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Research Article

IN-VITRO ANTI-OXIDANT ACTIVITY OF *CITRUS MACROPTERA* (VAR ANNAMENSIS) FRUIT PEELS 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 *Citrus macroptera (*var annamensis) fruit peels so as to find out the extract having highest anti-oxidant activity.

Methods: 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 anti-oxidant activities. The order of activities were ethanol> n-hexane> chloroform extracts of *Citrus macroptera* fruit peels. Ethanolic extract of *Citrus macroptera* fruit peels (EECM) in DPPH scavenging activity (IC_{50} 281.11 µg/ml), Hydrogen Peroxide scavenging activity (IC_{50} 216.49 µg/ml), Nitric Oxide scavenging activity (IC_{50} 182.89 µg/ml) and were comparable with standard Ascorbic acid. The total Phenolic content was highest in EECM (142.5±3.29 mg/gm Eq of Gallic Acid) and Total Flavanoid content was highest in EECM (333.0±36.06 mg/gm Eq of Quercetin).

Conclusion: *Citrus macroptera* fruit peels extracts possess anti-oxidant activities and the potency of anti-oxidant activities depends on the type of extract. The ethanol extract of *Citrus macroptera* fruit peels possess highest anti-oxidant activity *in-vitro.*

Keywords: *Citrus macroptera*, 1, 1-diphenyl-2-picryl hydrazyl (DPPH), Total Phenolic Content, Ascorbic acid, IC_{50} value.

INTRODUCTION

The importance of oxygen is known for all and for survival in this earth it is essential for every living being. Oxygen is utilized by all living organisms for normal physiological and metabolic activity. During utilization of oxygen in our body functions and metabolism around 5% of oxygen gets univalently reduced to oxygen derived free radicals like superoxide, peroxide, hydroxyl and nitric oxide free radicals [1-2]. All these are known as Reactive Oxygen Species (ROS) and exert oxidative stress towards the cell of human body rendering each cell to face 10000 oxidative hits per second [3]**.** Oxidative damage to cellular biomolecules such as lipids, proteins, and DNA is thought to play a crucial role in the development of degenerative diseases and pathogenesis of diabetes, cardiovascular diseases, nephrotoxicity, hepatotoxicity, neurological disorders, inflammations, cancer and in the process of aging [3-7].

It has been suggested that some natural plants, fruits and vegetables contain a large variety of substance called phytochemicals which are present in plants and are the main source of antioxidant in the diet, which could decrease the potential stress caused by reactive oxygen species [8-9]**.** Recently, it is observed that flavonoids and other polyphenolic compounds, Vitamin C, Carotinoids, tannins etc present in plants has antioxidant and free-radical scavenging activities[10-11].

Citrus macroptera var annamensis which belongs to the family of Rutaceae which is considered to be the native to the regions of Southeast Asia mainly Myanmar, Thailand, Indonesia, Malayasia, Papua New Guinea, Sylhet Division of northeastern Bangladesh and northeastern India mainly Manipur and Assam. Local in Bengali it is called "hatkora" or "shatkora" and in English known as Wild orange [12-13].

The fruit of *Citrus macroptera* var annamensis is edible and popular among the people of Bangladesh, Meghalaya and Assam of India. In Bangladesh both green matured fruits are used in cooking for flavoring curry mainly meat dishes, pickle preparation and oil in perfume production [14].

As per medicinal importance, some literature says that stem bark of *Citrus macroptera* posses antioxidant activity[15], essential oil of leaves posses antimicrobial activity[16] and traditionally fruits as appetite stimulant and treatment of fever [17]. Moreover, it is found that *Citrus macroptera* essential oil of leaves contains mainly terpenoids like limonene, and aromatic hydrocarbons [16, 18, 19] and Lupeol and Stigmasterol [15].

Except used as flavoring in curry and pickle there is few literature available for therapeutic value of fruit and hence the present investigation was conducted to study *In-vitro* antioxidant activities of various fruit peel extracts so as to make a clue for researcher for other pharmacological activities.

MATERIAL AND METHODS

Chemicals and Instruments

Ascorbic acid, quercetin, gallic acid, hydrogen peroxide, potassium ferricyanide, trichloroacetic acid, ferric chloride, folin - ciocalteu reagent, α-α diphenyl β picryl hydrazyl (DPPH), Griess reagent, were all purchased from SD-fine chemicals, India, all other reagents used were of analytical grade. Instruments UV/VIS Spectrophotometer (LABINDIA, UV 3000+), Microcentrifuge (REMI, RM-12 C)

Plant material and extraction procedures:

The fruits of *Citrus macroptera* Var. annamensis were collected from local areas of Assam state and and was authenticated by Prof. Dr. K. Madhava chetty, Taxonomist, ,SVU University, Chithoor, Andhra Pradesh (India). The air dried peels 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 *Citrus macroptera* Var. annamensis were tested for different phytoconsituents like alkaloids, glycosides, saponinins, tannins, terpinoids, phenolic compounds, protein, carbohydrates using standard procedures [20].

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 [21]. 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), while 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).

$$\%\ inhibition = \frac{Absorbance\ of\ control - absorbance\ of\ sample}{Absorbance\ of\ Control} X\ 100 \quad \textbf{eq. (1)}$$

Ferric Reducing Power Activity

The reducing powers of the extracts were determined by the method [22-23]. 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 $FeCl_3$(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 [24]. 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 [25]. 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 [26]. 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 [27-28]. 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 percentage yield for n-hexane , chloroform and ethanol extract of *Citrus macroptera* fruit peels were 16.2%, 1.2% and 22.4% respectably.

Result of Preliminary Phytochemical Analysis

The various extracts of *Citrus macroptera* fruit peel extracts 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 ethanol extract was found to contain more varieties of phytoconstituents and result is given in Table 1.

Table 1: Result of preliminary phytochemical analysis of *Citrus macroptera peels* extracts

S. No.	Phytoconstitutents	n-Hexane extract	Chloroform extract	Ethanolic extract
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

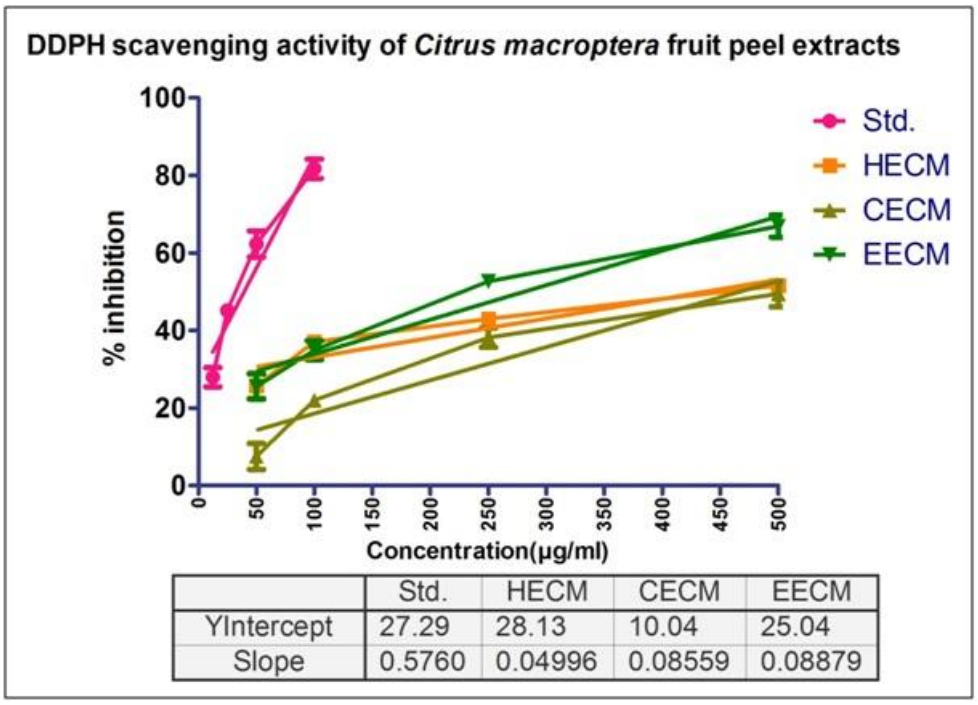
+ve- Present, -ve-Absent

Results of *In-vitro* Antioxidant Activities

Results of DPPH free radical scavenging activity

The DPPH radical scavenging activity of n-Hexane extract of *Citrus macroptera* (HECM), Chloroform extract of *Citrus macroptera*(CECM) and Ethanol extract of *Citrus macroptera*(EECM) fruit peels were detected and compared with Ascorbic acid and the results are given in Figure 1. The percentage inhibition (% inhibition) at various concentration (50- 500 µg/ml) of HECM, CECM and EECM as well as standard Ascorbic acid (12.5 -100 µg/ml) were calculated using

Graph pad prism version 5 by linear regression analysis .The IC_{50} values are calculated from graph and were found Ascorbic acid (39.42 µg/ml), HECM (437.75 µg/ml), CECM (466.87 µg/ml) and EECM (281.11 µg/ml).



	Std.	HECM	CECM	EECM
YIntercept	27.29	28.13	10.04	25.04
Slope	0.5760	0.04996	0.08559	0.08879

Fig. 1: DDPH scavenging activity of different *Citrus macroptera* var annamensis fruit peel extracts

Results of Reducing power activity

The reductive capabilities of n-Hexane extract of *Citrus macroptera* (HECM), Chloroform extract of *Citrus macroptera*(CECM) and Ethanol extract of *Citrus macroptera*(EECM) fruit peels were detected and compared with Ascorbic acid and the results are given in Figure 2. The mean absorbance at various concentration (50- 500 µg/ml) of HECM, CECM and EECM as well as standard Ascorbic acid (12.5 -100 µg/ml) were using Graph pad prism version 5 by linear regression analysis The reductive capabilities were found to increase with increasing of concentration in various extract as well as standard ascorbic acid.

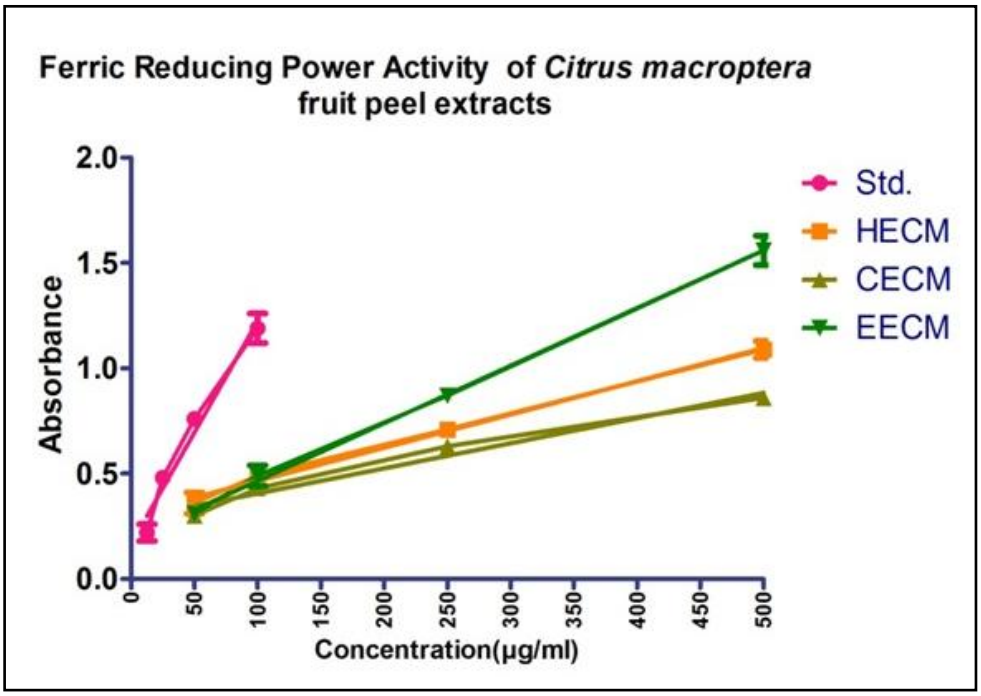
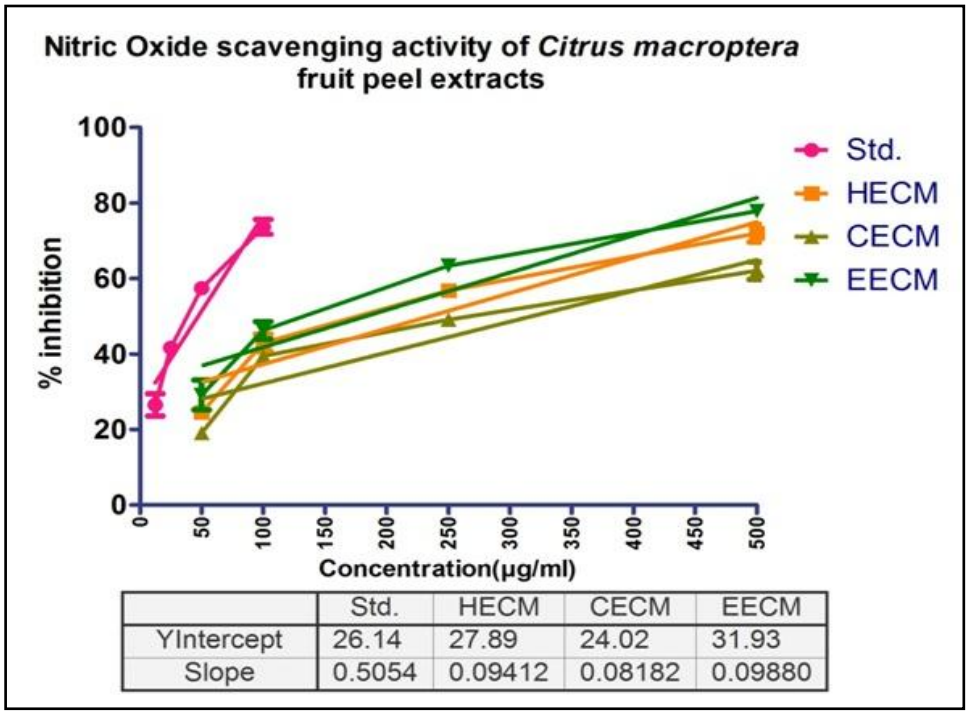


Fig. 2: Reducing power activity of different *Citrus macroptera* var annamensis fruit peel extracts

Results of Hydrogen Peroxide scavenging activity

The Hydrogen Peroxide scavenging activity of n-Hexane extract of *Citrus macroptera* HECM), Chloroform extract of *Citrus macroptera* (CECM) and Ethanol extract of *Citrus macroptera*(EECM) fruit peel extracts were detected and compared with Ascorbic acid and the results are given in Figure 3. The percentage inhibition (% inhibition) at various concentration (50- 500 µg/ml) of HECM, CECM and EECM as well as standard Ascorbic acid (12.5 -100 µg/ml) were calculated using Graph pad prism version 5 by linear regression analysis.. The IC_{50} values are calculated from graph and were found Ascorbic acid (43.23µg/ml), HECM (335.03 µg/ml), CECM (347.74 µg/ml) and EECM (216.49 µg/ml).

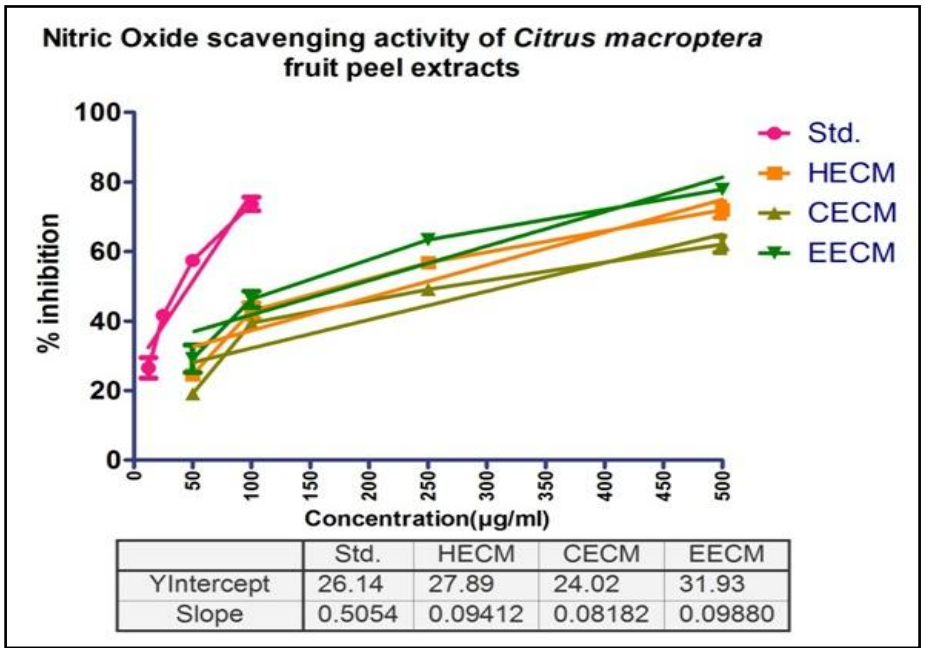


	Std.	HECM	CECM	EECM
YIntercept	26.14	27.89	24.02	31.93
Slope	0.5054	0.09412	0.08182	0.09880

Fig. 3: Hydrogen Peroxide scavenging activity of different *Citrus macroptera* var annamensis fruit peel extracts

Results of Nitric Oxide scavenging scavenging activity

The Nitric oxide scavenging activity of n-Hexane extract of *Citrus macoptera* (HECM), Chloroform extract of *Citrus macroptera*(CECM) and Ethanol extract of *Citrus macroptera*(EECM) fruit peels were detected and compared with Ascorbic acid and the results are given in Figure 4. The percentage inhibition (% inhibition) at various concentration (50-500 µg/ml) of HECM, CECM and EECM as well as standard Ascorbic acid (12.5 -100 µg/ml) were calculated and plotted using Graph pad prism version 5 by linear regression analysis. The IC_{50} values are calculated from graph and were found Ascorbic acid (46.69 µg/ml), HECM (234.91 µg/ml), CECM (317.52 µg/ml) and EECM(182.89 µg/ml).



	Std.	HECM	CECM	EECM
YIntercept	26.14	27.89	24.02	31.93
Slope	0.5054	0.09412	0.08182	0.09880

Fig. 4: Nitric Oxide scavenging activity of different *Citrus macroptera (*var annamensis) fruit peel extracts

Results for Total Phenolic contents

The Total phenolic contents in n-Hexane extract of *Citrus macroptera* (HECM), Chloroform extract of *Citrus macroptera*(CECM) and Ethanol extract of *Citrus macroptera*(EECM) fruit peels 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 Figure 5. The total phenolic contents for HECM, CECM and EECM were obtained for 1000 µg/ml of extracts from Total Phenolic content calibration of gallic acid and the result are given in Table-2. The Total phenolic content for HECM, CECM and EECM were calculated using standard calibration curve (y=0.007x+ 0.056, R^2=0.995) and found to have 98.68±7.32, 75.33±10.82, 142.5±3.29 equivalent of Gallic Acid respectably.

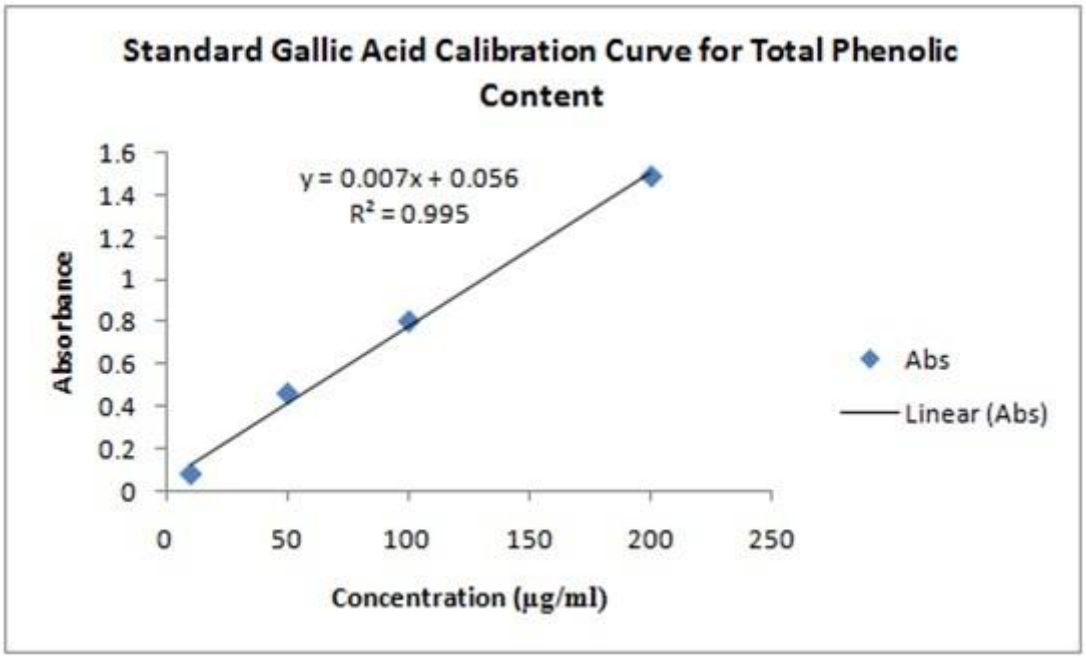


Fig. 5: Standard Calibration curve for Total Phenolic contents for standard Gallic Acid

Table 2: Total Phenolic contents of different *Citrullus macrotera* fruit peel extracts

Extract	Concentration	Total Phenolic Content (mg/g GAE)
HECM	1000(ug/ml)	98.68±7.32
CECM	1000(ug/ml)	75.33±10.82
EECM	1000(ug/ml)	142.5±3.29

Values are in Mean ±SD, for three readings

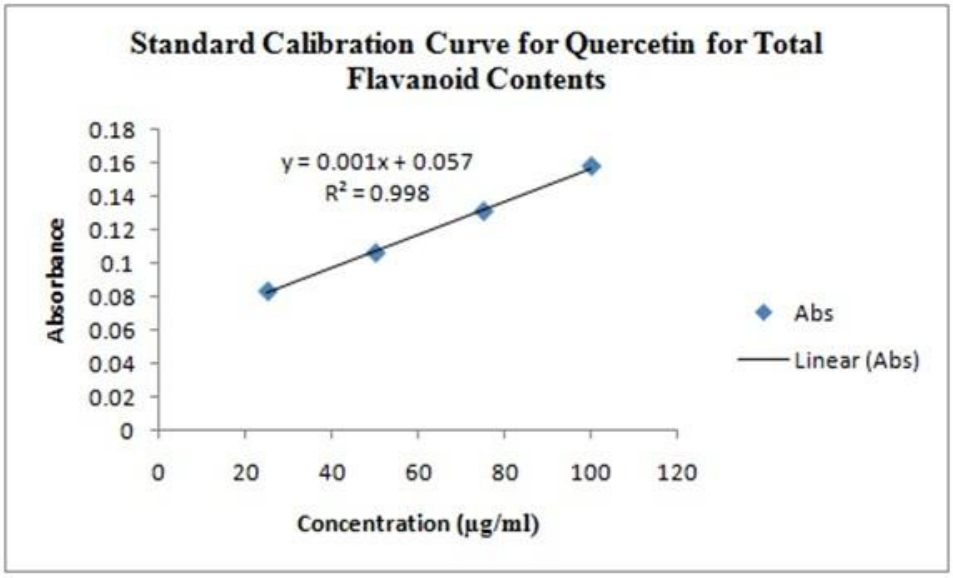


Fig. 6: Standard Calibration curve for Total flavanoid contents for standard Quercetin

Results for Total Flavanoid content

The Total flavanoid contents in n-Hexane extract of *Citrus macroptera* (HECM), Chloroform extract of *Citrus macroptera* (CECM) and Ethanol extract of *Citrus macroptera* (EECM) 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 Figure 6. The total phenolic contents for HECM, CECM and EECM were obtained for 1000 µg/ml of extracts from Total flavanoid content calibration of Quercetin and the result are given in Table 3. The Total flavanoid content for HECM, CECM and EECM were calculated using standard calibration curve (y=0.001x+ 0.057, R^2=0.998) and found to have 186.3±15.28, 99.67±15.28, 333.0±36.06 equivalent of Quercetin respectably.

Table 3: Total Flavanoid contents of different *Citrus macropters* fruit peel extracts

Extract	Concentration	Total Flavanoid Content (mg/g)
ECL	1000(ug/ml)	186.3±15.28
CECL	1000(ug/ml)	99.67±15.28
EECM	1000(µg/ml)	333.0±36.06

Values are in Mean ±SD for three readings

DISCUSSION

The fruit peels of *Citrus macroptera* were made coarse powder and extracted with using n-hexane, chloroform and ethanol as solvents using standard procedure. It is found that percentage yield for ethanolic extract is more than other solvent extracts. The difference in yield in ethanolic extract is due to presence of different phytoconstituents present in ethanolic extracts and different extractive values revealed the solubility and polarity particulars of the metabolites for particular solvent.

The various extracts of *Citrus macroptera* fruit peels 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 [29]. 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 [30-31]. 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* [32].

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) [33-34].

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 [35]. 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 *Citrus macroptera* (HECM), Chloroform extract of *Citrus macroptera*(CECM) and Ethanolic extract of *Citrus macroptera*(EECM) fruit peels were detected and compared with Ascorbic acid. The IC_{50} values for DPPH assay of for ethanolic extract was found maximum followed by hexane 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 phytoconstituents. Thus, choosing the appropriate solvent is one of the most important factors in obtaining extracts with a high content of bioactive compounds and antioxidant activity [37].

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 *Citrus macroptera*(HECM), Chloroform extract of *Citrus macroptera*(CECM) and Ethanolic extract of *Citrus macroptera*(EECM) fruit peels were detected and compared with Ascorbic acid. The ethanolic extract showed highest reducing power followed by hexane 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].

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 [39]. The hydrogen peroxide scavenging activity of n-Hexane extract of *Citrus macroptera*(HECM), Chloroform extract of *Citrus macroptera*(CECM) and Ethanolic extract of *Citrus macroptera*(EECM) fruit peels were detected and compared with Ascorbic acid. The IC_{50} values for hydrogen peroxide scavenging activity of for ethanolic extract was found maximum followed by hexane 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 [39].

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 [40]. The Nitric oxide scavenging activity of n-Hexane extract of *Citrus macroptera* (HECM), Chloroform extract of *Citrus macroptera* (CECM) and Ethanolic extract of *Citrus macroptera* (EECM) fruit peels were detected and compared with Ascorbic acid. The IC_{50} values for nitric oxide scavenging activity of for ethanolic extract was found maximum followed by hexane 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 *Citrus macroptera*(HECM), Chloroform extract of *Citrus macroptera*(CECM)

and Ethanolic extract of *Citrus macroptera* (EECM) fruit peels were estimated using standard Gallic acid equivalent of phenols. The ethanolic 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 [41].

In total Flavanoid content, it was found Ethanolic extract to posses maximum amount equivalent of Quercetin then other hexane and chloroform extracts. Flavonoids play some important pharmacological roles against diseases, such as cardiovascular disease, cancer, inflammation and allergy. Epidemiological studies have indicated the relationship between flavonoid intake and reduced risk of certain cancers [42].

From, above discussion, it was clear that the most powerful anti-oxidant extract is ethanolic extract of *Citrus macroptera*(EECM) fruit peels.

CONCLUSION

By performing the above work, it can be concluded that *Citrus macropter*) fruit peels extracts possess anti-oxidant activities and the potency of anti-oxidant activities depends on the type of extract. The is ethanolic extract of *Citrus macroptera*(EECM) possess highest anti-oxidant activity *in-vitro*. This anti-oxidant power depends on total phenolic and flavanoid contents on particular extract. Finally it can conclude that ethanolic extract can be used for evaluation of pharmacological activities and further research is in process for evaluation of pharmacological activities for ethanolic extract of *Citrus macroptera* (EECM).

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