QUANTIFICATION OF TOTAL POLYPHENOLIC CONTENT & ANTI FREE RADICAL POTENTIAL OF SELECTED TRADITIONAL PLANTS OF CHITRAKOOT REGION

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Abstract: Globalization of herbal medicine system comes with the better advancement. Herbal plants are used as alternative medicines since ancient time to treat various infectious as well as metabolic diseases. Accumulation of free radicals in the body results in many oxidative stress related diseases. Plants are rich in various chemical compounds such as polyphenols, flavonoids, tannins, alkaloids, amines, terpenoids, carotenoids, vitamins, which act as antioxidants and scavenge free radicals thus minimizing their deleterious effects. The aim of this study was to evaluate in vitro free radical activity of methanolic extract of some traditional plant parts (leaves/fruits) of chitrakoot area. The free radical scavenging capacity was estimated by DPPH (2, 2-diphenyl-1-picrylhydrazyl radical) method. The total phenolic contents were measured using a Folin–Ciocalteu assay. *Datura stramonium* was observed to be potent free radical scavenger i.e. 34.63 ±0.007 µg/ml and lowest polyphenolic contents were present in *S. lycopersicum* i.e. 0.087±0.002 µg/ml equivalent to catechol. However strongest antioxidant activity (IC_{50} value 27.56µg/ml) shown by *C. maxima* while lowest IC_{50} value was given by *S. lycopersicum* (106.94 µg/ml).

Keywords: polyphenols, flavonoids, Antioxidants, free radicals, DPPH.

Introduction: Herbal medicines play a significant role in the treatment of various common ailments and diseases of about 72-80% population of the world. The importance of herbal medicines has been realized in India since time immemorial. India has a great ancient heritage of traditional medicinal plants. The materia-medica of India provides much information on ethnic folklore practices and traditional aspects of therapeutically important natural products[1]. India gave Ayurveda, the oldest golden book in herbal medicin system.

"**Jagatyevamanoushadham**..."

Acharya Charaka has clearly said that there is no plant which is not medicinal in this universe which opens a door for research [2].

Ayurveda elaborates the nature as a whole as the tool of its treatment. It is further verified by Charaka in the following statement.

"***NanaushadhibhutamJagatiKinchit***"- **C. S. 1:69**

Charak proclaims that there is nothing on the earth which is not a medicine. Apart from this, it is equally famous saying of our ancient Maharshisthat:

"***AmantramAksharamNasti,***
NastiDravyamanaushadham
AyogyahPurushamNasti,
YojakastatraDurlabhah"

Which reflects that there is no matter in the universe which is not having medicinal property. There are two streams of traditional systems of medicines are prevalent in India. The one is well developed documented and codified system of traditional medicines like Ayurveda, Unani, Siddha, and Homoeopathy and the other stream is of the folklore or ethnomedicins which has not been documented and codified[3].

Chitrakoot is a holy place for Hindu pilgrimage, which is situated in the northern region of Satna district of Madhya Pradesh. It has a very rich wealth of medicinal plants, which has also been described in our epics like Ramayana.

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Plants are best source for developing alternative drugs, extensively studied by advanced scientific techniques and reported for various medicinal properties viz, anticancer, antibacterial, antifungal, antidiabetic, antioxidant, hepatoprotective, hemolytic, larvicidal and anti-inflammatory activity[4].

Developing as well as developed countries utilize traditional plant medicines which possess phytochemicals or bioactive compounds that can be derived from barks, leaves, flowers, roots, fruits and seeds. These isolated bioactive phytochemicals include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids which provide certain physiological action on the human body to prevent chronic diseases. According to some recent studies, compounds such as flavonoids, isoflavones, flavones, anthocyanin, phenols and catechin play an important role in antioxidant activity with mechanisms involving both free radical scavenging and metal chelation. Free radicals are those powerful oxidant species, which contain unpaired electrons and can randomly modify important cellular biomolecules,

thereby causing mutation[5, 6]. Continuous generation of reactive oxygen species (ROS) inside the human body is responsible for chemical modification of important cell components such as DNA, protein, and lipids and ultimately oxidative damage of the cell. This indicates the deterioration of antioxidant defense system of the body[7]. According to the recent scientific investigation, activated oxygen is known as a major factor of chronic disease and degenerative disease such as diabetic metabolic disorder, cancer, inflammation etc[8]. The potentially reactive derivatives of oxygen and nitrogen radicals continuously produced in cells, are detoxified by free radical scavengers and antioxidants. Antioxidant based drug formulations are used for the prevention and treatment of diabetes[9].

Diabetic vascular complications are directly correlated with increased concentration of oxygen derived free radicals. This increase in free radical generation and subsequent oxidative stress are supposed to be involved in complications of diabetes. Some evidences also suggest that over generation of free radicals is a key factor in the generation of insulin resistance, diabetes and cardiovascular diseases.Search of new specific antioxidants is still ongoing that will be useful in progression and treatment of diabetes[10].

According to Sharaka Diabetes Caused By[11]

आस्यासुखं स्वप्नसुखं बधीनि ग्राम्यौदकानूपरसाः पयांसि।

नवान्नपानं गुडवैकृतं च प्रमेहहेतुः कफकृच्च सर्वम्।।

-चरक संहिता

Several investigations have been indicated that the free radical scavenging activities of some vegetables were highly correlated with their total phenolic contents. Free radical scavenging property of these polyphenolic contents is due to redox properties which play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxidation[7]. Therefore, it is important to evaluate free radical scavenging activity of plants used as traditional medicinal system to elucidate the mechanism of their pharmacological action. The main objective of this study were to determine the total phenolic contents of ten selected traditional plants (*S. tuberosum*, *S. lycopersicum*, *S. melogena*, *C. fructnes*, *D. stramonium*, *L. siceraria*, *M. charantia*, *C. lanatus*, *C. maxima* and *L. acutangula*) and to assay their free radical scavenging activity.

Material And Methode:

Collection of Plant Materials: The plant leaves/fruits of Potato (*S. tuberosum*), Tomato (*S. lycopersicum*), Brinjal (*S. melogena*), Cilli (*C. annum*), Dhatura (*D. stramonium*), lauki (*L. siceraria*), karela (*M. charantia*), tarbuz (*C. lanatus*), kaddu (*C. maxima*) and taroi (*L. acutangula*) were collected in March 2013 from Chitrakoot region and identified. All plant leaves/fruits were collected, washed with fresh water and dried under shade at room temperature separately. The leaves were powdered and stored separately in sterile and air tight container for further use.

Chemicals: Methanol, Water, DMSO, Tris-HCl, Folin & Ciocalteu's Phenol Reagent (FCP), Sodium carbonate and ascorbic acid were obtained from SRL, India. While 2, 2- Diphenyl-1-picryl hydrazyl (DPPH), Catechol were purchased from Alfa acer, Great Britain. All solvents were HPLC grade while chemicals were AR grade and used without further purification.

Preparation of Plant extracts: 100 mg powdered plant samples of plant leaves/fruits were extracted with 10 ml HPLC grade methanol through open air reflux process at 40°C for 6 hours till dried than make the volume again 10 ml with methanol and reflux, this process was repeated several times. The extracts were filtered through filter paper (Watman no.1) to remove free un-extractable substances. The filtrates of plant extracts were evaporated at room temperature upto dryness, finally dissolved with 10 ml with DMSO and preserved at 4-5°C for further process.

Determination of Total Polyphenolic Content: Total polyphenolic content of extracts of plant leaves/fruits were measured using Folin-Ciocalteu reagent method, adopted as it is described by Tripathi et al.[1] (2013). The 25 µl of plant extract diluted with 125 µl water followed by addition of 150 µl of Folin-Ciocalteu reagent (1N) & 25 µl of Na_2CO_3 (20% w/v) incubated at 45°C for 60 min absorbance was measured spectrophotometrically at 765 nm (Synergy H_4 multimode micro plate reader, Biotek instrument, inc Winoosci, VT, USA). Quantification was performed with respect to the standard curve of Catechol. ($y= 0.003X+0.023$, $R^2 =0.964$) results were expressed as milligram of catechol equivalent per ml of extract.

Representation of therapeutic value and leading phytochemicals of selected ten plants					
S.No.	Plant (*Botanical name*)	Family	Therapeutic value	Leading phytochemicals	Ref.
1.	Brinjal (*S. malongena*)	Solanaceae	α-glucosidase inhibitory activity, antioxidants activity, .moderate to high angiotensin I-converting enzyme inhibitory activity, inmanagement of type 2 diabetes and hypertension.	**Phenolic compounds** - Ncaffeoylputrescine,5-caffeoylquinic acid, and 3-acetyl-5-caffeoylquinic acid **Flavonols compound**-quercetin-3-glucoside, quercetin-3-rhamnoside, and myricetin-3-galactoside are also reported in its pulp	22
2.	Lauki (*Ls. siceraria*)	Cucurbitaceae	Cytotoxic activity, antimicrobial activity, antidiabetic, antihyperlipidemic, antihepatotoxic, analgesic, CNS activity, anticancer, cardioprotective, anti-inflammatory, immunomodulatory and antioxidant	**Cucurbitacins**- B, D, G and H **Sterols** - campesterol and sitosterol **Other compound**- fibres, and Polyphenol	31 32
3.	Kaddu (C. maxima)	Cucurbitaceae	diabetic, antitumor, antihypertensive, anti -inflammatory, immunomodulatory and antibacterial agents	**carotenoid compounds**- lutein, zeaxanthin, β–cryptoxanthin, and β–carotene	35
4.	Tomato (S. lycopersicum)	Solanaceae	prevention of some chronic diseases, including cardiovascular diseases (CVD) by decreasing total cholesterol, lipid peroxidation and inflammation	**Lycopene**-(HLY 13, HLY 18 and Lyco 2), **phenolic compounds**- (chlorogenic acid, caffeic acid, rutin and naringenin	33 26
5.	Potato	Solanaceae	Suppressed lymph-node carcinoma of the prostate (LNCaP) and prostate cancer-3 (PC-3) prostate cancer cell proliferation and induced apoptosis via caspase-dependent and caspase-independent pathways	Anthocyanin, Chlorogenic acid, caffeic acid, gallic acid, catechin, malvidin, and glycoalkaloids (α-chaconine and solanine)	34
6.	Taroi	Cucurbitaceae	anti-proliferative effects, Cures vata, kapha anemia, asthma, jaundice, leucoderma, tumors useful as diuretic and in splenic enlargement	**colocynthin, saponins –** acutoside A, B, C, D, E, F, and G. **cucurbetacin**- B and E **Other compound**- oleanalic acid and 1,8 dihydroxy-4-methylanthracene 9,10-dione, luffin,	36 41
7.	Chilli	Solanaceae	Decreased risk of cancer, heart disease and degenerative disease associated with ageing, antioxidant, antimicrobial activity	**alkaloid** - capsaicinoids	37
8.	Dhatura	Solanaceae	anti-inflammatory property, stimulation of the central nervous system, respiratory econgestion, treatment of dental and skin infections, alopecia and also in the treatment of toothache7	**Tropane alkaloids**- hyoscyamine and scopolamine	24
9.	Karela	Cucurbitaceae	improvement in glucose tolerance against Type II diabetes and reduction in blood cholesterol, anticancerous and antileukemic activity, expel intestinal gas, for tumors wound treatment, rheumatism, malaria,vaginal discharge and the seeds are used to induced abortion	Polypeptide-p, triterpens, trehalose and momordin, p- insulin, v-insulin, lutein lycopene, cucurbitacin, and MAP30	38 39
10.	Tarbooj	Cucurbitaceae	Anti-Inflammatory Activity, Antimicrobial activities, Antigiardial activity, Anti-*Prostatic Hyperplasia* activity, Antisecretory Effects, Anti-diabetic activity, Anti-ulcerogenic property, anti- aging	Citrulline,	40

DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay: The assay for free radical DPPH was done by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method adopted as it is described by Tripathi et al.[1] (2013). In brief, a 96-well microplate, 25µl of various dilutions of methanolic extract 125 µl of tris –HCl buffer (0.1M, pH 7.4) and 125 µl of DPPH solution (0.004 in methanols) were added. The reaction mixture was shaken well. The DPPH decolorisation was recorded at 517 nm on a Biotek Synergy H_4 hybrid multimode micro plate reader after 30 min incubation in dark. The percentage of DPPH scavenging by plant extracts obtained in terms of ascorbic acid equivalent concentration. Quantification was performed with respect to the standard curve of ascorbic acid (Y= 0.733X + 14.6, R^2= 0.948). Results were expressed as milligrams of ascorbic acid equivalent per ml of the extract.

Statistical Analysis: Data reproduced during the experimental work, were analyzed using Origin Pro8.5 software. All parameters were triplicatly recorded for accurate result. Tables (1-3) show mean value and standard deviation (±) of reproduced data. Graphs (1-2) were also plotted using ANOVA software.

Result: It is known that numerous fruit and vegetables contain high amount of polyphenols which are beneficial for human helth[12]. Due to their scavenging properties, polyphenolic compounds present in fruits, vegetables, spices and hearbs are prominent source of antioxidants[13]. Folin-Ciocalteu reagent was used to conduct TPC assay in this study. Methanolic extracts of the tested samples showed noticeable amount of polyphenols. *D. stramonium* contains the highest amount of total polyphenolic contents i.e. 34.64 µg/ml, followed by *C. lanatus*, 27 µg/ml. *S. melongena* and *L. siceraria* expressed the same value of TPC i.e. 21 µg/ml. *C. annum* and *C. maxima* also found to contain approximately same amount of polyphenols i.e. 16 µg/ml whereasTPC value of *M. charantia* was12 µg/ml. *L. acutangula* showed 7 µg/ml, and and *S. tuberosum* showed 6.7 µg/ml total polyphenolic value. Least amount of total polyphenols was estimated in *S. lycopersicum* i.e. 0.0871 µg/ml. (**Table 2**)

DPPH scavenging activity is the most common and reliable method to assay antioxidants. Here, free radical scavenging detection is done with the help of the scavenging activity of a stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical. Among the studied plants, *C. maxima* found to possess strongest antioxidant activity with the IC_{50} value of 27.56 µg/ml. IC_{50} value of *L. acutangula* was 53.10 µg/ml followed by *C. lanatus* (IC_{50} value 57.23 µg/ml). IC $_{50}$ value of *M. charantia* was found to be 69.02 µg/ml, whereas *D. stramonium* showed 72.37 µg/ml followed by *C. annum* i.e. 88.88 µg/ml. Least antioxidant activity was shown by *S. lycopersicum* with the IC_{50} value of 106.94 µg/ml. In this study *S. melongena*, *L. siceraria* and *S. tuberosum* did not show any antioxidant activity (**Table-1 & Fig 1**).

Discussion: Plant derived medicines have made larger contributions to human health and well being by providing advance synthetic methodologies in developing several alternatives of several compounds with strong therapeutic potential[14]. Family Cucurbetaceae is primarily composed of vegetable crops and spices, consumed as food worldwide. Vegetables of cucurbetaceae are regularly taken as salad with daily meals[15]. *L. siceraria*, *L. acutangula*, *C. maxima*, *C. lanatus* and *M. charantia* are not only low cost and easily available vegetables but also have various medicinal uses. A wide variety of alkaloids is present in family solanaceae such as scopolamine, atropine, nicotine etc. which are being used in pharmaceuticals. Some widely used vegetables of this family are *S. lycopersicum*, *S. tuberosum*, *C. annum* and *S. melongena*, because of their availability and presence of many bioactive compounds with nutritional values[7]. The method of extraction was designed in such a way that maximum extraction of phyto-constitute (polyphenol) present in selected plant leaves/fruits, can be done with the help of methanol in an open reflection apparatus at 40 ^oC. Free radical scavengers have been reported to prevent oxidation damage cause by free radicals; it can interfere with the oxidation process by reacting with free radicals, chelating catalytic metals, acting as oxygen scavengers and thus helpful for the treatment of chronic diseases. Phenolic constitute of plants show various type of advantageous properties include antimicrobial, antiviral, anti-ulcerogenic and anti-inflammatory[16].

Percentage inhibition of DPPH scavenging activity of *L. Siceraria* fruit ethanolic extract were determined by S. L. Deora *et. al.* and they evaluated 79.12, 83.34, 91.03% inhibition at 20, 30, and 60 ml respectively. Total polyphenolic content of ethanolic extract of its fruit were found to be 79.43%[17]. Total polyphenolic contents present in methanolic extract of *C. maxima* was found 12mg/ml and its IC_{50} value for DPPH scavenging method was155 µg/ml[18]. Total antioxidant capacity by phosphomolybdenum method assay in aqueous extracts of *L. acutangula* and *M .charantia* were 6.5 µg and 29 µg of ascorbic acid /ml of extract respectively[19]. Whereas total polyphenolic contents present in methanolic extract of *L. acutangula* and *M. charantia* were calculated to be 28.10±0.30mg/g and 8.22±0.21mg/g respectively [20]. In an experiment, DPPH scavenging activity of *C. lanatus* fruit was calculated to be17±0.33 and total polyphenolic

contents were found to be 16.94±0.32(mg of Gallic acid equivalent/100 gm of fruit)[21].

Kaur C, et al., described that phenolic constituents in Brinjal posses antioxidant effect, which is identified as major bioactive compounds in this plant[22]. Sumitra Chandra et al obtained that 31.71± 0.06 mg/ml total poly phenolic content in extract of *S.malongena*. The degree of discolourization indicates the scavenging potential of the scavengers. Recent study showed that the calyx part of *S. malongena* show strongest antioxidant activity [7, 23].

Many phyto compounds such as alkaloids, carbohydrates, fat, proteins and tannins have been reported to be present in different parts of *D. stramonium* which have been reported for highly therapeutic value. Plant shows various types of activities such as analgesic and antiasthamatic activity which may be due to the presence of the investigated active chemical constituents[16]. All parts of the plant are toxic but the highest amount of the alkaloids is contained in ripe seeds. They act as competitive antagonist of acetylcholine at peripheral and central muscarinic receptor sites dhatura[24]. The primary biologically activesubstances in *Datura stramonium* are the alkaloids atropine and scopolamine. Atropine has been used in treating Parkinson's disease, peptic ulcers, diarrhea, and bronchial asthma[25]. Tomatoes contain several bioactive antioxidant compounds such as vitamin C and E, phenolic compounds, high content of hydrocinnamic acids (mainly caffeic acid and its ester chlorogenic acid) and flavonoids such as rutin and narigenin. Several studies have indicated these compounds responsible for the prevention of some chronic diseases, including cardiovascular diseases (CVD)[26].

Potato is rich in chlorogenic and caffeic acid, catechin and also contain glycoalkaloid compounds[27]. It is known that potatoes (mainly the pigmented varieties) are rich sources of anti-oxidants. Lamos et al., obtained clearly demonstrate the presence of bioactive compounds in the uncooked *Purple Majesty* potato and that cooking interferes with the level of these compounds. There are some contradictory results related to the effect of cooking on the levels of anthocyanins and total phenolics. Some investigations have shown that their consumption can decrease the risk of chronic diseases, such as heart disease, type2 diabetes and cancer[28].

C. annum are extremely popular for the vast content of vitamin C and total soluble phenolics higher than other vegetables commonly recognized as a source of these phyto compounds. Scientific research has established that, *capsicum annum*, is the only crop that produce alkaloid compound called capsaicinoids (phenolic pungent) Capsicum fruits have a great source of antioxidants compounds and contain capaicinoid (phenolic pungent) in higher amount[29, 30].

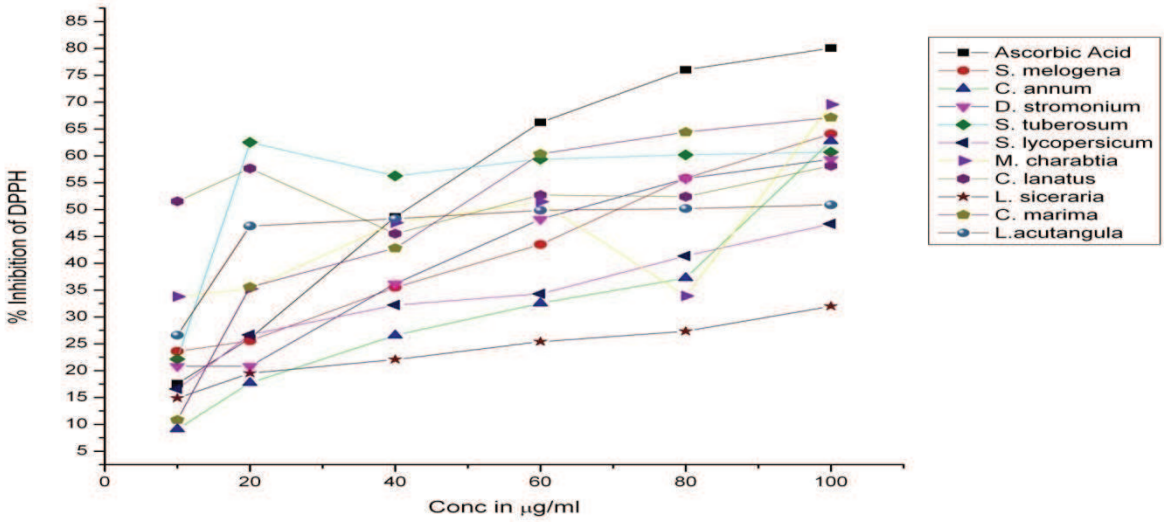
Table 1- IC_{50} values of fruit extracts

S. No.	Plants Name (Botanical name)	IC_{50} Values (µg/ml)
1	*L. siceraria*	-
2	*M .charantia*	69.02±0.004
3	*C. lanatus*	57.23±0.003
4	*C. maxima*	27.56±0.008
5	*L. acutangula*	53.10±0.03
6	*S. malongena*	Nill
7	*C. annum*	88.88 ±0.004
8	*D. stramonium*	72.37 ±0.006
9	*S.tuberosum*	Nill
10	*S. lycopersicum*	106.94 ±0.004
11	*Ascorbic Acid*	47.25±0.002

Table 2- Total polyphenolic contents present in fruit extracts

S. No.	Plants Name (Botanical name)	Total polyphenolic content value(µg/ml)
1	*L. siceraria*	21±0.003
2	*M. charantia*	12±0.001
3	*C.lanatus*	27±0.005
4	*C. maxima*	16±0.007
5	*L. acutangula*	7±0.001
6	*S. malongena*	21.34±0.004
7	*C. annum*	16.08±0.005
8	*D. stramonium*	34.64±0.007
9	*S.tuberosum*	6.7±0.005
10	*S. lycopersicum*	0.087±0.002

Conclusion: Various bioactive compounds such as polyphenols, flavonoids, terpenoids, tannins, vitamins alkaloids, amines, carotenoids, act as antioxidants and decrease the detrimental effects of free radicals. In present study Total Polyphenolic contents was evaluated as highest in methanolic extracts of *D. stramonium* (34.63 µg/ml±0.007) other than that *C. lanatus* (27 µg/ml), S. melongena (21.34 µg/ml±0.004), C. annum (16.08 µg/ml±0.005), *L. acutangula* (7µg/ml), S. tuberosum (6.7 µg/ml±0.005) and S. lycopersicum (0.0871 µg/ml±0.002). while lowest IC_{50} value was observed in *M. charantia* (60.02µg/ml).



Graph 1- Concentration v/s percentage inhibition of DPPH

C. maxima possess strongest antioxidant activity (IC_{50} value 27.56µg/ml) while lowest IC_{50} value was observed in S. lycopersicum (106.94 µg/ml). *S.melongena* did not show free radical scavenging activity but it contains medium amount of polyphenols. With the help of results obtained, we can conclude that traditionally used plants are richest source of free radical scavengers and may be efficient in preventing some chronic diseases. Further studies are needed to reveal some more medicinally important facts about these phytochemicals, thereby enhancing the chances of safe and natural drug discovery.

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