide radicals scavenging activity was calculated. The percentage inhibition of nitric oxide generated was measured by comparing the absorbance values of control and test samples using Eq. (1). IC_{50} values were estimated from the % inhibition versus concentration plot, using a non-linear regression algorithm.

Estimation of total phenolic content

Total phenolic content (TPC) were determined using Folin-Ciocalteu reagent.[28] Briefly, an aliquot of the sample extract (0.1 ml of 1000 µg/ml in ethanol) was mixed with distilled water (3 ml) and 0.5 ml of Folin-Ciocalteu reagent was added. After 3 min, 2 ml of 20% sodium carbonate was added and mixed thoroughly. The tubes were incubated in a boiling water bath for exactly 1 min, then cooled and the absorbance was measured at 650 nm using against the reagent blank. The calibration curve was prepared by gallic acid solution (0 - 100µg/ml) in ethanol. TPC was expressed as mg gallic acid equivalent (GAE)/100 g sample dry weight.

Total flavonoids determination

Aluminum chloride colorimetric method was used to determine Total Flavonoids contents in extracts. [29-30] briefly, an aliquot of 0.5 ml of 2% AlCl3 was added to 0.5 ml of sample solution. After 1 h at room temperature, the absorbance was measured at 420 nm at the final concentration of 1000 µ/ml). TFC was calculated as mg quercetin equivalent (QE) /100 g sample dry weight. The calibration curve was prepared by quercetin solution (0 - 100µg/ml) in ethanol.

RESULTS

Result of solvent Extraction

The successive solvent extraction was done using n-hexane, chloroform and ethanol using standard procedure. The behavior of various extracts like texture and colour and extractive yield were calculated. The results are given in Table 1.

Result of Preliminary Phytochemical Analysis

The various extracts of *Citrullus lanatus*seeds were tested for different phytoconsituents like alkaloids, glycosides, saponinins, tannins, terpinoids, reducing sugars, phenolic compounds, flavanoids, protein, carbohydrates and volatile oils using standard procedures and the results are given in Table 2.

Table 1: Yield and physical properties of *Citrullus lanatus*seed extracts

Solvent	Texture of Extract	Colour	% Yield
n-Hexane	Oily	Light yellow	16.43
Chloroform	Solid	Light brown	1.21
Ethanol	Waxy	Intense brown	7.32

Table 2: Result of preliminary phytochemical analysis of *Citrullus lanatus*seed extracts

Sl. No.	Phytoconstitutents	n-Hexane	Chloroform	Ethanol
1.	Alkaloids	-Ve	+Ve	-Ve
2.	Protein	-Ve	-Ve	-Ve
3.	Carbohydrate	-Ve	-Ve	+Ve
4.	Reducing sugar	-Ve	-Ve	-Ve
5.	Tannins	-Ve	+Ve	+Ve
6.	Saponins	-Ve	-Ve	+Ve
7.	Terpenoids	+Ve	+Ve	+Ve
8.	Glycosides	+Ve	-Ve	+Ve
9.	Flavanoids	-Ve	-Ve	+Ve
10.	Phenolics	+Ve	+Ve	+Ve
11.	Volatile oil	-Ve	-Ve	-Ve

+Ve-Present, -Ve-Absent

Results of *In-vitro* Antioxidant Activities

Results of DPPH free radical scavenging activity

The DPPH radical scavenging activity of n-Hexane extract of *Citrullus lanatus*(HECL), Chloroform extract of *Citrullus lanatus*(CECL) and Ethanol extract of *Citrullus lanatus*(EECL) seeds were detected and compared with Ascorbic acid. The percentage inhibition (% inhibition) at various concentration (50- 500 µg/ml) of HECL, CECL and EECL as well as standard Ascorbic acid (12.5 -100 µg/ml) were calculated and plotted in Fig 1 using Microsoft Office Excel 2007. The IC_{50} values are calculated from graph and were found Ascorbic acid (39.85 µg/ml), HECL (149.09 µg/ml), CECL (315.50 µg/ml) and EECL (183.52 µg/ml).

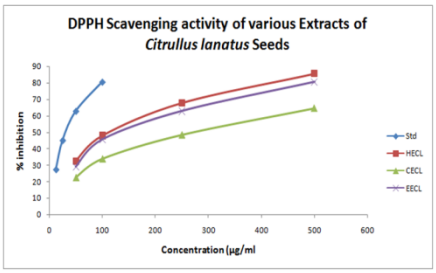

Figure1: DPPH scavenging activity of different *Citrullus lanatus* seed extracts

Results of Reducing power activity

The reductive capabilities of n-Hexane extract of *Citrullus lanatus*(HECL), Chloroform extract of *Citrullus lanatus*(CECL) and Ethanol extract of *Citrullus lanatus*(EECL) seeds were detected and compared with Ascorbic acid. The mean absorbance at various concentration (50- 500 µg/ml) of HECL, CECL and EECL as well as standard Ascorbic acid (12.5 -100 µg/ml) were calculated and plotted in Fig-2 using Microsoft Office Excel 2007. The reductive capabilities were found to increase with increasing of concentration in various extract as well as standard ascorbic acid.

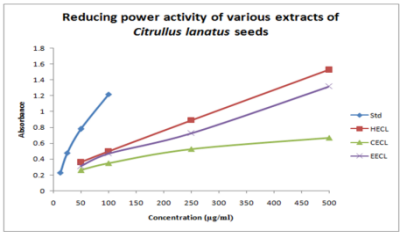

Figure 2: Reducing power activity of different *Citrullus lanatus* seed extracts

Results of Hydrogen Peroxide scavenging activity

The Hydrogen Peroxide scavenging activity of n-Hexane extract of *Citrullus lanatus*(HECL), Chloroform extract of *Citrullus lanatus*(CECL) and Ethanol extract of *Citrullus lanatus*(EECL) seeds were detected and compared with Ascorbic acid. The percentage inhibition (% inhibition) at various concentration (50- 500 µg/ml) of HECL, CECL and EECL as well as standard Ascorbic acid (12.5 -100 µg/ml) were calculated and plotted in Fig 3 using Microsoft Office Excel 2007. The IC_{50} values are calculated from graph and were found Ascorbic acid (43.12 µg/ml), HECL (164.13 µg/ml), CECL (339.88 µg/ml) and EECL (227.17 µg/ml).

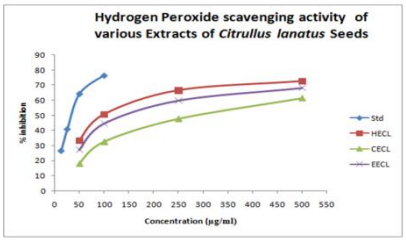

Figure 3: Hydrogen Peroxide scavenging activity of different *Citrullus lanatus* seed extracts

Results of Nitric Oxide scavenging scavenging activity

The Nitric oxide scavenging activity of n-Hexane extract of *Citrullus lanatus*(HECL), Chloroform extract of *Citrullus lanatus*(CECL) and Ethanol extract of *Citrullus lanatus*(EECL) seeds were detected and compared with Ascorbic acid. The percentage inhibition (% inhibition) at various concentration (50- 500 µg/ml) of HECL, CECL and EECL as well as standard Ascorbic acid (12.5 -100 µg/ml) were calculated and plotted in Fig-4 using Microsoft Office Excel 2007. The IC_{50} values are calculated from graph and were found Ascorbic acid (46.59 µg/ml), HECL (174.44 µg/ml), CECL (309.13 µg/ml) and EECL (238.75 µg/ml).

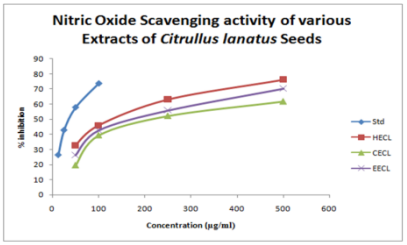

Figure 4: Nitric Oxide scavenging activity of different *Citrullus lanatus*seed extracts

Results for Total Phenolic contents

The Total phenolic contents in n-Hexane extract of *Citrullus lanatus*(HECL), Chloroform extract of *Citrullus lanatus*(CECL) and Ethanol extract of *Citrullus lanatus*(EECL) seeds were estimated using standard Gallic acid equivalent of phenols. The various concentration of Gallic acid (10-200 µg/ml) calibration curve was plotted using Microsoft Office Excel 2007 and the results were given in Table 3 and in Fig 5. The total phenolic contents for HECL, CECL and EECL were obtained for 1000 µg/ml of extracts from Total Phenolic content calibration of gallic acid and the result are given in Table 4. The Total phenolic content for HECL, CECL and EECL were calculated using standard calibration curve (y=0.007x+ 0.056, R^2=0.995) and found to have 76.28±0.13, 27.71±0.11 and 42.34±0.21 mg/g equivalent of Gallic Acid respectably.

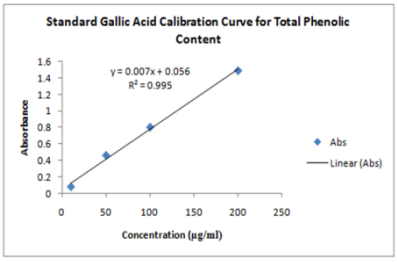

Figure 5: Standard Calibration curve for Total Phenolic contents for standard Gallic Acid

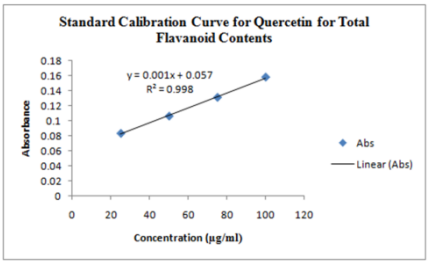

Figure 6: Standard Calibration curve for Total flavaoid contents for standard Quercetin

Table 3: Total Phenolic contents for calibration of standard Gallic Acid

Sl. No.	Concentration (µg/ml /ml)	Absorbance (mean ±SD)
1	10	0.083±0.02
2	50	0.463±0.01

3	100	0.803±0.02
4	200	1.486±0.04

Table 4: Total Phenolic contents of different *Citrullus lanatus*seed extracts

xtract	Concentration	Total Phenolic Content (mg/gGAE)
HECL	1000(ug/ml)	76.28±0.13
CECL	1000(ug/ml)	27.71±0.11
EECL	1000(ug/ml)	42.34±0.21

Values are in Mean ±SD for three readings

Results for Total Flavanoid content

The Total flavanoid contents in n-Hexane extract of *Citrullus lanatus*(HECL), Chloroform extract of *Citrullus lanatus*(CECL) and Ethanol extract of *Citrullus lanatus*(EECL) seeds were estimated using standard Quercetin equivalent of phenols. The various concentration of Quercetin (25-100 µg/ml) calibration curve was plotted using Microsoft Office Excel 2007 and the results were given in Table 5 and in Fig 6. The total phenolic contents for HECL, CECL and EECL were obtained for 1000 µg/ml of extracts from Total flavanoid content calibration of Quercetin and the result are given in Table 6. The Total flavanoid content for HECL, CECL and EECL were calculated using standard calibration curve (y=0.001x+ 0.057, R^2=0.998) and found to have 83.12±0.13, 43.09±0.11 and 113.53±0.21mg/g equivalent of Quercetin respectably.

Table 5: Total Flavanoid contents calibration of standard Quercetin

Sl. No.	Concentration (ug/ml)	Absorbance (mean ±SD)
1	25	0.085±0.001
2	50	0.106±0.001
3	75	0.133±0.002
4	100	0.158±0.002

Table 6: Total Flavanoid contents of different *Citrullus lanatus* seed extracts

Extract	Concentration	Total Flavanoid Content (mg/gE of Quercetin)
HECL	1000(ug/ml)	83.12±0.13
CECL	1000(ug/ml)	43.09±0.11
EECL	1000(ug/ml)	113.53±0.21

Values are in Mean ±SD for three readings

DISCUSSION

The *Citrullus lanatus*seeds were made coarse powder and extracted with using n-hexane, chloroform and ethanol as solvent using standard procedure. The behavior of various extracts like texture and colour and extractive yield were calculated. It is found that percentage yield for n–hexane extract is more than other solvent extract. The difference in yield in hexane extract is due to presence of oils in hexane extracts and different extractive values revealed the solubility and polarity particulars of the metabolites for particular solvent.

The various extracts of *Citrullus lanatus*seeds were tested for different phytoconsituents like alkaloids, glycosides, saponinins, tannins, terpinoids, reducing sugars, phenolic compounds, flavanoids, protein, carbohydrates and volatile oils. The Knowledge of the chemical constituents of plants is desirable because such information will be valuable for synthesis of complex chemical substances and to screen for biological activities.[31, 44, 45] The phenolic and flavanoids are widely distributed secondary metabolites in plants having anti-oxidant activity and have wide range of biological activities as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities.[32-33] Recent studies have shown that many dietary polyphenolic constituents derived from plants are more effective antioxidants *In-vitro* than vitamins E or C, and thus might contribute significantly to the protective effects *in vivo.*[34]

In-vitro antioxidant studies are widely carried to screen various plant containing phenolic and flavanoids constituents. Plant derived antioxidant compounds, flavonoids and phenolics have received considerable attention because of their physiological effect like antioxidant, anti-inflammatory, antitumor activities and low toxicity compared with those of synthetic phenolics antioxidant such as BHA (Butylated Hydroxyanisole), BHT (Butylated Hydroxytoluene) and Propyl Gallate(PG).[35-36]

DPPH is a purple colored stable free radical; when reduced it becomes the yellow-colored diphenyl-picryl hydrazine. DPPH radicals react with suitable reducing agents and then electrons become paired-off and the solution loses colour stoichimetrically with the number of electrons taken up. [37] Such reactivity has been widely used to test the ability of compounds/plant extracts to act as free radical scavengers. [36] In this present study, the DPPH radical scavenging activity of n-Hexane extract of *Citrullus lanatus*(HECL),

Chloroform extract of *Citrullus lanatus*(CECL) and Ethanol extract of *Citrullus lanatus*(EECL) seeds were detected and compared with Ascorbic acid. The IC_{50} values for DPPH assay of for hexane extract was found maximum followed by ethanol extract and for chloroform extract was minimum. Though the extracts showed good DPPH scavenging activity but it was less effective than standard Ascorbic acid. The difference of activity is due to presence of phenolic components in different extracts. Thus, choosing the appropriate solvent is one of the most important factors for obtaining extracts with a high content of bioactive compounds and antioxidant activity. [38]

In ferric reducing antioxidant power assay (FRAP),a yellow colour of the test solution changes to various shades of green and blue is depending upon the reducing power of each compound. The presence of radicals (ie antioxidant) causes the conversion of the Fe^{3+} / ferricyanide complex used in this method to the ferrous form. Therefore by measuring the formation of pearls Prussian blue spectroscopic ally, the Fe^{2+} concentration can be monitored; a higher absorbance indicates a higher reducing power. The reductive capabilities of n-Hexane extract of *Citrullus lanatus*(HECL), Chloroform extract of *Citrullus lanatus*(CECL) and Ethanol extract of *Citrullus lanatus*(EECL) seeds were detected and compared with Ascorbic acid. The hexane extract showed highest reducing power followed by ethanol and then chloroform extracts. The increased reducing power in the extracts indicated that some components in the extract were electron donors that could react with the free

radicals to convert them into more stable products to terminate radical chain reaction. Antioxidants are strong reducing agents and this is principally based on the redox properties of their hydroxyl groups and the structural relationships between different parts of their chemical structure. [38-39]

Hydrogen peroxide (H_2O_2), a biologically relevant, non-radical oxidizing species, may be formed in tissues through oxidative processes. Hydrogen peroxide (H_2O_2) which in turn generate hydroxyl radicals (•OH) resulting in initiation and propagation of lipid peroxidation.[40] The hydrogen peroxide scavenging activity of n-Hexane extract of *Citrullus lanatus*(HECL), Chloroform extract of *Citrullus lanatus*(CECL) and Ethanol extract of *Citrullus lanatus*(EECL) seeds were detected and compared with Ascorbic acid. The IC_{50} values for hydrogen peroxide scavenging activity of for hexane extract was found maximum followed by ethanol extract and for chloroform extract was minimum. Though the extracts showed good hydrogen peroxide scavenging activity but it was less effective than standard Ascorbic acid. The ability of the extracts to quench OH^- seems to be directly related to the prevention of the lipid peroxidation and appears to be moderate scavenger of active oxygen species, thus reducing rate of chain reaction.[40]

Nitric oxide (NO) is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signalling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities.[41] The Nitric oxide scavenging activity of n-Hexane extract of *Citrullus lanatus*(HECL), Chloroform extract of *Citrullus lanatus*(CECL) and Ethanol extract of *Citrullus lanatus*(EECL) seeds were detected and compared with Ascorbic acid. The IC_{50} values for nitric oxide scavenging activity of for hexane extract was found maximum followed by ethanol extract and for chloroform extract was minimum. Though the extracts showed good nitric oxide activity but it was less effective than standard Ascorbic acid.

The Total phenolic contents in n-Hexane extract of *Citrullus lanatus* (HECL), Chloroform extract of *Citrullus lanatus*(CECL) and Ethanol extract of *Citrullus lanatus*(EECL) seeds were estimated using standard Gallic acid equivalent of phenols. The Total phenolic content for HECL, CECL and EECL were found to have 76.28, 27.71and 42.34mg/g equivalent of Gallic Acid respectably. The hexane extract was found to have maximum phenolic components and which may be one the reason of its to posses maximum antioxidant activity then other two extracts. As previously, it was reported that Polyphenolic compounds contribute significantly to the total antioxidant capacity of plants. [42]

But in total Flavanoid content, it was found Ethanolic extract to posses maximum 113.53 mg/g equivalent of Quercetin then other hexane (83.12 mg/g Eq) and chloroform (43.09 mg/g Eq). Flavonoids play some important pharmacological roles against diseases, such as cardiovascular disease, cancer, inflammation and allergy and other oxidative stress related diseases.[43]

From, above discussion, it was clear that the most powerful anti-oxidant extract is hexane exact of *Citrulus lanatus* (HECL) seed.

CONCLUSION

By performing the above work, it can be concluded that *Citrullus lanatus*seed extracts possess anti-oxidant activities and the potency of anti-oxidant activities depends on the type of extract. The n-hexane extract of *Citrullus lanatus*seeds possess highest anti-oxidant activity *in-vitro*. This anti-oxidant power depends on total phenolic and flavanoid contents on particular extract.

Conflict of Interest: No

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